EXCRETORY PROBLEMS

IN FRESH-WATER FISH CULTURE

IN CIRCULATING SYSTEMS

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639.93WAT
203640 21 MAR 1977

A thesis
submitted to the University of Aston in Birmingham
for the Degree of Doctor of Philosophy

September 1976

SUMMARY

Increasing interest in intensive fish culture in tanks has led to a need for reliable estimates of excretory productivity by fish, especially with regard to ammonia, to facilitate the design of efficient water treatment equipment for recycling or effluent disposal. Two main aspects of this are examined: (a) realistic assessment of excretory productivity in the limited but multivariable tank situation; (b) criteria for allowable persistence of dissolved excretory products in closed systems, consistent with maintenance of fish growth.

Assessment of ammonia productivity was investigated using rainbow trout. Laboratory facilities were designed and built, and an approach to a fuller understanding of the problem worked out using multivariate analysis, resulting in a multiple regression model which satisfactorily described the experimental situation.

Under experimental conditions, the specific excretory rate of young rainbow trout could be related to environmental conditions by the following equation (parentheses denote 95% confidence limits for one observation):-

$$y = 5.7522 + 0.0427x_1 + 0.0002x_1^2 + 0.0414x_2^2 + 0.4165x_2x_3 - 0.0014x_1x_4 (\pm 4.9783)$$

- where y = specific excretory rate, x_1 = fish number, x_2 = temperature, x_3 = mean free path, x_4 = stocking (mass/volume)

The implications of the technique are discussed, and it is proposed that the method used to derive the relationship is worthy of further application and refinement.

Under similar experimental conditions, a preliminary study was conducted, of the growth tolerance of trout to simulated recycled fish effluent, and a basis provided for

future investigation thereof. Small differences in growth patterns were found between treated and control fish for two strengths of simulated effluent, lending limited support to the concept of growth stimulation at low concentrations of otherwise apparently toxic substances. The details and implications of this are discussed.

The overall context of the work is discussed, and related to the concept of stress in fish culture. Suggestions are made for future experiments.

This work is dedicated to the love and memory of my mother.

ACKNOWLEDGMENTS

Many people have helped me, both directly and indirectly, in the completing of this work. At Aston, my thanks are due to Professor Matty for originating the project; and to Messrs. R. Burns, A. Lawrence and J. Berwick, who gave much technical assistance. Acknowledgments are also due to the Science Research Council, for financial support; to Shearwater Fish Farming Ltd. (B.O.C.) for their generous financial support in the latter stages; and to Dr. John Forster, of M.A.F.F. and Shearwater, for advice and encouragement.

Special thanks must go to my supervisor, Dr. Chris Thornburn of the Biology Department, for his cheerful encouragement and assistance for so long, especially in the latter stages of production of this thesis; and also to Dr. John Aston, of the Metallurgy Department, for his invaluable aid with statistical and computing techniques, and unstinting sacrifice of the hours involved.

No acknowledgment here can possibly repay what I owe to my main colleagues in the Fish Culture Unit: Philip Smith, whose drive, dedication and comradeship from the beginning was directly responsible for most of what we achieved in establishing the Unit; and John Roberts, whose wit and companionship brightened the weary path of recurrent problems and frustrations. I am privileged to have worked with them both.

I must warmly thank Mrs. B. Smith, of Bramley, for typing the thesis with such despatch; and extend grateful thanks to all others who have helped in any way at all.

Lastly, my especial gratitude goes to my wife Christine, for her continual support and encouragement, and for suffering many more tribulations than I during the course of this work.

C O N T E N T S

Contents (continued)

ABBREVIATIONS AND CONVENTIONS USED

SI units or units derived from them are used throughout*; time of day is given in the 24-hour convention.

Standard biochemical abbreviations are used for well-known compounds (e.g. ATP), oxidation states, and stereo-isomers.

In statistical discussion:-

P	=	probability of result occurring due to chance: 1.0 represents 100% chance.
NS	=	not significant
*	=	significant at 5% level
**	=	significant at 1% level
***	=	significant at 0.1% level

(according to standard tables given in Bishop 1966)

r	=	simple correlation coefficient
R	=	multiple correlation coefficient

Simple abbreviations sometimes occur in Tables (e.g. TEMP for temperature, EXPT for experiment).

Most symbols used are explained in the text; the following list emphasises the most important ones:-

PO1, PO2, PO3	pilot experiments 1, 2 and 3
EO1... EO9	E-series experiments 1 - 9
TO1, TO2	T-series experiments 1 & 2
Lot	fish batch (as purchased)
FCAT	fish category (see Table 1)
HC	holding conditions

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* except for dimensions in Figures depicting developmental work in Chapters 2 and 3; materials were ordered and built using feet and inches, hence these units are retained.

Abbreviations continued

ST(S)	-	small)
MT(S)	-	medium) tank (system)
LT(S)	-	large)
SER	-	specific excretory rate
SREF	-	simulated recycled effluent

General abbreviations :-

(e.g.)	1.1	-	chapter and paragraph number
	Fig.	-	Figure
	para.	-	paragraph
	TMAO	-	trimethylamine oxide
	Q_{10}	-	rate change coefficient for 10 deg C rise
	LC-50	-	lethal concentration (50% mortality)
	EC-50	-	effective concentration (50% affected)
	P_i	-	inorganic phosphate radical
	K_a	-	equilibrium constant

$$(K_a = \frac{[H^+].[NH_3^o]}{[NH_4^+]} \text{ where}$$

a = dissociation constant)

SEM	-	standard error of the mean
ℓ	-	litre(s) [to avoid confusion with l]

PART 1

INTRODUCTION

1. GENERAL INTRODUCTION

BACKGROUND

1.1 The culture of fish for food is a practice undertaken by man in many ways and places, and one can accept that its earliest occurrence was probably a consequence of man's transition from a hunting style of life to the settled conditions of agriculture. China provides an example of a very ancient settled situation, and correspondingly furnishes the oldest records of fish culture (475 BC), concerned with the common carp (Hickling 1971).

1.2 Yet in another sense fish culture, especially in the form of fish farming, is a very modern asset in the struggle to supply man's food requirements; its occurrence and importance is rapidly growing, and in the United Kingdom alone interest, research and commercial commitment have sharply increased over the last decade, principally utilising high market-value species which will bear the required research and development costs.

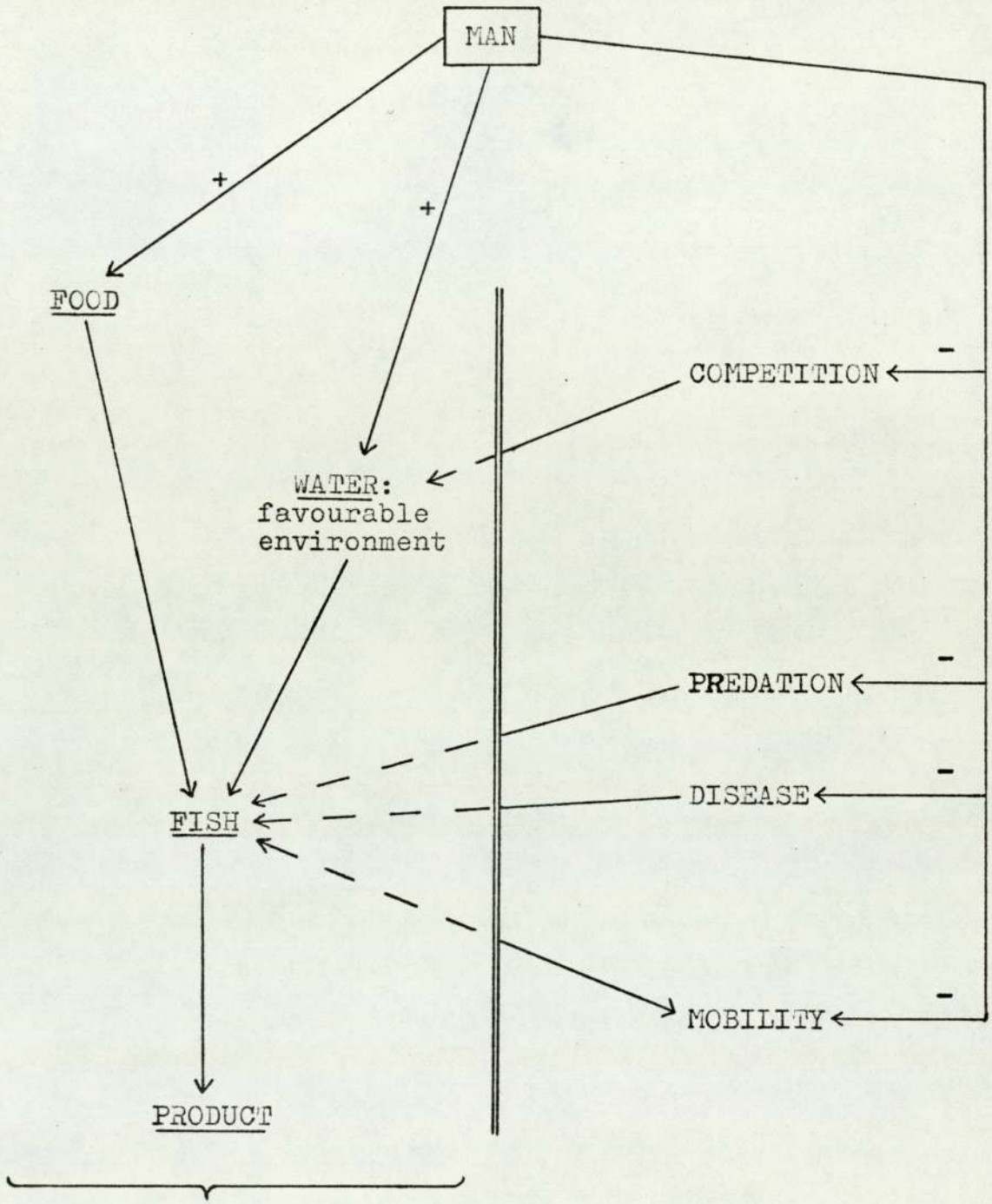
1.3 Much of the drive behind the recent surge in interest has come from the application of improved methodology and technology, some imported from the United States and some due to indigenous ideas and new equipment, together with a spirit of trial and investigation.

1.4 While fish culture may cover a wide-ranging group of activities (Hickling 1971, Fish Farming International 1973-1975), the concern of these studies is with one particular type of culture; the intensive rearing, in tanks, of salmonid fish. The exact definition of "intensive" is difficult, but for the present I shall take it to imply the production of large numbers of fish in an enclosure whose land area is considerably smaller than that which a "natural" distribution might require (where general behaviour would be unaffected by man). Salmonids have probably the fullest development history of all intensively cultured fish, largely due to the United States Bureau of Sport

Fisheries and Wildlife, whose research has generated salmonid rearing information for most of this century. All experiments documented here utilise the rainbow trout (Salmo gairdneri RICHARDSON), to which much American information applies, and which is currently the subject of the largest-scale British intensive fish-farming developments. One such development, that of Shearwater Fish Farming Ltd., is based on the employment of circular tanks at all stages of the rearing process, and it is with circular tanks that the work described has been carried out.

1.5 Foremost among the problems that the fish-farmer faces are the supplies of two vital ingredients, water and food. Like all animal culture, fish farming is essentially a function where a simplified path is chosen through a network of ecological interactions, resulting in man attempting to prevent certain interactions, while enhancing others (Fig.1.1). This simplistic model disguises a host of lesser problems, but emphasises man's particular role; he may enhance natural supplies or remove the natural supply and substitute it from another source. Food is frequently seen as the fish-farmer's first priority,and in many cases where the natural supply is substituted or greatly supplemented, it is his greatest commercial cost, although all of his activities may bear incidental costs.

1.6 Thus it is not surprising that nutrition has occupied a major place in fish culture research. Transition from early wet fresh-food diets for trout to modern dry pelleted feeds with easier storage, transport, handling and feeding requirements, has only been possible because of extensive research on fish nutritional requirements and correct diet formulations, spurred by the background of rising prices. Growth rates have been maintained or enhanced, but the principal cost involved remains the high protein requirement of salmonids, supplied largely from a world stock of fish meal which has recently become erratic and increasingly expensive (Smith 1976). Consequently a search has begun, in animal feed production generally,



+ = supply or enhancement effect

- = prevention effect

Figure 1.1 Some major considerations in the fish-farming situation

for substitute proteins which will yield equally good results at lower cost. Thus in trout nutrition at least, the emphasis has shifted from the basic ability to supply a convenient food, to a refining of technique, and while this is less true for the many other fish species at present under consideration for intensive farming, it is possible to feel that the major groundwork in this field has been performed.

1.7 However, when attention is turned to a favourable fish environment expressed in the form of water, it soon becomes clear that there are several topics of interest involved, and that the pattern of knowledge is frequently lacking or indeterminate. The position is further complicated because different systems of intensive rearing may require different husbandry rules. Thus information generated for use in the American raceway-based industry (Piper 1972, Liao 1970), may be much less useful in a high-flow circular tank system, particularly if oxygen enrichment is used to boost fish-loading levels.

1.8 Oxygen is usually the first limiting factor in trout production environments. Fish which are actively growing require abundant oxygen supplies, and the fish-carrying capacity will be limited at the point where fish oxygen consumption cannot be met by the oxygen present in the water supply.

1.9 Given that an environment suitably rich in oxygen can be provided, it has been increasingly felt that the next limiting factor involved is an inhibitory or "toxic" component (see Chapter 8) due to the fish themselves, identified with their excretory products. A variety of information has led to the conclusion that ammonia (the major excretory compound) is the offender. (Brockway 1950, Kawamoto 1961, Burrows 1964).

1.10 If excretory material is important in limiting fish-carrying capacity, it is clear that a second function of an abundant water supply for trout (besides oxygen supply) is the

removal of excreta as swiftly as possible so that each fish's local environment is continually renewed, and inhibition prevented. In this respect, in order to match water supply to requirement, the fish farmer needs to be able to estimate excretory production in his tanks, and manipulate either water supply or fish stocks to achieve the correct balance.

1.11 Since intensive rearing may entail great numbers of fish densely packed into relatively small volumes, it is clear that in many cases trout farming requires a large supply of water; the quantitative aspect of this will be discussed later, but the essential point at this stage is the setting of this requirement into a national background of a limited number of potential farm sites in the UK. Within the geographical limitation, the administrative limitations involved in operating a fish farm have been clearly stated by Cracknell (1974).

1.12 The recent response to these problems both in the USA and the UK has been to look forward to farms where water is recycled and the supplementary water requirement is hence minimised. This process carries many important implications for fish farms, but the aspect of greatest concern to this study is the waste product accumulation which is implied in such a system. At this point, a clarification of terminology may be useful; in this study, all systems where water is re-used (conventionally called recycled, recirculated or closed-circuit) are referred to as closed systems. The alternative, which may be referred to elsewhere as a single-pass, flow-through, open-circuit or discharge system, is here termed an open system.

1.13 While solid waste is the more visually-obvious problem of closed systems, the unseen problems of dissolved waste may be essentially more important. With the possible harmful effects of excretory products born in mind, it is clear that a fish farmer using recycled water must know the excretory productivity of his tanks. Only then can he efficiently design a water-treatment unit for incorporation into his system, knowing the

excretory loading it must deal with, and by reference to the water standard required for re-use, knowing the degree of efficiency required.

1.14 Unfortunately, the fish in a farm tank do not constitute a stable system; at the very least the farmer is promoting growth, and a range of other variables may be involved, possibly the most important ones having to do with the level of stocks carried. Thus a meaningful assessment of exretory productivity will only be one which takes into account these variables, and hence is able to express the prediction of excretory levels in operational terms. For many years such an approach was not forthcoming, but recently work at the Salmon Cultural Laboratory in Washington (Burrows 1964) and the Bozeman Fish Cultural Development Center in Montana (Piper 1972) has resulted in simple numerical guides for fish farmers. It is my submission that as trout farming begins to take a more scientific turn, and problems of the micro-environment of a fish-rearing tank begin to merit detailed study, it is of fundamental importance to attempt a fully scientific and comprehensive assessment of excretory productivity in the multivariable situation involved. The major part of this study is directed towards this goal.

1.15 With water re-use borne in mind, a secondary excretory problem is implied; as previously suggested, the filter efficiency required must be matched to some standard for the water delivered back to the fish. How clean must the filtered water be; or, what kind of water quality can the fish tolerate, consistent with the desired productivity? (Fig.1.2). Knowledge of the acute (lethal) toxicity of ammonia is well developed, but this information is too extreme: the interest is rather in the kind of excretory concentration where growth is affected. The terms become those of tolerance rather than toxicity, and the concept of median effective concentration (EC-50) affecting growth is more relevant (Webb & Brett 1972). Thus an attempt has been made during this study to provide basic guidelines, by

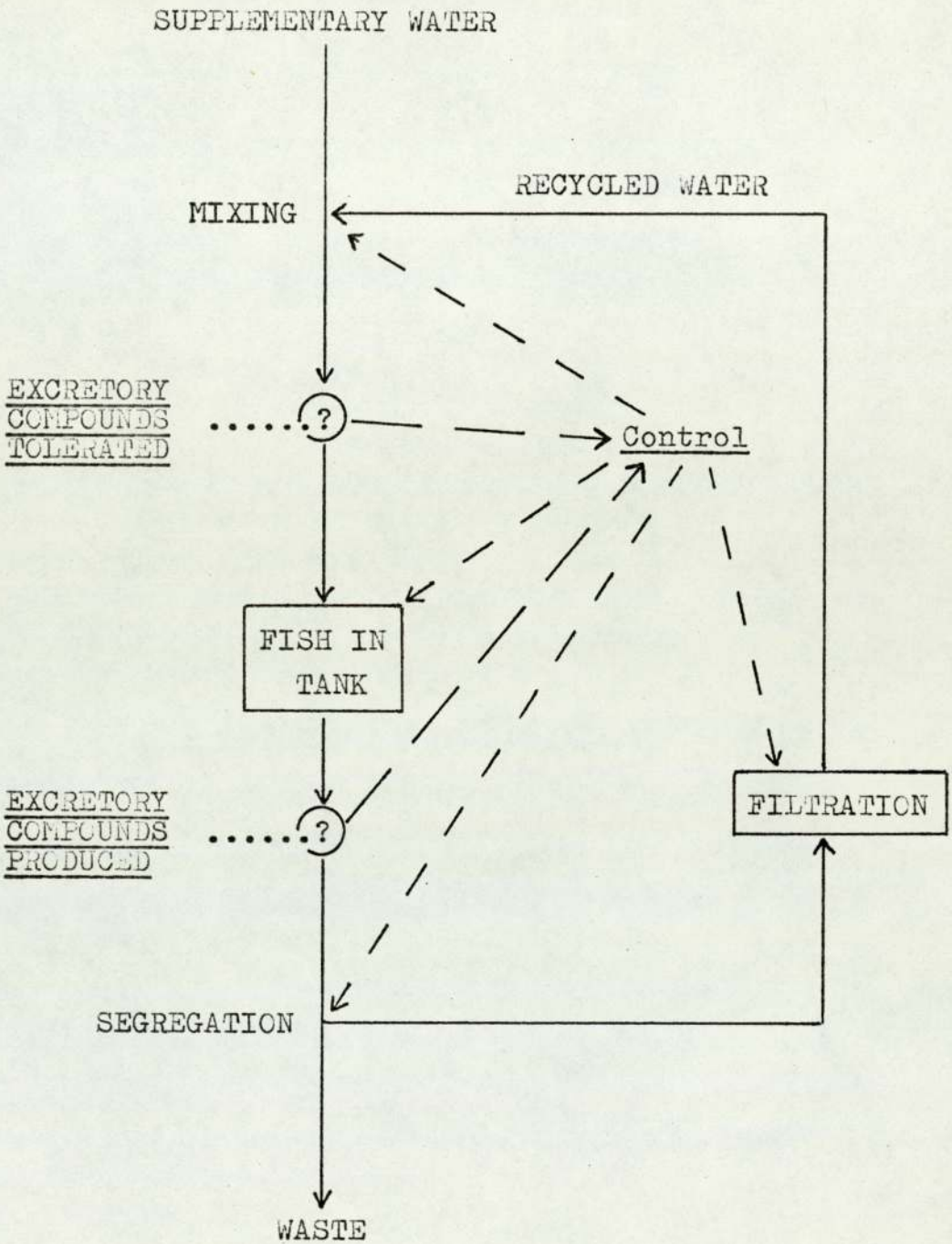


Figure 1.2 Fundamental control required in closed system operation

means of preliminary experiments, for an experimental approach to this problem.

1.16 Finally, this study discusses these problems in the general contexts of circulating systems (those where water is directed round a tank peripherally), closed systems, and the general fish culture environment. It is important to point out that small-scale work in the laboratory can be integrally different from the farm situation; the magnitude differential is expressed in many ways and the potentials of the two situations for measurements are also different. For this reason the aim of this work is to produce ammunition for theoretical discussion (supported by illustrative data), rather than to put forward the generated data in the form of authoritative statement. It is to be hoped that consideration of the ideas involved, in partnership with future experiments on full-scale research systems, will be of industrial or commercial benefit, while scientific interest may lie in the study of the production fish-tank environment as worthy of academic attention in its own right.

FISH AND HOLDING CONDITIONS

The rainbow trout

1.17 The experimental animal used throughout was the rainbow trout, Salmo gairdneri RICHARDSON. This spelling is used in preference to "S.gairdnerii" for simplicity, following McPhail & Lindsey (1970). This is a non-migratory fish, in contrast to the anadromous steelhead trout of the same species. Under experimental conditions the rainbow can be converted to living in sea-water, under which conditions it is said to grow faster. All work in this study was carried out in fresh-water.

1.18 This animal was used for a variety of reasons:-

a) its ease of availability,

b) its importance in commercial fish-farming, especially in current investigations into water-recycling,

c) its prominence in physiological, biochemical and fish-culture literature, providing a high level of general knowledge of the animal,

d) the importance of the salmonids as the fishes most sensitive to water pollution, and the extensive literature arising from toxicity studies.

1.19 The salmonid family belongs to the Order Salmoniformes, a sub-group of the Superorder Protacanthopterygii according to the classification of Greenwood et al (1966) (quoted in Alexander 1967). This Superorder represents a fairly primitive teleost group which may have given rise to most of the others. The primitive nature of the body shape is emphasised by the possession of an adipose fin without fin rays behind the main dorsal fin: this is typical of the Superorder. In contrast to more specialised fish which probably evolved later in other groupings, the paired fins of the salmonids are in the primitive positions; pectorals low down on the sides posterior to the gills; and pelvic fins just anterior to the vent. The salmonids in general are a group of relatively non-specialised, carnivorous, fresh-water and anadromous fish; the anadromous capability might also be considered fairly primitive.

1.20 Salmo gairdneri is readily identifiable from the other common British Salmo species, S.trutta (brown trout) and S. salar (Atlantic salmon), by its possession as adult of an irridescent streak along the flanks, a black-spotted green-brown mottled dorsal surface, and a red lateral band when in spawning condition (often clearest on the operculum). This contrasts with the silver appearance of the smolted S.salar, and the conspicuous red or orange dorsal spots on the brown trout. The parr of these species are less easy to distinguish (see McPhail & Lindsey 1970).

1.21 The rainbow trout is a native of the rivers of the North-West American seaboard, first described from the Columbia River; but it has in recent times been spread by man to many of the

temperate waters of the world, also into high altitude regions in lower latitudes (MacCrimmon 1971). The fish is said to have several intra-species varieties, the most commonly quoted being "Idaho" and "Shasta". The latter is characterised by autumn spawning (as opposed to spring), and Bernhart (1969) claimed to find haematological differences between these strains. Nevertheless, for most purposes the common strains are indistinguishable (save in spawning), and since no guarantee of strain could be given by the suppliers for the fish used in this study, no account will be taken of strain as a source of variability.

Holding conditions

1.22 Fish used were bought in as alevins, usually of between 3 and 7 cm length for growing up prior to experiments. Transportation was in large tied-off plastic bags placed inside plastic dustbins, half-filled with water from suppliers, and aerated continuously. On arrival, fish were placed in holding tanks in their own water, supplied with extra aeration via diffusers, and over a period of time, Birmingham tap-water was gradually allowed to flow in and displace the water in which the fish had travelled. In this way, minimum stress was associated with the transfer from hard, alkaline hatchery water to soft Birmingham water, and temperature shock was avoided. This routine kept initial losses of fish to a very few, and was usually followed by good feeding behaviour on the next day. Fish were retained in holding conditions for several weeks before being used for experiments.

1.23 Two major types of holding circumstances were used, designated HC1 and HC2 respectively. The main points of these conditions are summarised in Table 1.1.

1.24 During holding, populations in different tanks varied according to size, state of grading, cleaning activity, etc. but loading was usually maintained below 1.0 kg min l^{-1}, and reduced when high temperatures were encountered (following Burrows 1972).

Table 1.1 Details of holding conditions

DETAIL	CONDITIONS	
	HC1	HC2
Shelter	Outside (2nd-floor roof) but some protection from clear plastic "greenhouse" mounted over tanks	Inside (unheated wet laboratory)
Water supply	From main University building reservoirs, via reservoir tank on roof	From tap-water main, via constant head reservoir tank
Lighting Photoperiod	Natural	Artificial (controlled)
Tanks	3 x circular (250 ℓ), or 1 x rectangular (1000 ℓ)	6 x circular (250 ℓ)
Feeding	By hand, usually twice per day	Automatically, to schedule, five times per day
Disturbance	Occasional; by wind effects, drop in water flow, people	Rare (tanks screened); only by experimenter
Siting	Large distance from holding tanks to experimental system	Experimental system in same room
Grading	Occasional	Regular

1.25 Birmingham tap-water is chemically soft (see below), and originates mostly from the Elan Valley catchment area in Wales. It is brought directly to Birmingham with a small proportion (7%) of River Severn water added. Thus water treatment before addition to the mains supply is minimal; in particular the water is low in chlorine content. At all times fish were found to live and grow well in tap-water whose only form of treatment was a boost in aeration by spray discharge into a header tank before being conducted to the fish. In this respect the timing of the work was fortunate, since in the future greater proportions of Severn water will be required, necessitating more treatment and higher chlorine levels. The chemical nature of the water was described by the local Water Authority as in Table 1.2; periodic tests at Aston are in good agreement, but regular measurement of pH normally gave values between the minimum and mean values quoted.

1.26 Water supplies were at the approximate rates of 8 $\ell\ min^{-1}$ (HC1) and 9 $\ell\ min^{-1}$ (HC2), but this was liable to fluctuation in HC1. A constant-head tank kept the flow steadier in HC2, but in both cases the flows to individual tanks were adjusted according to the populations held at any particular time.

1.27 In the case of the 1000 ℓ tank in HC1, flow was introduced at one end, and the water was lost by over-flow from an exit port at the other end, thus creating a linear flow. In the 250ℓ circular tanks water was peripherally introduced at an angle, circulated round the tank, and left by a central exit hole, sweeping out faeces and any uneaten food in a self-cleaning action; the water was brought up by a U-tube beneath the tank, to overflow from the U-tube beside the tank, at a height which governed water level (and hence volume) inside the tank. U-tubes were regularly cleaned out to prevent debris accumulation. Fish normally swam against the direction of flow; this is well shown in Fig.3.14 in Chapter 3.

Table 1.2 Chemical nature of Birmingham tap-water

(Results in mg ℓ^{-1} except for pH) (from CBWD 1973)

CHEMICAL	MEAN	MAXIMUM	MINIMUM
Ammonia -N	0.042	0.208	nil
NO_2^- -N	< 0.001	0.004	nil
NO_3^- -N	< 0.5	2.4	trace
pH	7.65	8.60	6.80
free CO_2	1.3	3.0	nil
Carbonate) Hardness	12	16	9
Total) (as $CaCO_3$)	23	29	18
Calcium)	16	20	10
Fe	0.17	0.46	< 0.04
Cu	0.005	-	-
Zn	0.02	-	-

1.28 Fish were restrained in the 1000ℓ tank by a hinged wooden lid, opened for feeding. (The translucent tank sides admitted light from outside). The circular tanks were fitted for HC1 with flat covers of 6 mm square mesh green Netlon (see Chapter 3) bolted to the flange round the tank lip. Food could be dispensed through the mesh, or a small hole cut in it would allow demand feeder operation. For HC2 restraint was as described in Chapter 3.

1.29 Photoperiod, or day-length, was natural for HC1. Since the tanks were outside, daylight effect would last for a time varying from about 8h minimum during December to about 16 maximum during June, with gradual daily shifts between these points. Combined with seasonal temperature changes, this fluctuation would allow any seasonally-controlled metabolic effects in the fish to vary fully, and would, further, cause disjunction on the removal of fish to the indoor experimental system with its controlled photoperiod. In the improved HC2, fish were held indoors under a controlled 12h photoperiod with artificial lights. Since all main experiments were performed under this regime, no disjunction occurred. However, the HC2 photoperiod was displaced from a natural photoperiod; it began at 0800 and finished at 2000, whereas the natural 12h photoperiod would be from about 0600 to 1800. Thus the displacement was by 2h in a late direction, with "mid-day" occuring at 1400.

1.30 The effect of the HC2 photoperiod on fish would be to suppress the daily "re-adjustment" which is postulated to occur in natural inherent biological clocks (Lofts 1970). However seasonal information would still be partly available by means of the water temperature. Under these conditions, photoperiod was removed as a possible source of variability, and seasonal effect was minimised.

1.31 Feeding in holding tanks was conducted according to Table 1.3. This is a chart for use with CNP feeds, adapted from Deuel et al (1952), and is based on increases in % of body weight fed

Table 1.3 Feeding chart for CNP Beta Salmon and Trout feeds

FCAT	1	2	3	4	5	6	7	8	9	10
WEIGHT (g)	<1.5	1.5-5.1	5.1-12.0	12.0-23.0	23.0-39.1	39.1-61.7	61.7-91.7	91.7-130.6	130.6-179.2	179.2+
LENGTH (cm)	2.54-5.08	5.08-7.62	7.62-10.16	10.16-12.70	12.70-15.24	15.24-17.78	17.78-20.32	20.32-22.86	22.86-25.40	25.40+
TEMP (°C)										
3	2.4	2.0	1.7	1.3	1.2	0.95	0.8	0.7	0.65	0.6
4	2.5	2.0	1.7	1.4	1.2	1.0	0.8	0.75	0.7	0.6
5	2.7	2.2	1.8	1.5	1.3	1.1	0.9	0.8	0.75	0.65
6	3.0	2.4	2.0	1.6	1.4	1.2	1.0	0.9	0.8	0.7
7	3.3	2.6	2.2	1.7	1.5	1.3	1.1	1.0	0.9	0.8
8	3.5	2.8	2.3	1.8	1.6	1.4	1.2	1.1	1.0	0.9
9	3.9	3.1	2.6	2.0	1.8	1.5	1.3	1.2	1.1	1.0
10	4.2	3.3	2.7	2.1	1.9	1.6	1.4	1.3	1.2	1.1
11	4.6	3.6	3.0	2.3	2.0	1.8	1.5	1.4	1.3	1.2
12	4.9	3.9	3.1	2.4	2.2	1.9	1.6	1.5	1.4	1.3
13	5.3	4.2	3.4	2.6	2.4	2.1	1.8	1.6	1.5	1.4
14	5.5	4.5	3.6	2.8	2.5	2.2	1.9	1.7	1.6	1.5
15	6.0	5.0	3.9	3.1	2.7	2.4	2.1	1.9	1.8	1.7
16	6.5	5.3	4.2	3.4	2.9	2.5	2.2	2.0	1.9	1.8
17	5.5	4.5	3.6	2.9	2.5	2.2	1.9	1.7	1.6	1.5
18	4.5	3.8	3.1	2.3	2.1	1.8	1.5	1.4	1.3	1.2

Notes:-

a) All figures in FCAT columns in the lower part of the table are % BWD (% of body weight fed per day).

b) During measurement, length readings were taken to the nearest o.5 cm, thus FCAT 1 embraced 3.0, 3.5, 4.0, 4.5, 5.0 cm, FCAT 2: 5.5, 6.0, 6.5, 7.0, 7.5 cm, etc.

per day (%BWD) with temperature to an optimum (16°C) and thereafter a fall, for any single size-category of fish (FCAT). With increase in size, %BWD at any given temperature falls. Thus the highest %BWD is fed to the smallest fish at the optimum temperature. It should be noted that the commercial chart prepared by CNP recognises a smaller size-category (not encountered in this study) and a separate brood fish category (also not encountered).

1.32 CNP also recommends which sizes of foods (Beta Salmon and Trout diets) should be fed to different sizes of fish. In practice the best test was acceptability to the fish, and the No.4 (floating) Trout diet was generally acceptable to fish in all categories from FCAT 3 upwards. All main experiments utilised this diet. Under normal circumstances, trout will feed both at the surface and off the bottom, so the sinking diets used for small fry (Beta Salmon No.2 and No.3) were not a drawback. Major characteristics of the diets (as published by CNP) are listed below:-

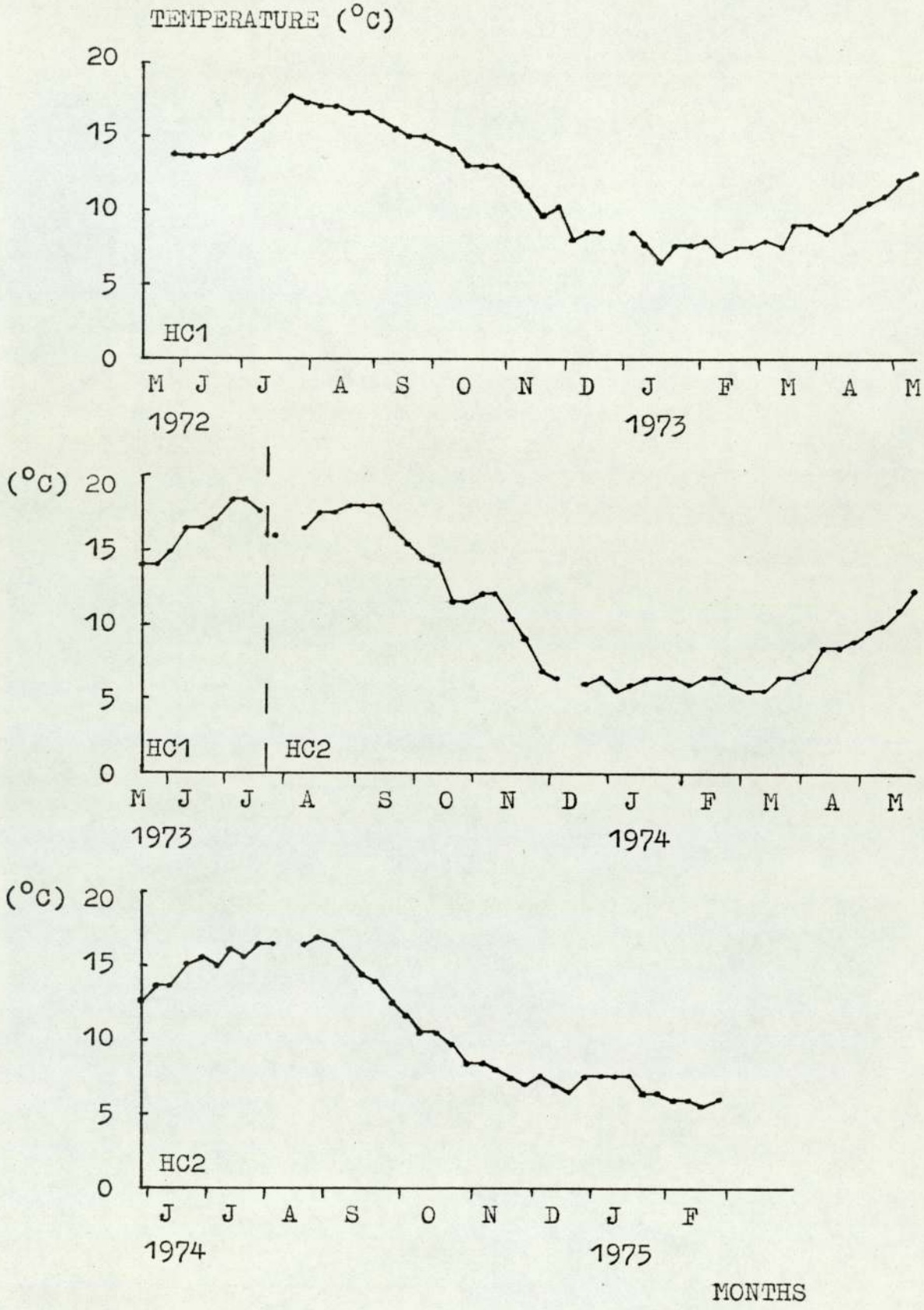
a) Salmon No.2 and No.3: sinking granules, 7.5% oil, 58% protein, 1.5% fibre, 2000 iu kg^{-1} vitamin A, 2000 iu kg^{-1} vitamin D, 36 iu kg^{-1} vitamin E.

b) Trout No.4: floating pellets, 4.5% oil, 40% protein, 4.5% fibre, 1000 iu kg^{-1} vitamin A, 2000 iu kg^{-1} vitamin D, 30 iu kg^{-1} vitamin E.

Trout No.4 pellets are cylindrical, approximately 3 mm long by about 2 mm diameter.

1.33 Since %BWD is controlled by temperature and size, records of temperature (and periodic re-grading of fish) were essential in determining feed rates. Average weekly water temperature was recorded throughout all work, and is shown in Graph 1.1.

1.34 Four batches of fish were used during the study, each designated by a Lot number (01,02,03,04). Table 1.4 gives details of utilisation of each Lot; all fish were supplied by Vortex (Donnington) Ltd. trout farm.



Graph 1.1 Temperature records

Table 1.4 Details of fish Lots and utilisation

DATE (wb)	LOT	No.of FISH	FCAT	HC		EXPERIMENTS USED FOR
22/5/72	01	480	1	1	until wb 16/7/73	P01,P02,P03
2/4/73	02	126	5	1		
16/7/73				2	Lots 01 & 02 pooled	BMP
3/12/73					Lots 01 & 02 written off	
10/12/73	03	1350	1	2		E00,E01,E02 E03,E04,P04, E05
26/8/74	04	1500	2	2		
11/11/74				2	Lots 03 & 04 pooled	E06,E07,E08, E09,T01,T02
24/2/75					Lots 03 & 04 written off	

Notes:-

a) wb indicates "week beginning".

b) Codes quoted in the final column (P01,etc.) refer to individual experiments, and are explained in Chapters 6,7 and 9.

c) "written off" indicated that fish were becoming too large for laboratory holding, or were otherwise finished with, and were passed on to colleagues for use in other work.

1.35 Fish handling was by different-sized hand nets of fine nylon mesh which were kept clean and wetted before contact with fish. Similarly, for manual handling of fish, wetted rubber gloves were worn: these precautions avoided damage to fish epidermis.

PART 2

DEVELOPMENT OF

EXPERIMENTAL SYSTEMS

2. DEVELOPMENT OF PRELIMINARY SYSTEMS

INTRODUCTION

2.1 An integral part of the work described in this account was the planning and construction of experimental facilities. At the commencement of the studentship (October 1971), facilities available comprised one 1000 ℓ rectangular fibreglass holding tank in an outdoor location supplied by a (shared) water line with maximum delivery about 16.5 ℓ min^{-1}; and half-share in an indoor area measuring 3.3 m x 2 m. Thus available building space was about 3.3 m x 1 m x 3 m height.

2.2 Before acquisition of a suitable laboratory in June 1973, an exploratory pilot system was built to cater for three small-scale pilot experiments, in order to provide background experience for the subsequent larger-scale facility.

2.3 In October 1971, a colleague, Philip Smith, also commenced work on applied fish culture research, and throughout the planning and building periods there was much common discussion, simultaneous usage of fish batches and mutual assistance, especially in designing the laboratory and common facilities required by both. However, all experimental work and decisions of experimental policy regarding this study, were carried out by this author alone, after due consultation with the research supervisor.

2.4 Planning, acquisition of equipment and building accounted for at least one-third of the time spent on the project work, with frequent uncontrollable delays in delivery of equipment and also the problems consequent upon the industrial 3-day week of early 1974.

PILOT SYSTEM

Basic plan

2.5 The pilot system took a proportionally longer period of time to construct than the later larger-scale system, due to

its necessarily evolutionary nature.

2.6 Requirements were for:-

a) controlled water supply to each of 8 small tanks,
b) controlled overflow-levelled drainage from each tank,
c) imposition of a controlled photoperiod,
d) isolation from as many uncontrolled external stimuli as possible,
e) ease of access to tank water for sampling,
f) containment of fish within tanks,
g) some ability to control, or at least modify, temperature variation.

2.7 The tanks were positioned in a row inside a rectangular trough which formed a water bath, and a water line from the outside supplied each tank from the mains. The central tank exits were connected through the trough bottom to U-tubes whose longer sides emerged outside the trough, where the U-tube overflows governed the tank water levels. (Fig.2.1) The whole system, except for U-tube overflows, was inside a light-proof enclosure whose roof housed the lighting system. Overflow drainage was to a pipe and drain-cup. A separate water circuit maintained the water bath at a controlled temperature: temperature exchange was by means of a Pyrex heat exchanger.

Tanks

2.8 Two small tank (ST) types were used in pilot experiments. ST Type 1 was a modified polythene bucket (Plysu brand) 25.5 cm high and of diameter tapering downwards from 28 cm to 20 cm. The tank was 10ℓ in capacity, coloured blue, with handle removed and bottom modified; the trough accommodated eight tanks, but only four were used at any one time. ST Type 2 was a modified polythene circular frozen-food contained (Ekco-ware brand), of 12ℓ total capacity, 15 cm high and 33 cm in diameter. It was translucent-white, and the tank rim was well below the rim of the trough when in position, making careful control of water-

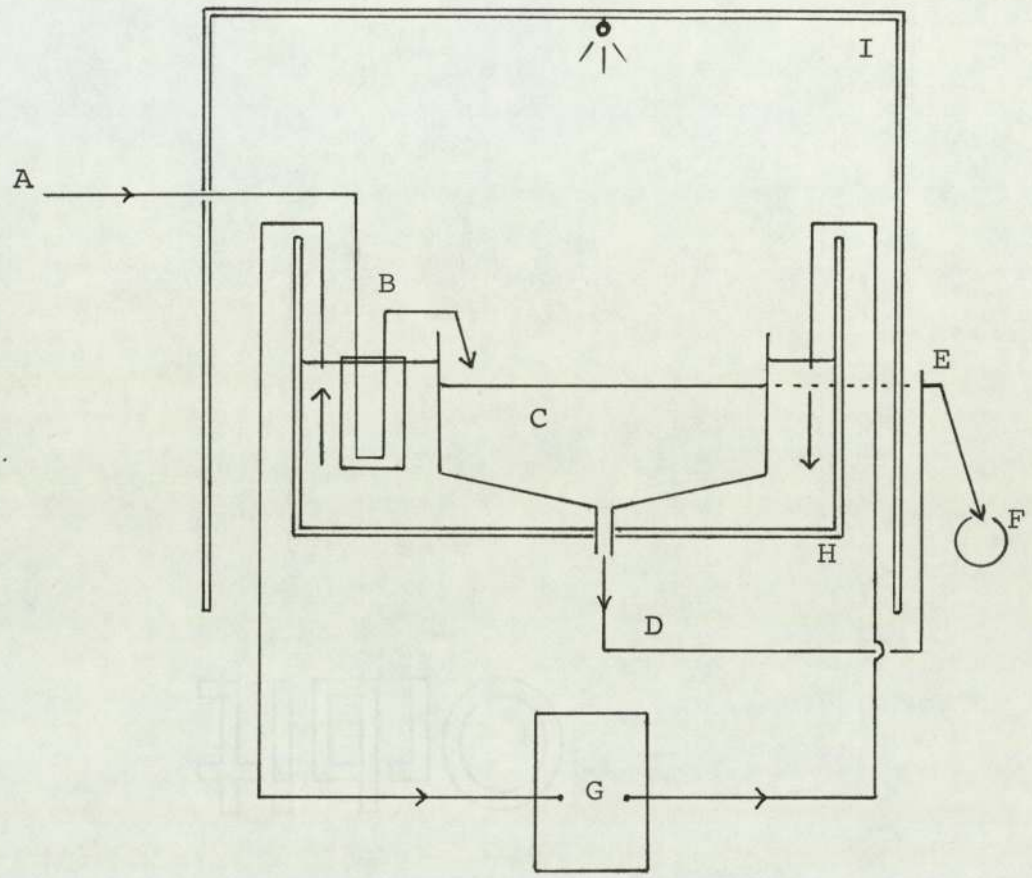


Fig. 2.1 Pilot system basic plan : cross-section

A = mains supply
B = heat exchanger
C = tank
D = U-tube
E = overflow controlling tank level
F = drainpipe
G = Temperature control unit
H = trough water bath
I = light-proof enclosure

bath level obligatory. Four suitably modified ST Type 2 could be accommodated. ST Type 1 were used for the first pilot experiment, but their lack of stability, restricted swimming room for fish, and poor hydraulic pattern (incomplete circular flow due to greater height than diameter), suggested that ST Type 2 were more suitable.

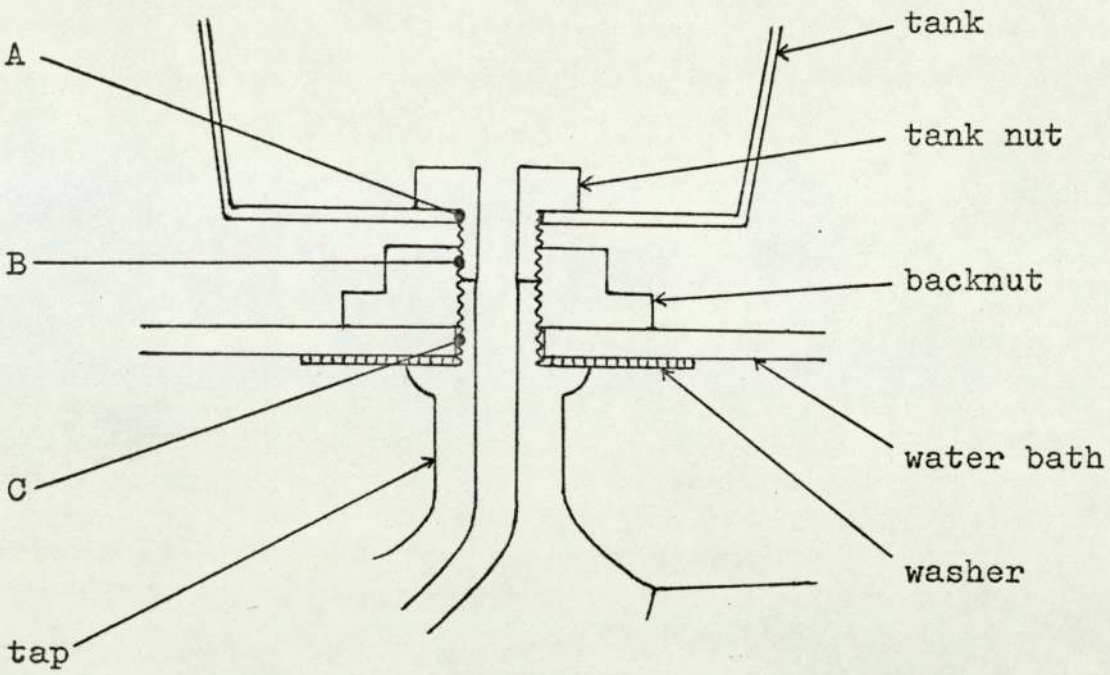
2.9 Each tank was fixed in position by a central tank sealing joint with a hole in the water-bath floor. Water left the tank through this joint, which also connected with the effluent U-tube. Since tanks were intended to be removable and interchangeable, the joint was necessarily complex, and Fig.2.2 shows both the first type (for use with ST Type 1) and the later, strengthened type used for ST Type 2. The early type allowed the tank to be unscrewed directly from a mounting, but potential leakage at points A, B and C suggested that a joint in which tank and trough-floor were clamped together was superior.

2.10 Fish were confined in the tanks by means of transparent hoods (large plastic bags) placed over the tank tops and secured (by waterproof adhesive tape) around the rims. For feeding and general inspection, tank access was via a hole in the top of the hood, otherwise the hood was tied off below the hole. Periodic checks were made of the surrounding water bath to ensure that no fish escaped. Figure 2.3 shows an ST Type 2 in position with hood furled back.

Arrangement of system

2.11 Fig.2.4 shows the system layout for pilot experiments; in this case P01, using ST Type 1.

2.12 Tanks were positioned, suitably spaced, inside the water bath so that their central drain points were in the mid-line of the trough's long axis. The fibreglass trough was internally 2.44 m long x 18 cm wide x 9 cm high, and was internally lined



a) EARLY TYPE (for ST Type 1) ↑

(not to scale)

b) LATER TYPE (for ST Type 2) ↓

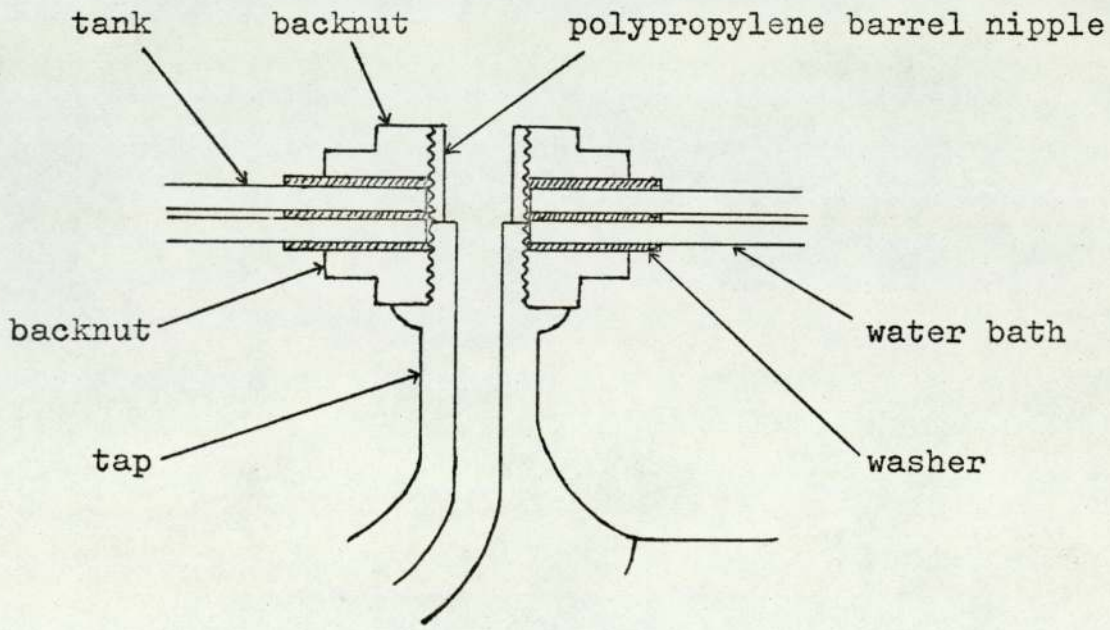


Figure 2.2 Pilot system tank sealing joints

Figure 2.3 (p.15)

ST Type 2 during pilot experiments

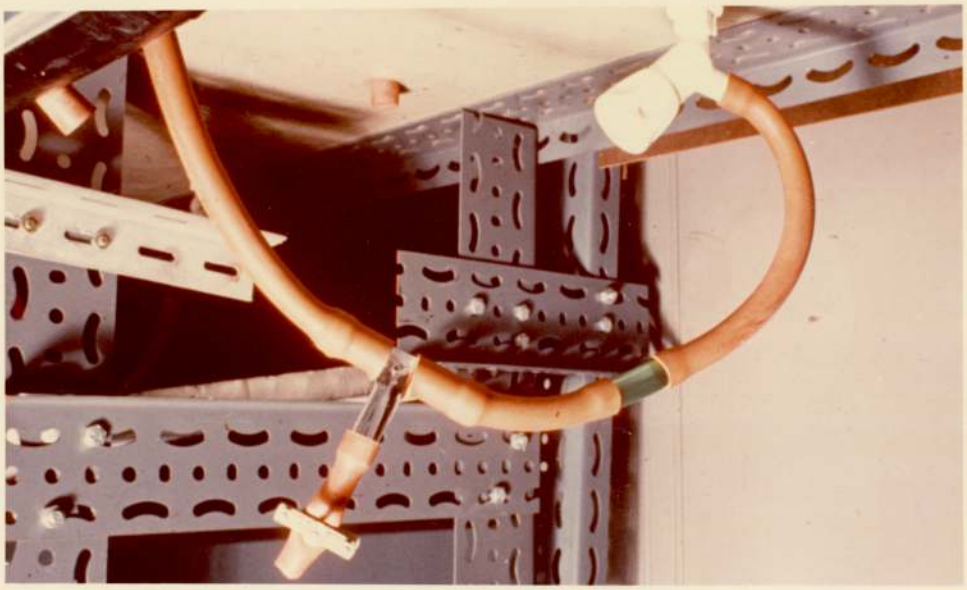
Figure 2.5 (p.16)

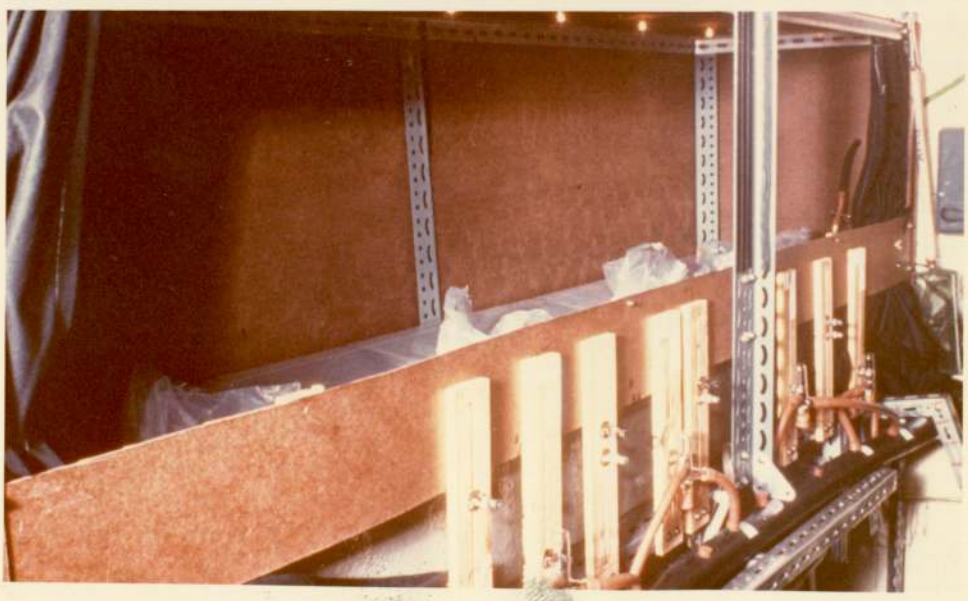
STS lower plumbing (pilot experiments)

Figure 2.7 (p.16)

Pilot system, front view

(overflow levelling controls at front)





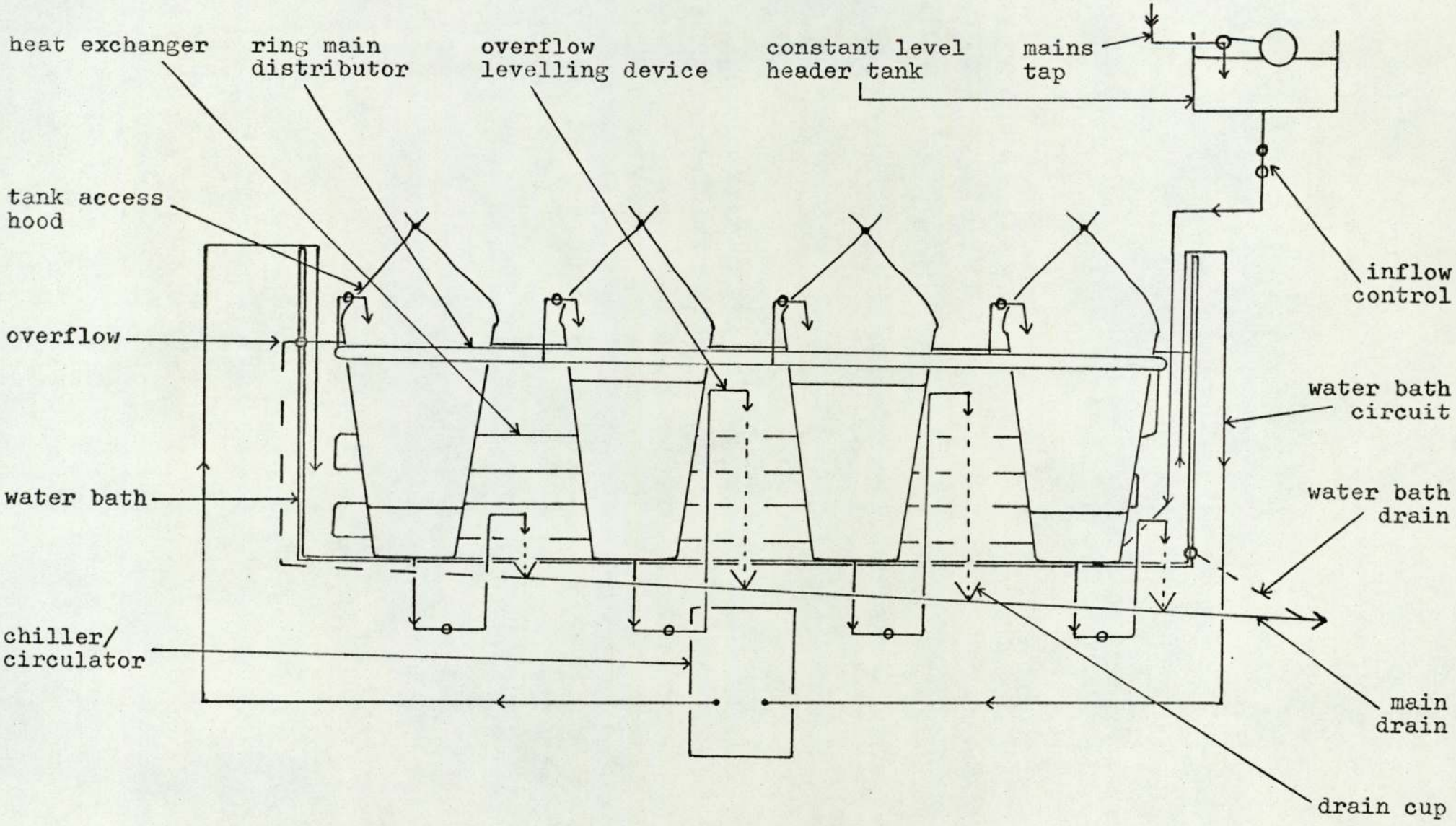


Figure 2.4 P01 apparatus layout

with PVC; it had two apertures at opposite ends, at suitable heights for overflow and drainage respectively (Fig.2.4).

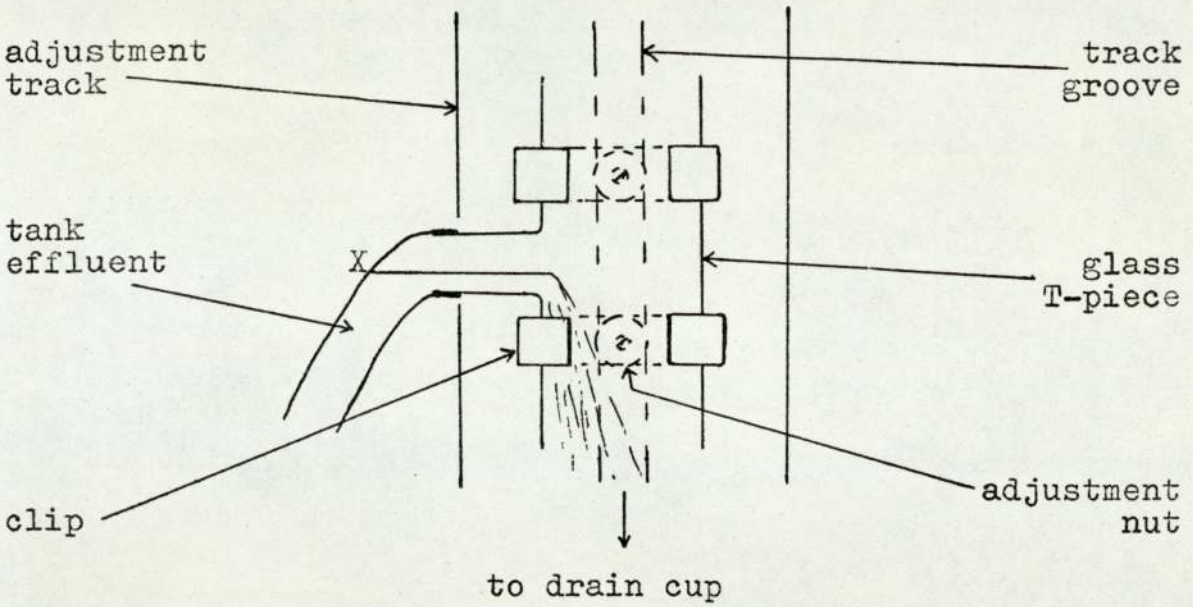
Plumbing

2.13 Mains supply from a 9ℓ ballcock-controlled header tank, placed on an angle-iron scaffolding, had a water-head of about 1.6 m above the tank inlets. Water passed via the heat exchanger (submerged in the trough) and ringmain distributor into the experimental tanks, with a branch tube for inlet water sampling just before the heat exchanger. The heat exchanger was constructed of 8 x 1.5 m lengths of Pyrex tubing, in two manifolds of 4, placed along each side of the trough, interconnected by green plastic tubing, and held in place by short lengths of PVC piping drilled through and stood on end (Fig.2.3).

2.14 Individual tank inlets left the ring main by glass T-pieces, and plastic tubing directed the influent water over each tank side to provide angled drive for the tank circular flow (Fig.2.3). The tubing was attached to the tank side by means of rigid plastic tubing.

2.15 Each tank effluent passed outside the water bath, round the U-tube upwards to a levelling outlet for volume control and then passed into a large main drain and so to waste. Fig.2.5 shows the outlet from below, with nylon control tap (open during experiment) and U-tube bleed-off (for removing any accumulated debris). Fig.2.6 shows the overflow levelling control in its two design stages, and Fig.2.7 shows the levelling controls in front of the full system. Tank water-level control was accomplished by sliding the glass tree up or down the groove in a fixed wooden track, and locking its position by means of two tubing-clips with wing nuts. The tree top aperture allowed a thermometer or pH electrode to be inserted to measure water parameters.

2.16 Pipe dimensions were as follows:-



a) EARLY DESIGN

(not to scale)

Adjustment nuts allow T-piece or tree to move in vertical plane along groove in track.

Height of side-arm X controls water level in tank.

b) LATER DESIGN

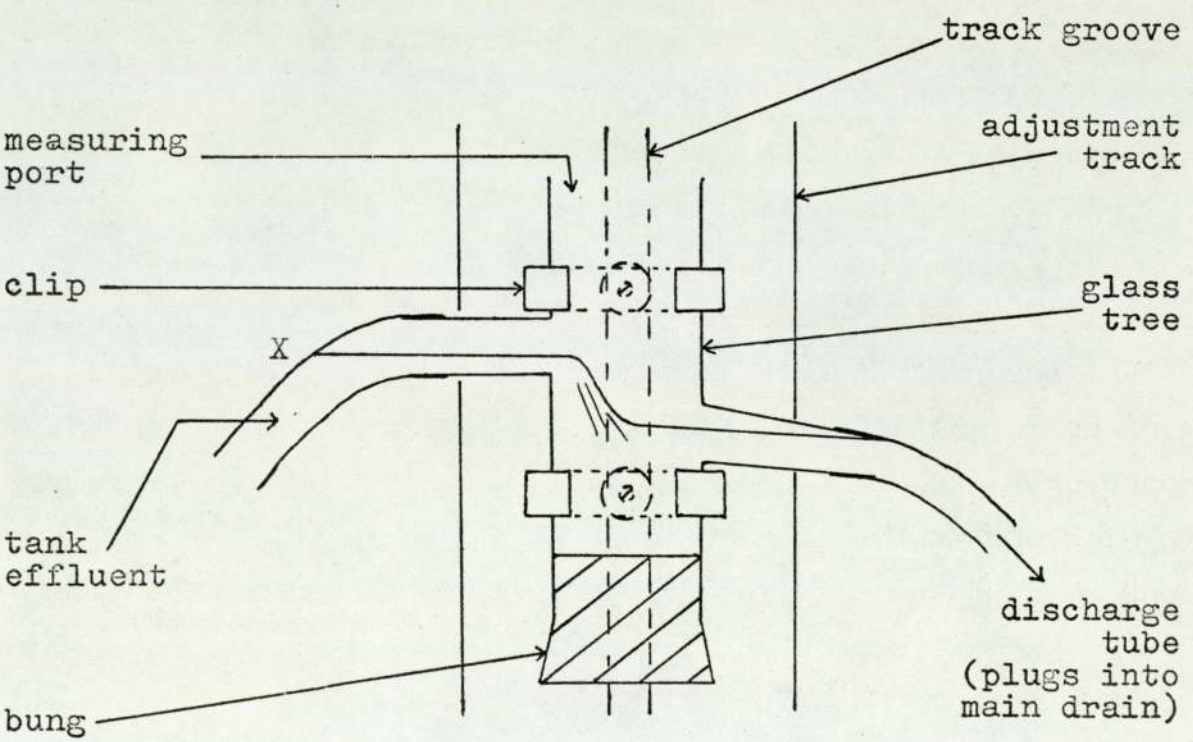


Figure 2.6 Overflow levelling device

a) 13 mm: main inflow,
heat exchanger

ring main distributor

individual tank outlets to overflow levelling controls

b) 6.5 mm: individual tank inlets

c) 9 cm: main drain pipe

2.17 Flow control along flexible tubing was by means of Hoffman clips, except as in Fig.2.5 below the tank.

Illumination control

2.18 As Fig.2.7 shows, the tanks and trough were enclosed inside a light-proof hardboard box (with angle-iron framework) and rested on an angle-iron trestle. The front portion of the box was closed off by means of black cloth curtains during experiment; these allowed easy access when required.

2.19 Set in the roof of the box was an array of 8 light bulbs (Fig.2.7), each of 6W @ 12V AC, positioned one above each tank in MES screw fittings. Control of the lights to the desired photoperiod was automatic, by means of a simpler version of the system described in Chapter 3.

Temperature control

2.20 In the 80 cm high space below the system, which provided for access to U-tubes and tank sealing joints, a Churchill chiller/heater circulator was installed. This received water from the water bath by means of 13 mm tubing, imposed temperature control, and pumped water back to the water bath via a further length of tubing. A thermometer built into the chiller allowed temperature monitoring. In practice the heat exchanger was found to be limited in value. Tank water temperature was governed largely by the inflow temperature, since this was an open system, and the water bath was only sufficient to buffer minor fluctuations.

Aeration

2.21 Dissolved oxygen was kept non-critical, and any chlorine in the inflow was removed, by vigorous aeration in the header tank using 2 laboratory air-pumps with air-stones, (one during experimental dark periods).

Pilot equipment sources

2.22 The following list details the manufacturers of important apparatus.

ST type 1 (buckets)	- Plysu Products Ltd., Bletchley
ST type 2 (containers)	- Ekco Plastics Ltd. Southend-on-Sea
Fibreglass/PVC trough (to specification)	- Cago Ltd., Birmingham
Chiller thermo-circulator	- Churchill Instrument Co., Perivale
Pyrex tubing	- James A.Jobling Ltd. Laboratory Division, Stone, Staffs.
6W light bulbs	- Vitality Ltd., Bury St. Edmunds
Glass overflow-levelling control trees (to specification)	- J.A.R. & M.K.Hill, Walsall

INTERIM PLANS

2.23 During construction and operation of the pilot system, plans were laid for a larger-scale laboratory. Six circular tanks of diameter 91 cm and depth 46 cm were designed, and built to order by a local firm. They were of translucent white polypropylene, each with a lip-flange round the top and mounted on three tubular PVC legs with the central drain-hole 30 cm off the ground. Each tank floor was slightly sloped for self-cleaning drainage, and the drain-hole was of 2 cm diameter with a short length of PVC piping sealed into the hole. The tank held about 250ℓ and was designated LT (large tank).

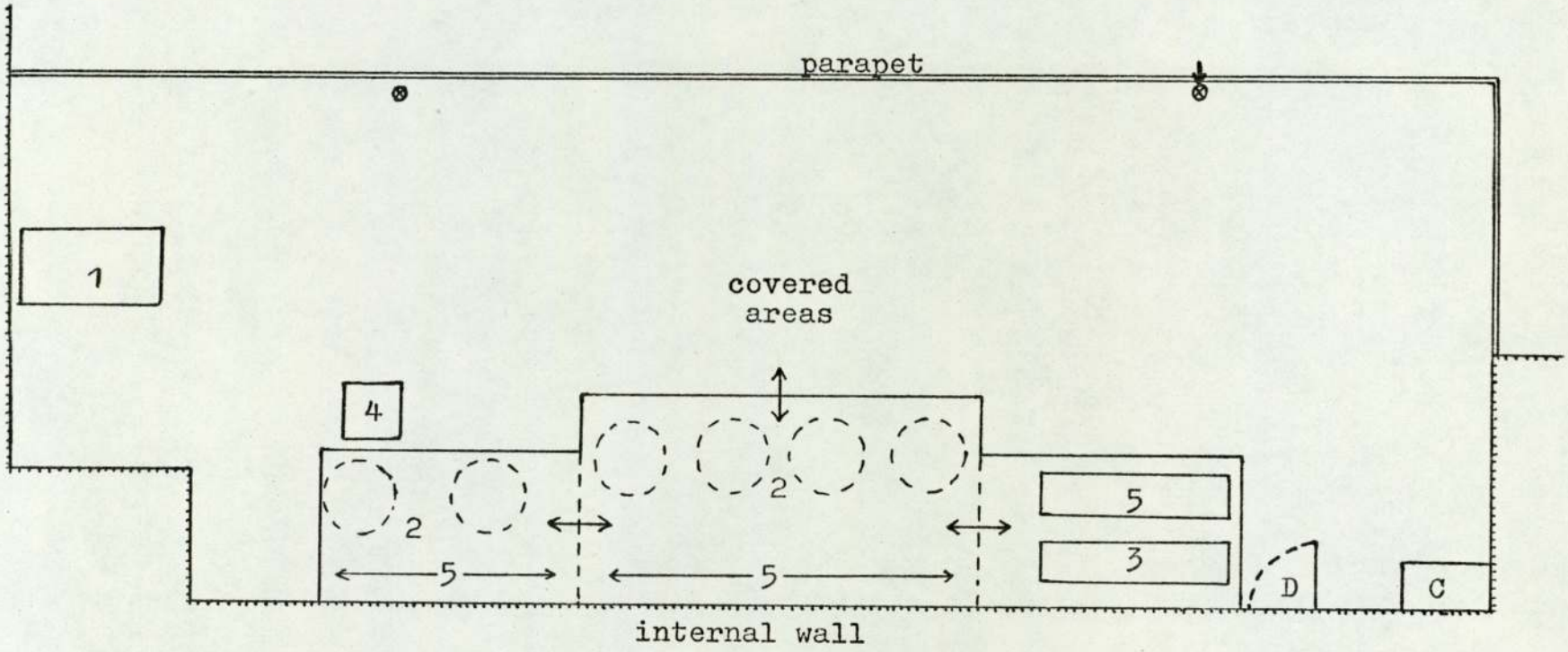
2.24 The first plan for a larger laboratory was based on conversion of a roof-area (Figure 2.8). The plan was disallowed by University authorities on structural grounds.

2.25 Following this planning session, the merits of a fully indoor working area and an independent water supply were becoming apparent, and attention for the next plan was focussed on a similar area in the basement of the main University building, following the example of the MAFF (Ministry of Agriculture, Fisheries and Food) Salmon and Freshwater Fish Laboratory in London. After consideration by the University, this plan also had to be abandoned, and it became clear that a site outside the main University building would stand most chance of success.

2.26 Eventually a promising site was found at the rear of a fully walled-in outdoor area. Plans envisaged either a prefabricated construction or the setting up of ready-made huts on the site (Fig.2.9). A firm commitment by those responsible had not been made when another nearby site was discovered which was superior to all those so far examined.

2.27 The last site, which eventually was turned into an experimental fish facility, was a two-storey shed-type building behind a laboratory complex, with a concreted base floor, two brick walls and two corrugated-sheeting walls, and a wooden-beam and floor-boarded first storey with a sloping roof. Windows were present in both storeys. When first encountered the building was in some disrepair (Fig.2.10).

2.28 A new series of plans was formulated, and after approval and confirmation, the initial technical drawing for basic conversion work was prepared by the University Estates division in December 1972.



1 = 1000 l rectangular tank 2 = 250 l circular tank

3 = STS (trough) 4 = 350 l square tub

5 = other research tanks (colleague)

C = compressor housing (air supply)

D = access door to interior

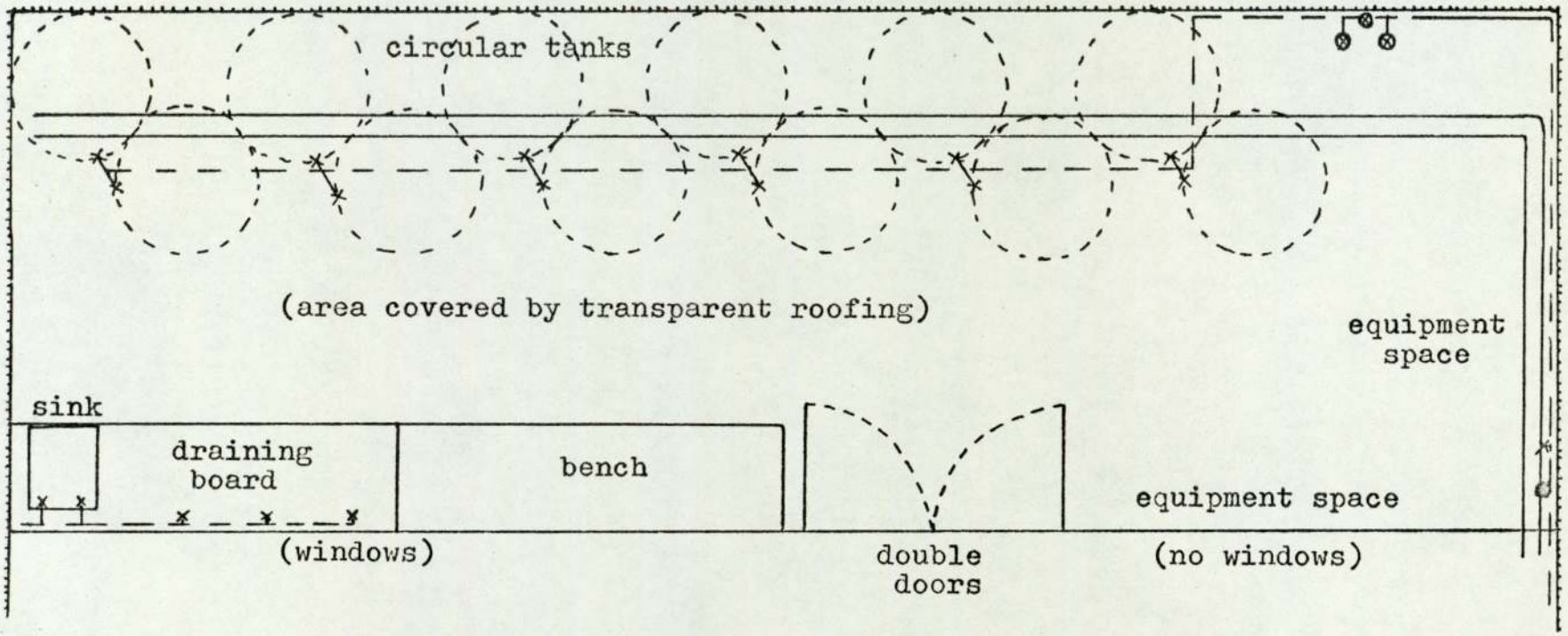
⊗ = drain

↕ = access to covered areas

↓ = water supply tap (supplying other fish holding as well)

Scale: ¼ inch rep. 2 feet

Figure 2.8 Plan for roof-area conversion



All facilities shared with colleague.

x = tap ⊗ = stopcock

= gutter — — — = water line

Scale: $\frac{1}{4}$ inch rep. 1 foot

Figure 2.9 Plan for outdoor area conversion

2.29 The necessary conversion work, including removal of old fittings, repair of walls and ceiling, installation of systems and benches as in Appendix B, was carried out by contractors during early 1973. A steel walkway was included, allowing access to the upper storey from an adjacent fire-escape; the trapdoor connecting the two storeys was supplemented by a vertical steel ladder; and the lower storey windows were boarded over to make it light-proof.

2.30 Water was supplied by a branch off the University main supply, and was made to empty via a large ballcock into a 1000 ℓ fibreglass rectangular tank mounted on wooden trestles in the upper storey of the building, at a point where the wooden floor of the prefabricated part of the building gave way to a concrete floor (part of a rather larger building immediately adjacent). The stone-floored section was that detailed on the plan (Appendix B) as "header tank room". Contractors installed the header tank and supply, and finished its plumbing with four PVC 13 mm outlet pipes descending to the lower storey, terminating above head height with angle-seat valves. Water entrance to the header tank was by cascade from the valve, and the mains pressure during operation caused an effective jet-spray ensuring excellent aeration.

2.31 Conversion work was finished in June,1973 and by July it was possible to instal the 6 LT and to institute fish holding under HC2. The laboratory facility was shared with P.Smith, and for experimental purposes one half of the lower storey was allotted to each project. This was designated as a wet lab., with facilities for water drainage, while the upper storey accommodated bench work and desk space.

3. DEVELOPMENT OF FULL EXPERIMENTAL SYSTEMS

PLANNING

3.1 After laboratory conversion the following facilities were available:-

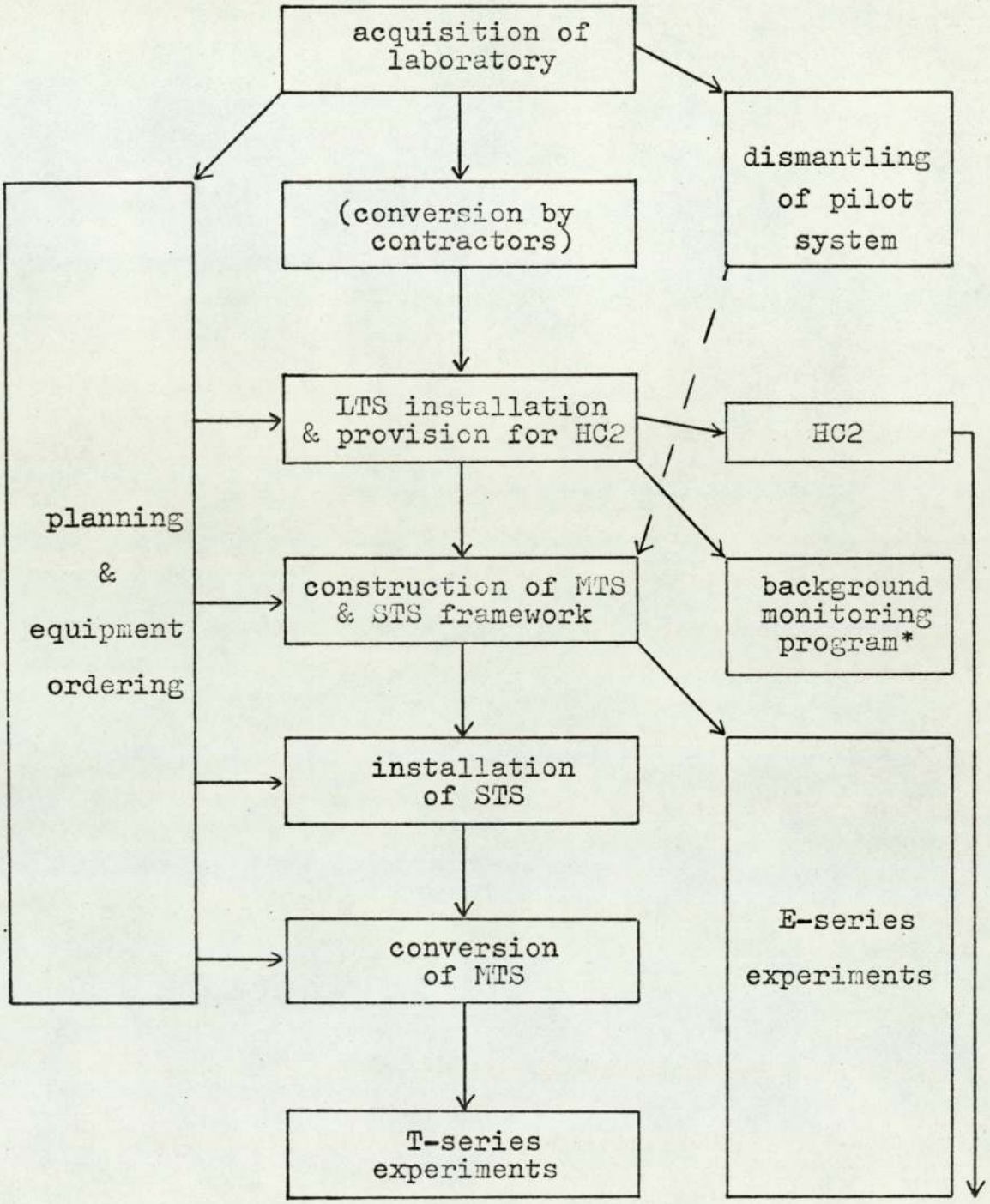
a) 2.5 cm diameter main water supply issuing by ballcock to a 1000 ℓ fibreglass constant-head reservoir tank with four separate 13 mm outlet pipes (with control valves), and suitable overflow, drain-tap and hinged lid attachments. Two of the outlets were available for this project work.

b) Building-space of 6.4 m long x 2.5 m height x 1.2 m depth. At one end the trapdoor ladder formed a boundary; at the other, a corner space was available. The floor had a drainage gulley, and there were twin electric points at 1.2 m above the floor at each end of the long wall; also, the end wall nearest the trap door had supplementary electric points. Illumination was by a single standard fluorescent light. Supplementary water supplies (hand taps) were provided, one at each end of the wet lab.

c) Half-share in the upper storey (dry) laboratory space including bench, cupboard, sink, water and electricity facilities.

3.2 Figure 3.1 show the activities scheme pursued during the project after laboratory acquisition. Development, construction and experimental work had to be organised for simultaneous operation.

3.3 Fig.3.2 shows the elevation view of the wet lab building space, and in this space 3 separate systems were accommodated, the small (STS), medium (MTS) and large tank systems (LTS). The STS and LTS used the tanks previously described, but for the MTS a number of fibreglass tanks were purchased. These were of 15 ℓ capacity, coloured light green, with diameter 49 cm and depth 10 cm; they were modified from the circular hatchery tray design used by Vortex Ltd., and in place of the standard central fittings had a well of 12.5 cm diameter and



*see Chapter 7

Figure 3.1 Chart of major developmental activities

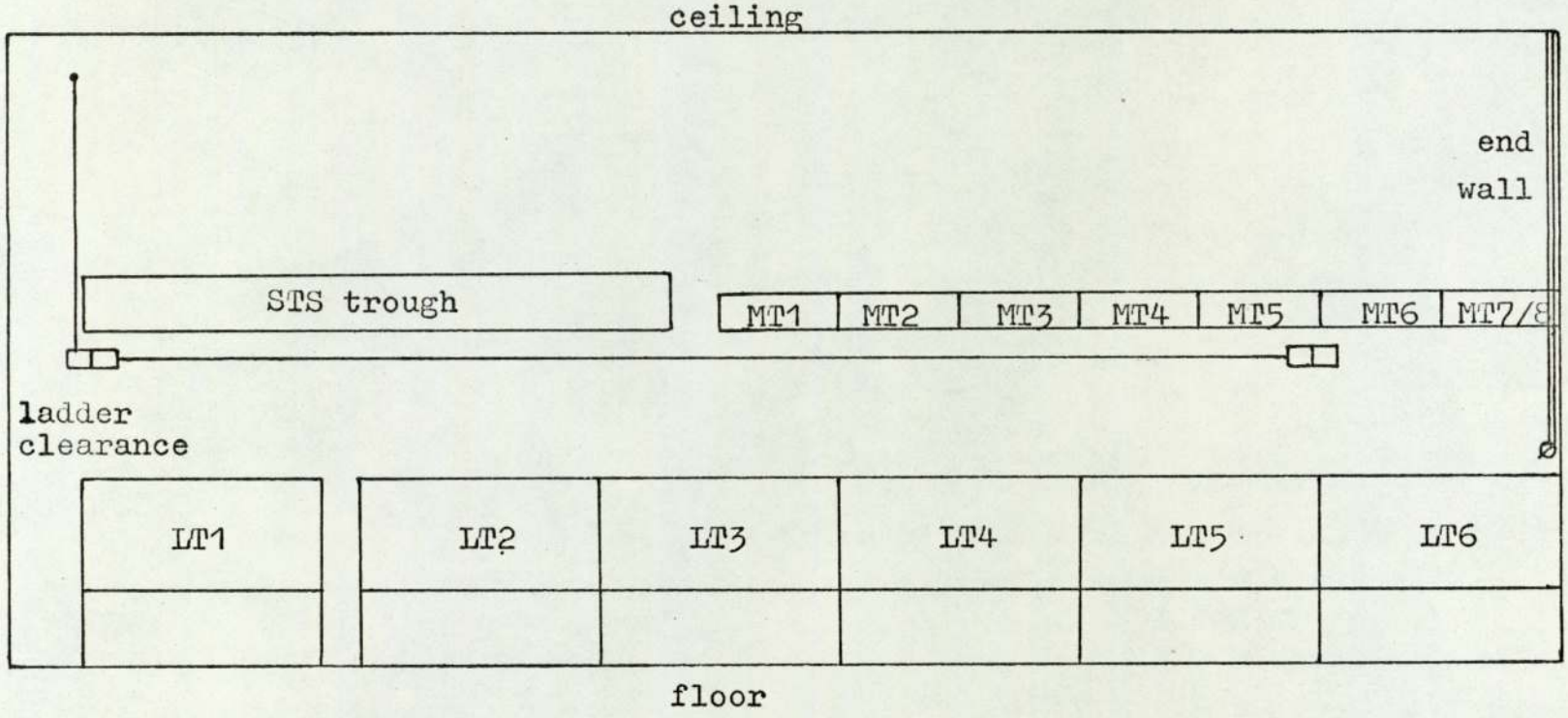


Figure 3.2 Elevation view of wet lab building space

2.5 cm depth, leading to a central exit hole of 2.5 cm diameter. A rim-ledge allowed a mesh to be placed across the well, and the upper rim of the tank was rolled to allow stacking and cover attachment.

3.4 Assembly work was performed in the following order:-

a) Main plumbing to all systems.

b) Angle-iron framework to support MTS and STS, and enclose LTS.

c) Installation of LTS (6 tanks) and LTS drainage.

d) MTS angle-iron superstructure installation and drainage; STS trough installation, superstructure and drainage.

e) Lighting systems and controls.

f) Feeding systems and controls.

g) LTS and MTS tank tops.

h) Blackout.

3.5 Items (a) to (c) were required before fish could be held in the LTS, and items (e) and (f) were required before HC2 could be instituted.

3.6 Systems were designed, profiting from pilot experiment, with several major objectives in mind:-

a) to allow simultaneous fish holding and experimentation with a dual-purpose LTS;

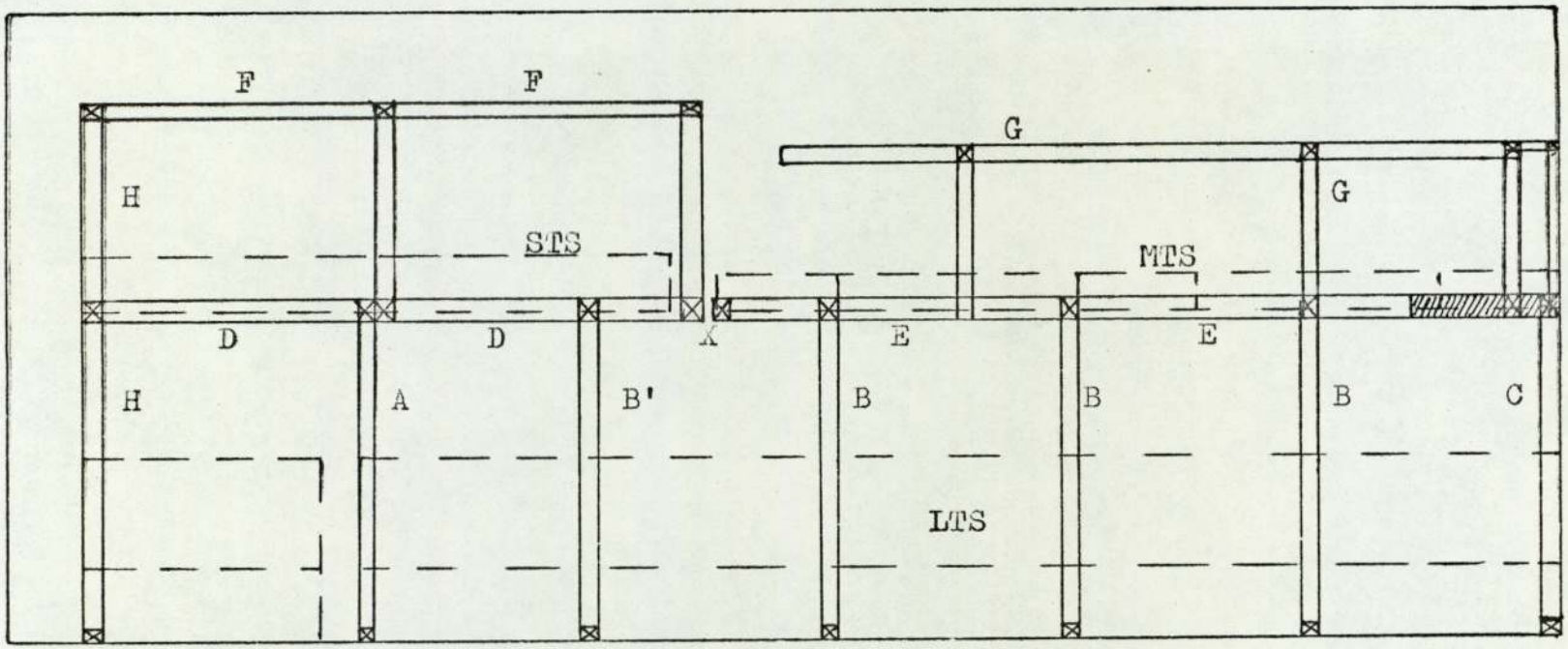
b) to provide an MTS for the bulk of the excretion/environment work (E-series experiments), comparable in arrangement with the LTS when used for the same work, but also readily adaptable for tolerance work;

c) to allow easy access to all systems, so far as was compatible with space limitations;

d) to provide an STS specifically geared to tolerance work (T-series experiments);

e) to provide ease of cleaning and removal of components;

f) to provide automated controlled lighting and feeding systems for each tank;



= angle iron frame = angled cross strut to support MTS

⊠ = cross strut A = arch of depth 2'6"

B = B' = arch of depth 2'3" C = arch of depth 3'6"

D = STS trestle E = MTS trestle

F = STS superstructure G = MTS superstructure

H = end frame; arch depth 2'9" X = break between sections

Figure 3.3 Elevation view of angle-iron framework

(Scale: 1 cm rep. 1 foot)

g) to exclude unwanted external stimuli, particularly visual. In view of considerations (c) and (e), open gutters were preferred to drain pipes; and in order to fulfil condition (g), blackout curtaining was preferred to box enclosures. Blackout was necessary since it would allow other work in the laboratory and use of external lights without fish disturbance.

3.7 A complete system for air supply to all tanks was originally planned. This would have required a large compressor, and despite preliminary negotiations, such a supply was not in practice authorised and purchased. All systems received excellent initial aeration from the header tank, and small laboratory air pumps were used in emergency, at the introduction of new fish Lots, during experimental anaesthesia and sorting, and for air supply in T-series experiments.

3.8 After the major assembly work and progress on the E-series of experiments, a later assembly stage was involved in preparation for T-series experiments, (see Chapter 9). In their final configuration the three systems were in use for fish holding, E-series and T-series experiments simultaneously.

EQUIPMENT AND CONSTRUCTION

Framework

3.9 Surrounding the LTS, and supporting the STS and MTS, an angle-iron framework was built in two sections; Fig.3.3 shows all basic framework.

3.10 The STS section consisted of 3 arches (H,A,B') on which rested longitudinal trestle runners (D). Arch (H) was continued to form an end frame, and superstructure (F) was erected on top of the trestle to enclose the STS in box-like fashion. This was similar to the pilot system construction, and allowed similar arrangements to be made for curtains, overflow levelling and internal fixtures.

3.11 The MTS section abutted closely to the STS section (X) and also employed an arch-supported trestle (E; arches B,C). However, the MTS superstructure was formed of two arches surmounted by runners (G) forming girders for fixture attachment. At the wall end, the girders were continued at 90° to the general direction, and a larger arch (C), and supporting cross-strut and stanchions, allowed space for an extra MT. Thus seven MT were accommodated along the long wall and one against the short wall. A girder-type superstructure was used in preference to a box for ease of access to, and removal of, MT and tank services.

3.12 Both superstructures were curtained-off, as was the LTS beneath the trestles, by use of blackout attachments at the walls, ceiling, and suitable points on the framework.

3.13 Angle-iron was of the stove-enamelled Bartangle variety, and according to strength required and position, use was made of the three width-sizes, 38 mm, 64 mm and 89 mm. The framework was levelled and bolted together with suitable strengthening corner struts where necessary, to give rigid scaffolding.

3.14 Arches (H,A,B,C) stood out further from the long wall than the superstructures above. This allowed access and removal space for the LTS, support for drainage systems, and protection of superficial equipment.

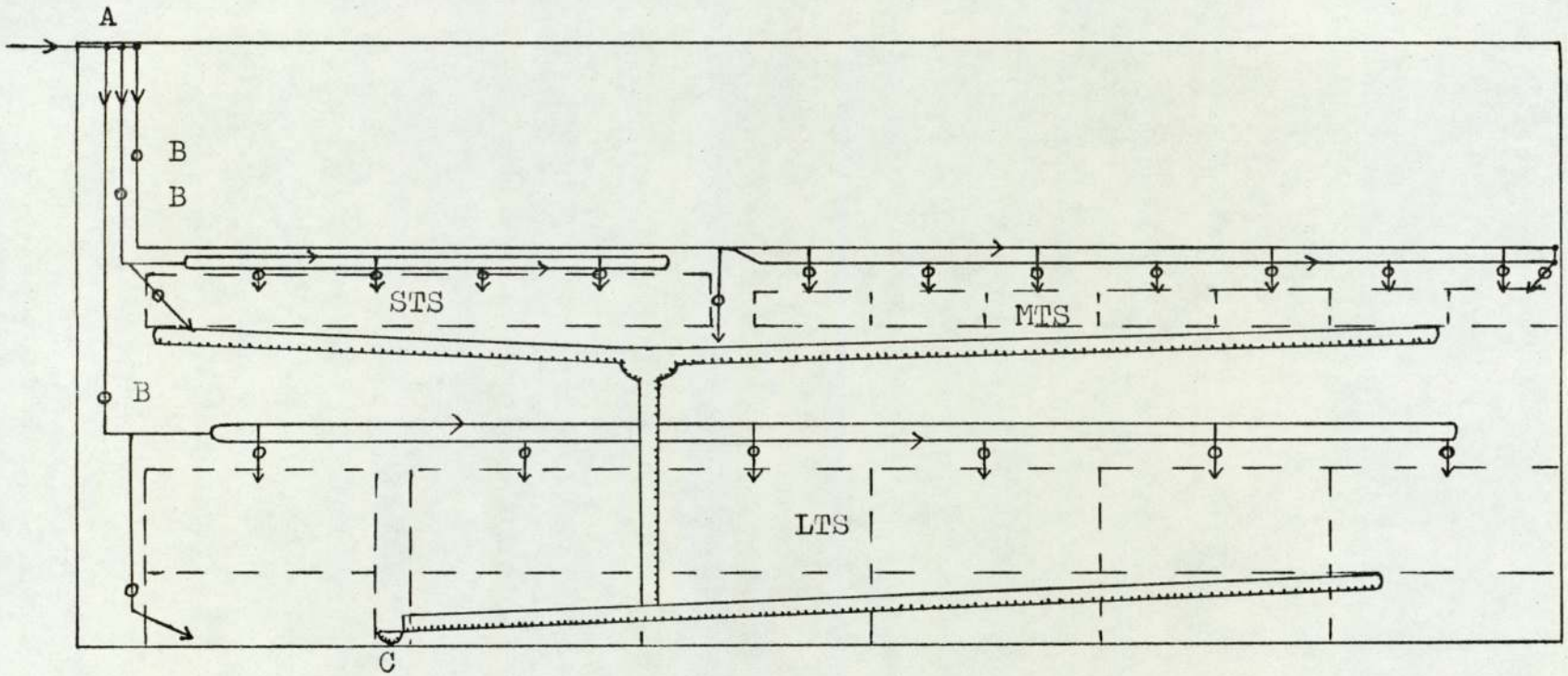
Plumbing and drainage

3.15 Water supplies for the MTS and LTS were taken from the two available outlet pipes from the header tank. Green transparent plastic tubing of 13 mm diameter was used to convey water supplies to arrive at the experimental system area at ceiling height. Water for the STS was brought from the header tank drainage tap (by similar tubing) through the ceiling next to the MTS and LTS supplies. Water-head above the STS/MTS was 180 cm.

3.16 Inlet plumbing and drainage are shown in Fig.3.4. Water supplies from point A were distributed by the green tubing with occasional short sections of rubber tubing (e.g. at the main shut-off controls). Each supply line had a main shut-off (large Hoffman clip), and subsequently split into a ring main to supply the tanks, allowing greater facility in individual inlet control. Just before each line split, it was provided with a clip-controlled side branch for inlet water samples. Supply lines were held in place by spring clips attached to wooden battens fixed on the wall. The STS supply rested on the front trough flange to allow ease of access to control clips.

3.17 15 cm above each MT or LT, the ring main branched by a glass T-piece to give a 6 mm clip-controlled inlet supply. This passed through a 90^{o} glass bend to enter the tank at an angle, thus providing circular tank drive. MTS inlets were suspended in position by cord from above; each LTS inlet was held in a groove through a small wooden block fixed onto the end of an angle-iron support arm (see Fig.3.14 later). Thus the inlet was held at a constant position. The connection between T-piece and inlet bend was made with flexible 6 mm tubing, which supported the control clip; inlet sample supplies were similarly furnished, with greater lengths of tubing to allow easy manipulation and stowage. The U and Y-tubes delimiting the ring main were 13 mm glass tubing. STS inlet supplies were similar to those of the pilot system.

3.18 Outflow from STS and MTS, and trough overflow and drainage, passed from the overflow level controls or trough outlet holes via short lengths of rubber tubing to the appropriate drainage gutters; these were mounted in the angles of the framework arches, and discharged into a central saddle fitment and down a 94 cm length of 6 cm diameter vertical PVC pipe. At the lower end, waste water entered the main LTS gutter (Fig.3.4)



ϕ = Hoffman clip ↓ = tank inlet or inlet sample tube ⎓ = drainage

A = arrival of water supplies from header tank C = cross-gutter to gulley

B = supply main shut-off control

(Scale: 1 cm rep. 1 foot)

Figure 3.4 Elevation diagram of inlet plumbing and drainage

and flowed into a cross-gutter discharging into a drainage gulley. These arrangements allowed the lab floor to be kept fairly dry when required. Guttering was of 10 cm diameter PVC.

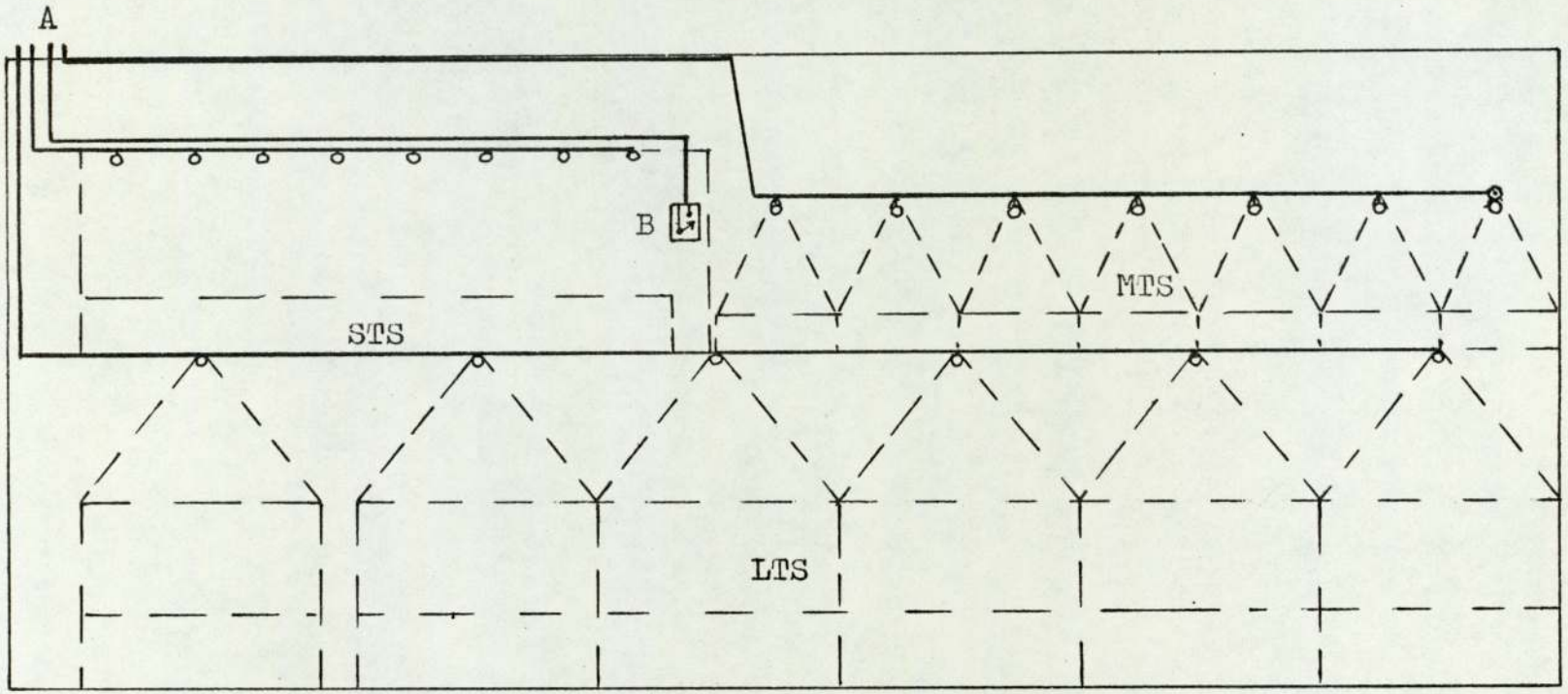
Electric fitments

3.19 All systems had electric lighting; one bulb per tank for MTS and LTS, and 8 bulbs in the STS system (Fig.3.5). There was also a remote override switch for the feeder system (see below) mounted on the side of the STS superstructure, and sealed into a wooden block.

3.20 Electricity control was performed by means of a master control box mounted for safety on the upper (dry) laboratory wall; cables passed through a special aperture in the wet lab. ceiling.

3.21 Since LTS lighting was below the MTS and STS water level, it was necessary to protect LTS lights from flooding. This was done by mounting, sealing and covering light fitments on hardboard plates positioned on angle-iron girders just below the STS and MTS trestles. An inverted length of guttering served as roof for each fitment (Fig.3.6). MTS lights were similarly mounted on the superstructure girders, and STS lights were set in a hardboard roof over the box superstructure.

3.22 Lighting control was accomplished as in Fig.3.7. The mains supply directly powered a 24h Venner timer, imposing a 12h-on, 12h-off photoperiod routine by means of control pointers. The regulated mains (240V) supply was then transformed to 24V for safety, and fed three supply circuits (independently switched) serving the tank systems. The bulbs were 12W pearl bus interior lamps, with SBC fittings and holders, mounted in parallel to prevent failure of one from extinguishing the rest. A master switch allowed the lighting system to be switched off without affecting the feeders (see below), if required.



= double cable

= 12W bulb

A = cables to control box

B = remote manual override (for feeders)

Figure 3.5 Elevation diagram of wet lab electric cables

(Scale: 1 cm rep. 1 foot)

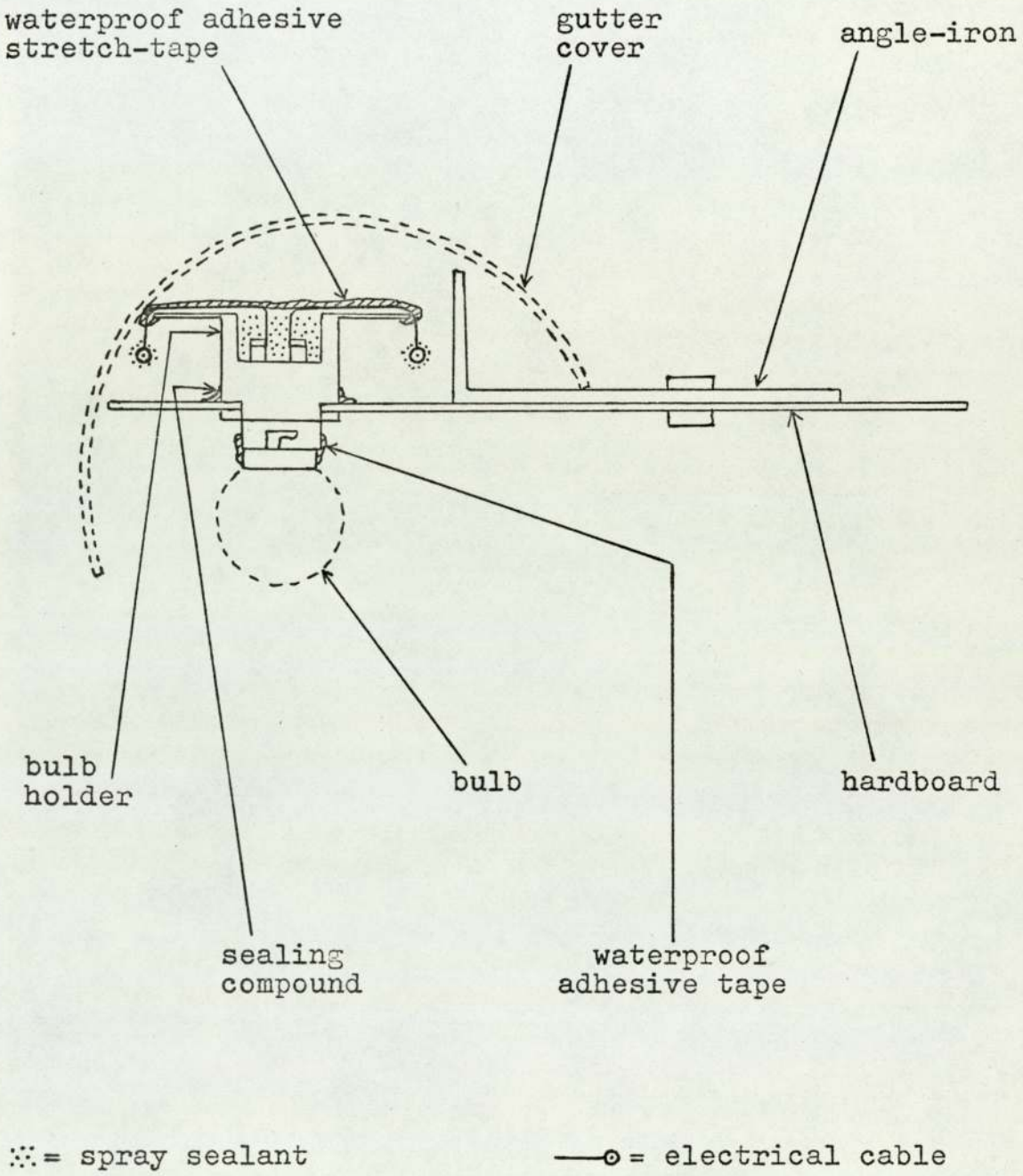
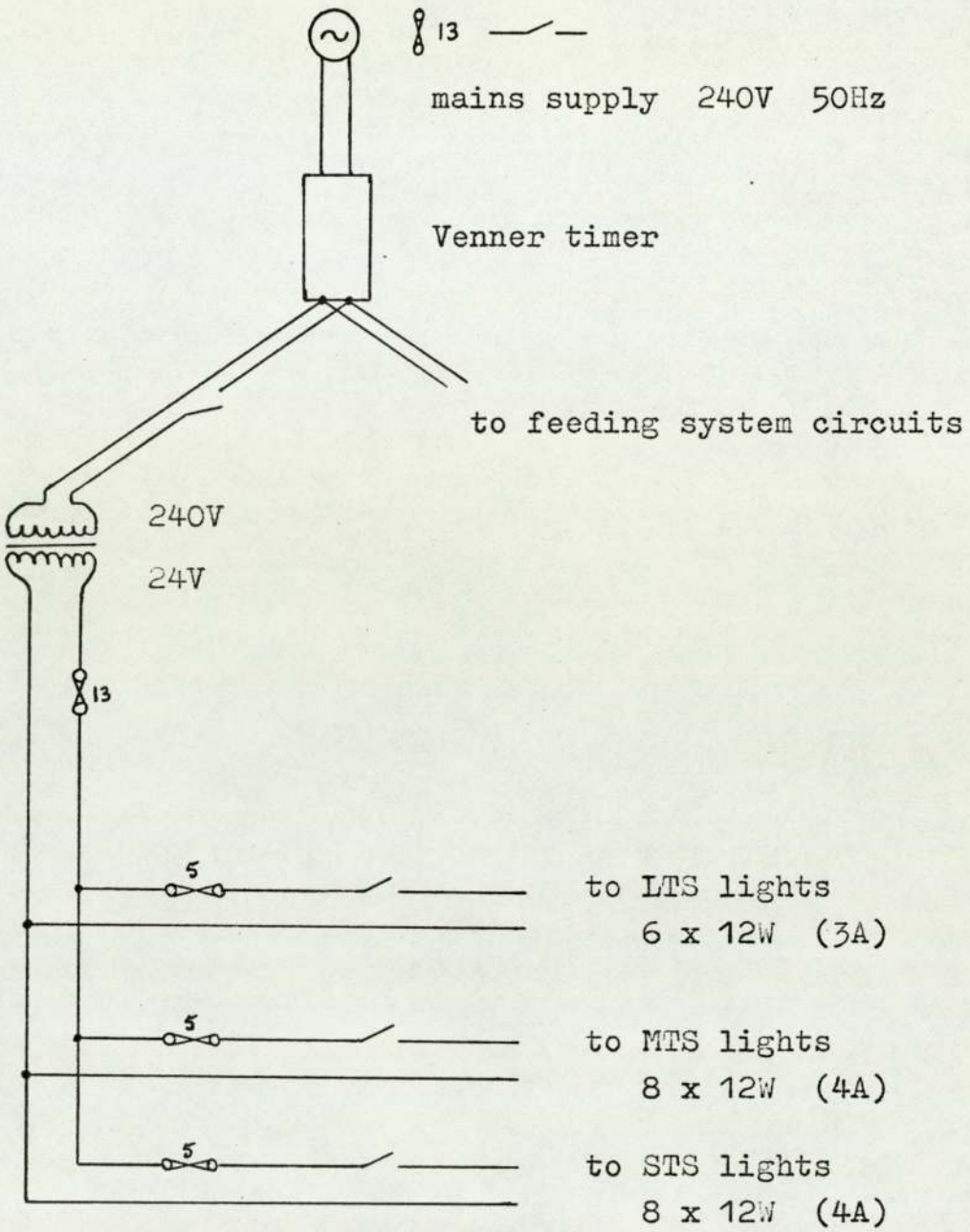


Figure 3.6 MTS & LTS: light-mounting and sealing



(number by fuse indicates current rating)

Figure 3.7 Diagram of lighting control circuit

3.23 Electrical components for the feeders were contained in the master control box. The feeder general supply also derived from the Venner timer controlling photoperiod but was independently switched (Fig.3.8). The 12h supply was then further regulated by No.1 process timer, delivering a 1.5 min pulse at the end of each 2h cycle. This pulse was the power for a feeding session. The photoperiod control switched on at 0800 and pulses occured at 1000, 1200, 1400, 1600 and 1800. The 2000 pulse was just prevented by careful setting of the Venner. The 1.5 min pulse powered No.2 process timer, delivering a 6s pulse at the end of each 1 min cycle. Thus for each 2-hourly feeding session a 6s pulse of power reached the feeder discharge mechanisms. Since No.2 timer only received a 1.5 min power supply, it was cut off during its second cycle. As the process timers were automatically reset to zero when switched off, this ensured a routine of 6s of firing every 2h. (Fig.3.9)

3.24 The 6s pulse fired a solenoid, which operated a release valve built into the feeder system (see below), and caused food to be dispensed. During feeding, a red neon light, mounted on the control box and wired across the feeder solenoid, was lit to allow remote visual check of feeder operation.

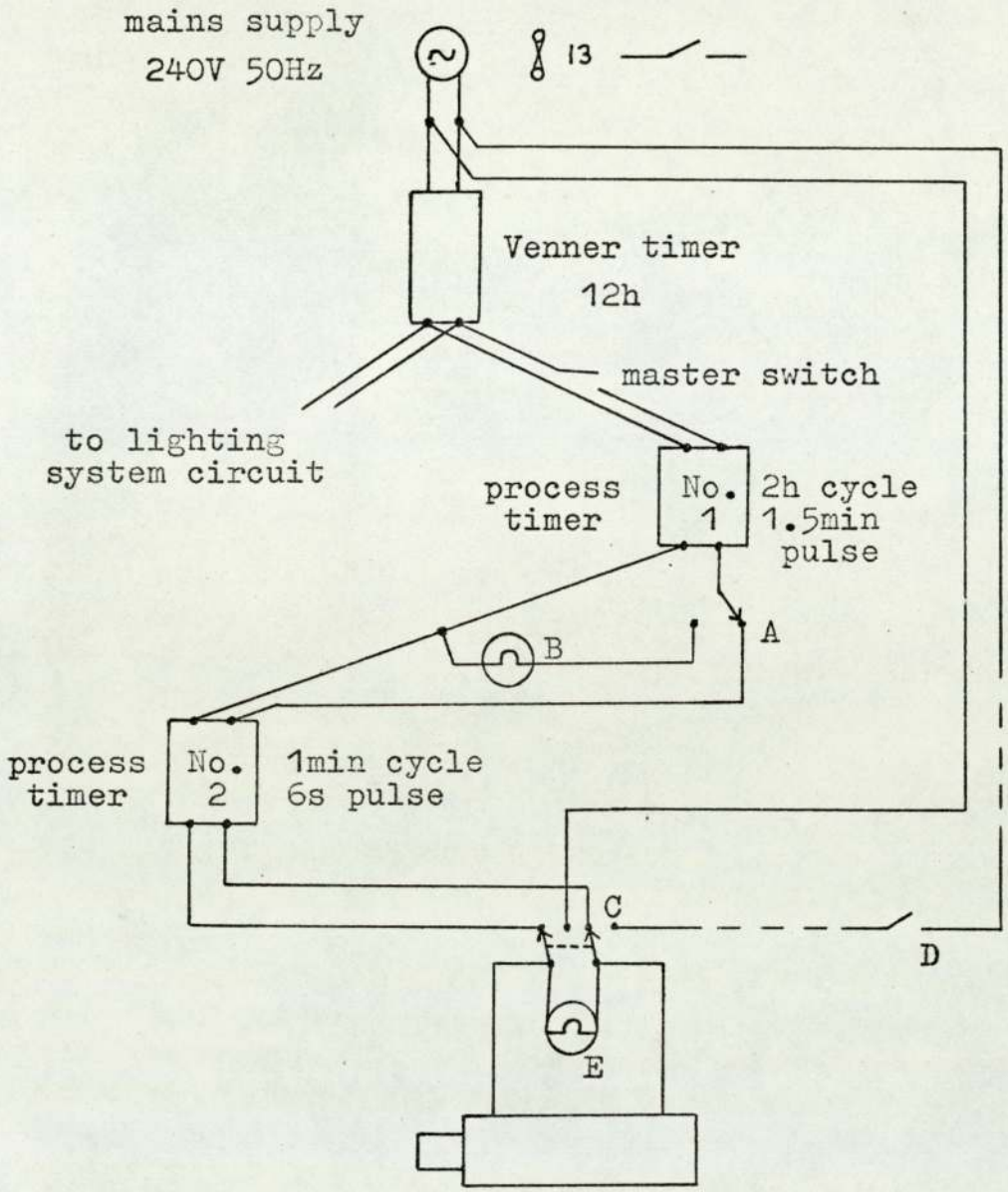
3.25 For convenience, especially when calibrating feeders at the beginning of an experiment, and when sampling overran its time, it was desirable:

a) to be able to prevent a feed manually by delaying it until the control pulse had passed;

b) to be able to feed whenever required by manual override without affecting the timing circuits; and

c) to be able to feed by manual override either at the control box, or by a remote control mounted in the wet lab.

These objects were accomplished by the circuitry of Fig.3.8, which also incorporated a warning light (orange) as a visual reminder of delay switch action. Thus complete flexibility



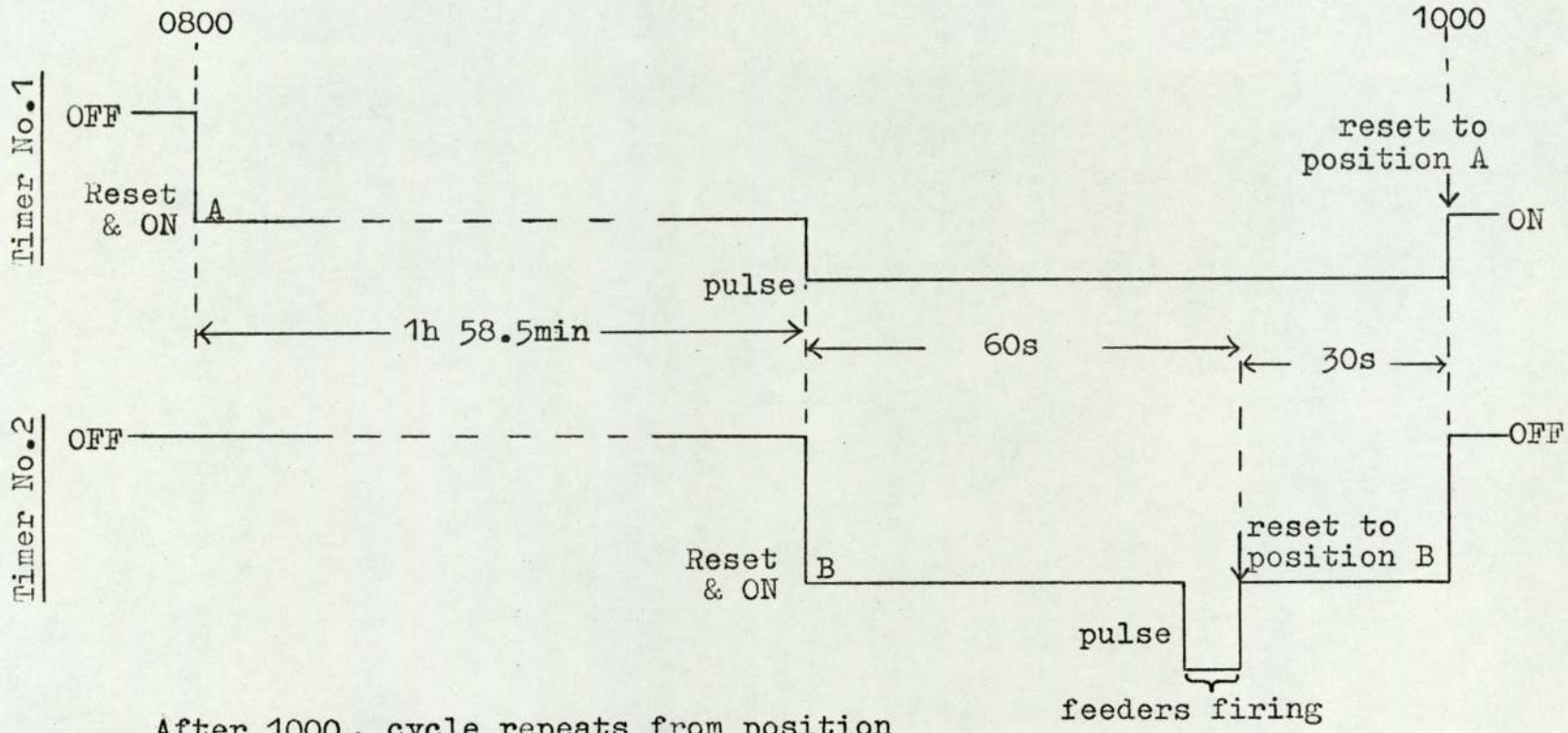
A = manual delay switch

B = delay warning light

C = manual override switch)
) - used in conjunction
D = remote override switch)
(in wet lab)

E = feeding monitor light

Figure 3.8 Diagram of feeding control circuits



After 1000, cycle repeats from position A four times (to 1800); but in the next cycle, the 12h control turns all timers off just before position B at about 1957.

Figure 3.9 Automatic timing cycle for feeders

was obtained over the lighting and feeding systems, allowing automatic or manual control as required.

3.26 The control box was constructed of hardboard panels with a wooden backboard (2 cm thickness) to which the components were fixed. All components were suitably connected by soldered wiring, earthed, fused and insulated, and a hinged door allowed access. The faces of the process timers, and the control switches were mounted on the front face of the box for operation, with neon lights alongside, but for safety the Venner timer could only be operated by opening the door. The box was served from a mains electric point with switch kept on all the time except when components were being checked.

Individual tank fittings

3.27 Each of the 8 MT had an array of fitments as detailed below (see Fig.3.10).

3.28 A short length of 2.5 cm diameter PVC pipe was inserted tightly into the tank exit hole and sealed with a non-toxic sealing compound. 19 mm diameter rubber tubing formed a U-tube below the trestle in the inter-LT spaces, with one end fitted over the PVC pipe and the other end rising to the front of the trestle, inboard of the MTS gutter. At the bottom of the U bend a glass T-piece and bleed-off tube allowed debris removal as in the pilot system.

3.29 The U-tube front end was inserted into a PVC 2.5 cm diameter T-piece which formed an overflow level control, held in 2 spring clips on a track formed of two short vertical pieces of Bartangle strip; it was adjustable over the height of the track by means of wing nuts on the clips. The T-piece side-arm controlled level, and rubber tubing conveyed the effluent water to the gutter.

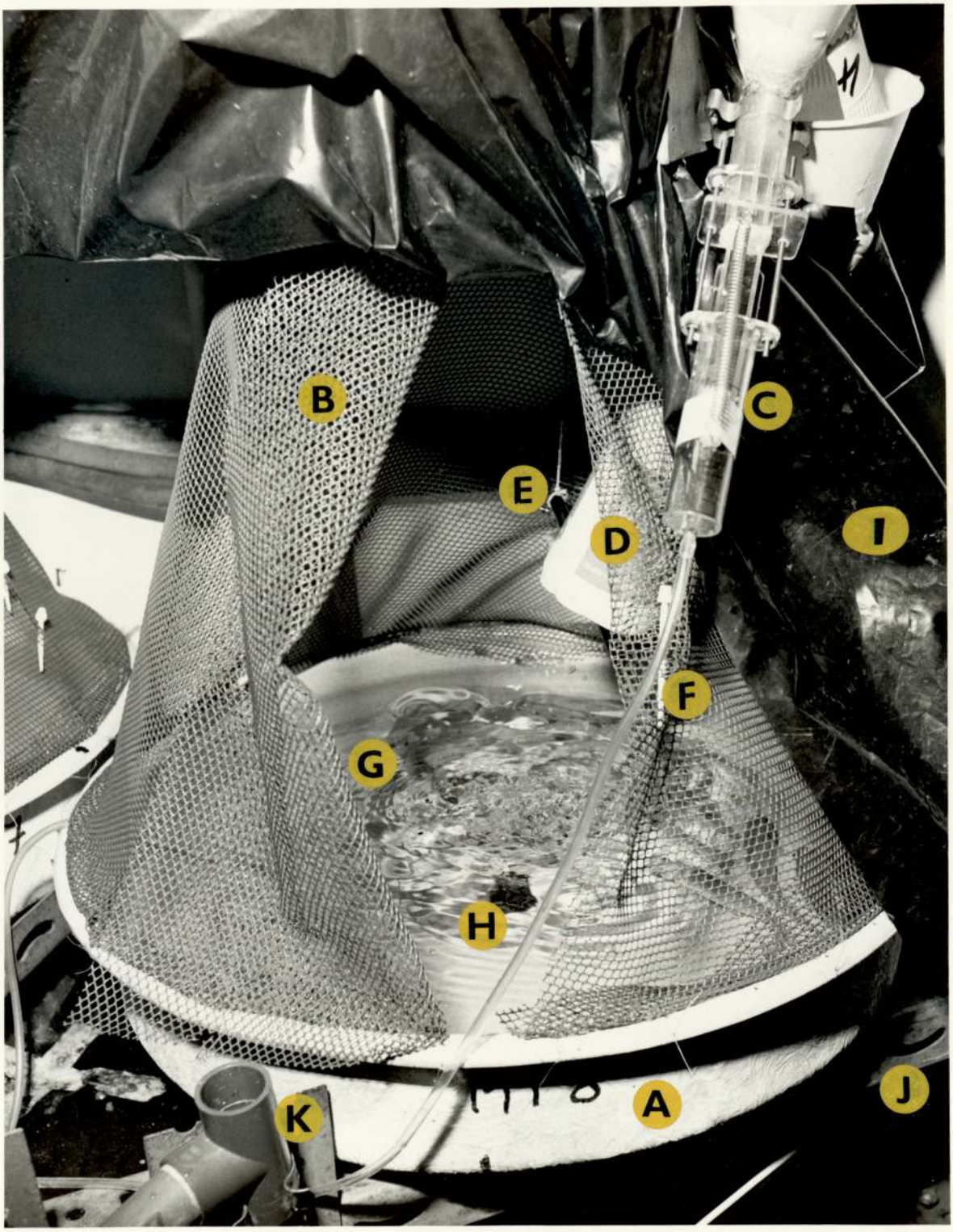
Figure 2.10 Laboratory before conversion

A) Upper laboratory/office, with header tank room beyond partition and trapdoor in floor at right.

B) Lower (wet) laboratory, with future experimental space along right-hand wall.

Figure 3.10 MT in E-series configuration

A MT8 in position
B Tank shroud pinned back
C Feeder in operation (spring extended)
D Delivery tube protruding through blackout and shroud
E Tank inlet suspended in place
F Shroud pin
G Swirling denotes feeding activity
H Mesh plug in tank exit hole
I Blackout panel
J MTS trestle cross-strut supporting MT8
K Overflow level control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3.30 The well of each MT could be screened either by a circular disc of 6 mm hard square green plastic mesh fitted tightly into the well ledge, or by a short roll of the same mesh forming a plug in the hole (as in Fig.3.10). In either case the purpose was to allow removal of faeces or uneaten food but prevent loss of small fish; the latter method proved better since the former caused a "dead space" in the well and inhibited self-cleaning of the MT.

3.31 A length of flexible 6 mm plastic tubing was led over the tank rim to attach to a 30 cm length of 4 mm bore straight glass tubing mounted on a diagonally placed graduated scale (card and board assembly) across the front of the tank as in Fig.9.4 (Chapter 9). This formed the volume indicator. Water was sucked through the tube by the operator (to form a siphon over the rim) and left to find a level in the external indicator. By prior calibration of the indicator with known water volumes, tank volume readings could be obtained. The diagonal scale allowed more accuracy than a vertical scale would have done due to its greater length. The volume indicator was fixed in place to the trestle, and to the overflow level control Bartangle supports.

3.32 Fish were retained in the MT by means of a conical tank shroud of soft green plastic mesh (3 mm diamond), rolled round so that the larger end of the cone fitted over the tank, and the smaller end fitted over the light bulb hardboard plate (see Fig.3.6). Holes were drilled around the MT perimeter, and the cone sewed in place with nylon fishing line of 4 kg breaking strain. The cone overlapped at the front of the tank and one side was left free to allow for handling of fish into and out of the tank. During experiment, the overlap was closed by pinning through the mesh. Apertures through the mesh allowed water inlet and feeder discharge tubes to protrude inwards.

3.33 The LTS was equipped with similar tank shrouds to the MTS, attached to the tank sides by nylon bolts and nuts. When necessary (with small fish) mesh plugs were inserted into LT exit holes. The LTS was not equipped with volume indicators: instead the water level was observed through the translucent tank side and its height measured from the tank bottom. Prior calibration thus allowed experimental volumes to be measured. LTS overflow level controls were similar to those of the MTS but were mounted at convenient points on the framework. No U-tube bleed-offs were present because of the lack of room for operation below an LT; satisfactory debris removal was accomplished by "pumping" by stamping the U-tube against the floor several times.

3.34 The STS was equipped similarly to the pilot system, employing ST type 2. The U-tubes below the tanks hung in the inter-LT spaces and the only major modications were deletion of the plastic tap below the tank, and the use of horizontal fitted plastic lids for the tanks. All later modifications of the STS and MTS for T-series experiments, are discussed in Chapter 9.

Feeders

3.35 The feeding system which supplied all tanks was made to a design* by P.Smith, and was powered by compressed air cylinder. The basic plan, as in Fig.3.11, was a ring main compressed air circuit supplying a feeder at each tank. Firing of the control solenoid pulled open the master supply valve for 6 seconds. This allowed compressed air at about 1520 millibars to enter the ring supply, and at each feeder a piston discharged food from a barrel, acting against a calibrated stop nut. When the solenoid ceased pulling, the valve closed again, the pistons withdrew, and the compressed air was exhausted at the valve.

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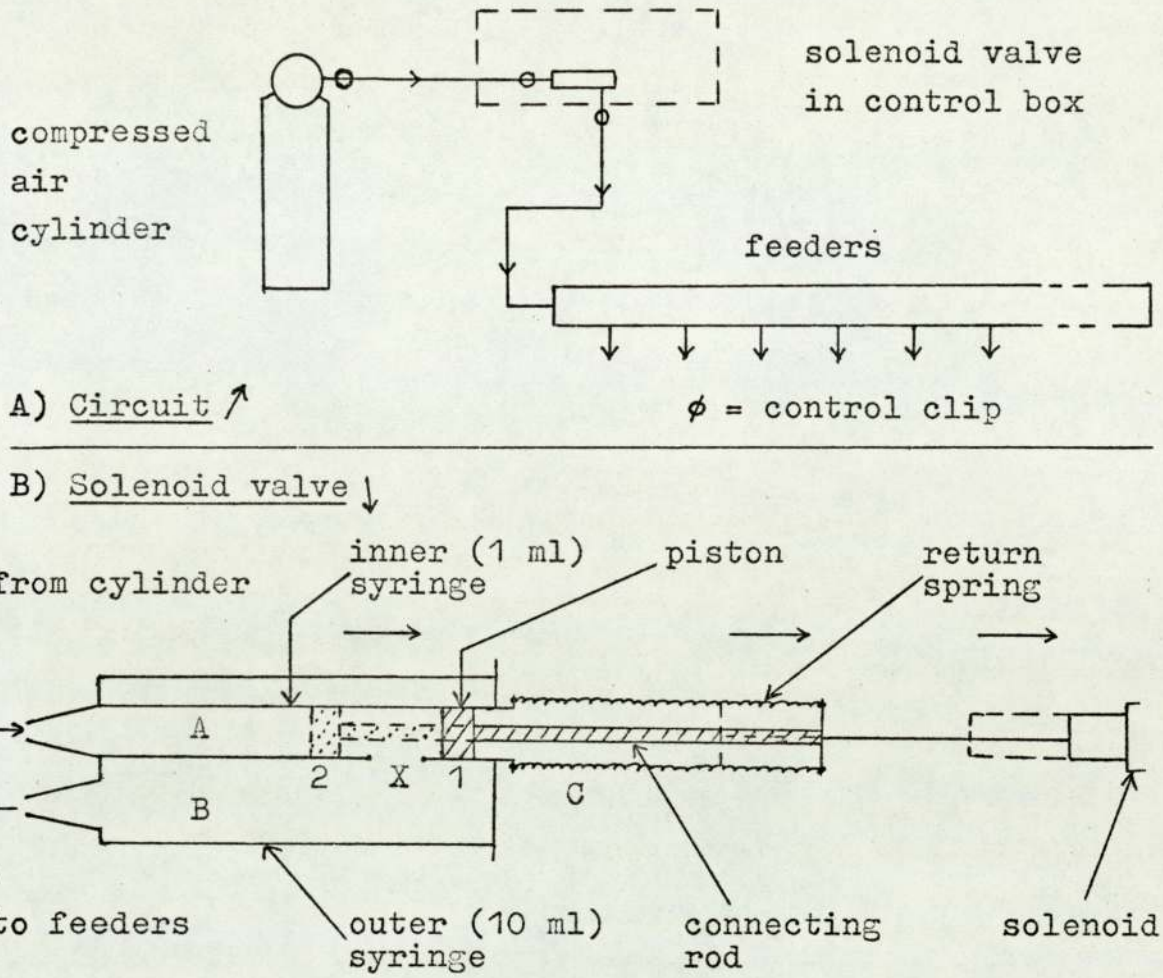
* currently under consideration for patent



Valve open: Solenoid pulls piston to position 1 and compressed air passes from cylinder via inner syringe (A) through valve aperture (X) into outer syringe (B) and so to feeders.

Valve closed: Return springs pull piston to position 2, shutting off air supply (A) and allowing air from feeders to exhaust from B through X past the connecting rod to outside of the valve (C); return springs maintain position 2 against the compressed air supply.

Figure 3.11 Compressed air feeding system

As each feeder was on a branch supply off the ring, individual feeders could be taken out of use as required by means of clips. The compressed air lines were of 3 mm flexible plastic tubing.

3.36 Each feeder unit was built of inexpensive materials, based on the use of plastic syringes, with plastic cups for supply hoppers. The basic design was as in Fig.3.12, and this was modified for the LTS by substituting 50 ml syringes for the 20 ml ones, and using plastic bottles for the hoppers. The hoppers were covered by plastic (petri-dish) lids to prevent water from entering.

3.37 Fig.3.13 shows an LTS feeder; the top of the barrel was protected from spray by an inverted plastic cup which deflected discharged food downward into the receiver cup, whence food fell via an angled delivery tube protruding through the tank shroud as in Fig.3.14 (delivery tube at top centre). This 2-stage operation facilitated calibration of the feeders, as a blind cup could be placed in the receiver cup to allow observation of discharge but prevent food delivery.

3.38 LTS feeder units were mounted on the framework between the tanks, for easy access. MTS feeders were mounted on hardboard plates bolted to the superstructure girders. STS feeders were mounted in pairs on angle-iron support arms, just above the tank lids.

3.39 Feeder calibration was required at the beginning of each experiment. First the amount to be fed daily was calculated (see Chapter 7), and the feeder hoppers filled at some convenient time after the day's last automatic feed at 1800 (supplying the fish on HC2). Blind cups were inserted into the receiver cups to prevent food delivery. The calibration nut was then set on each feeder to a likely position and the feeders

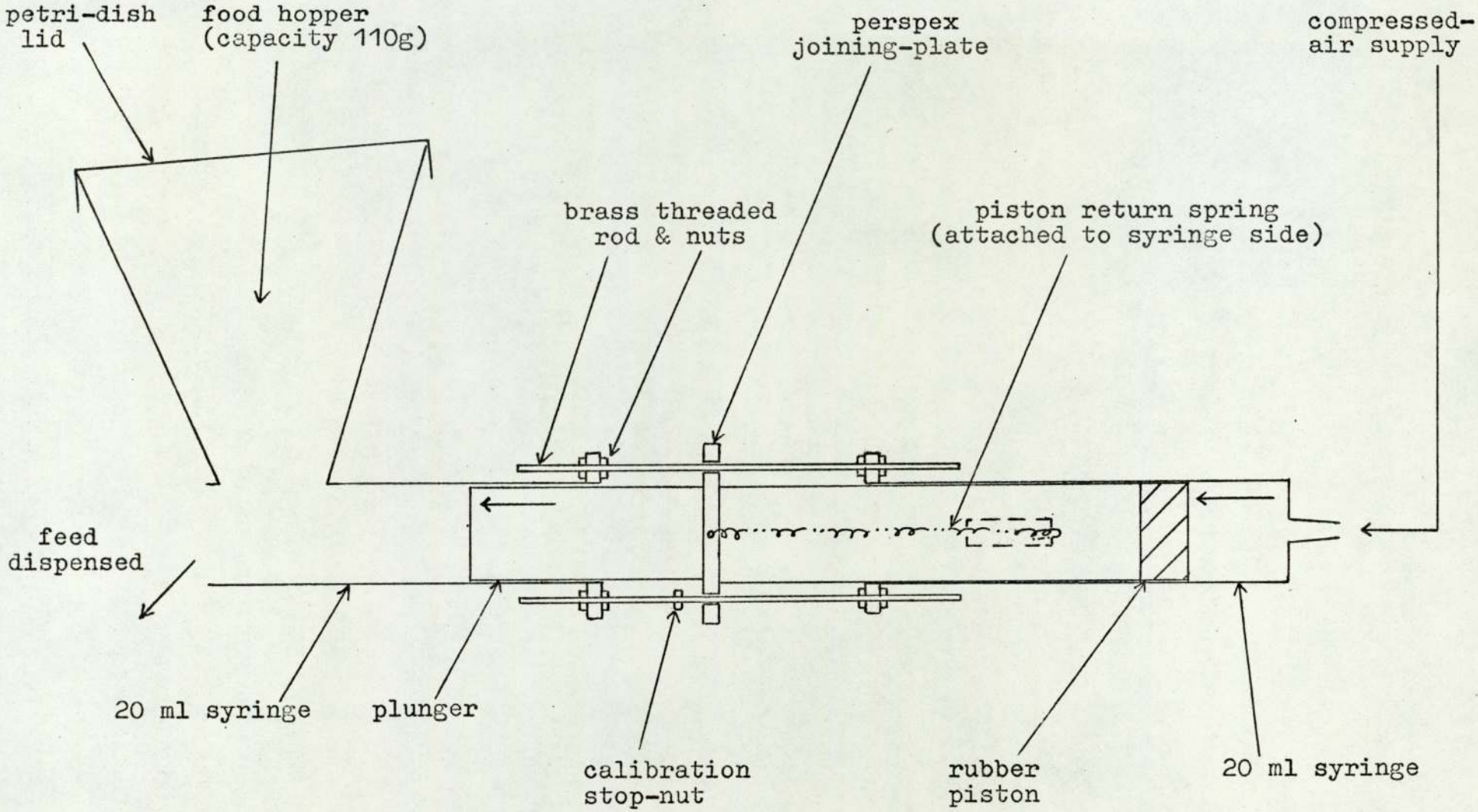


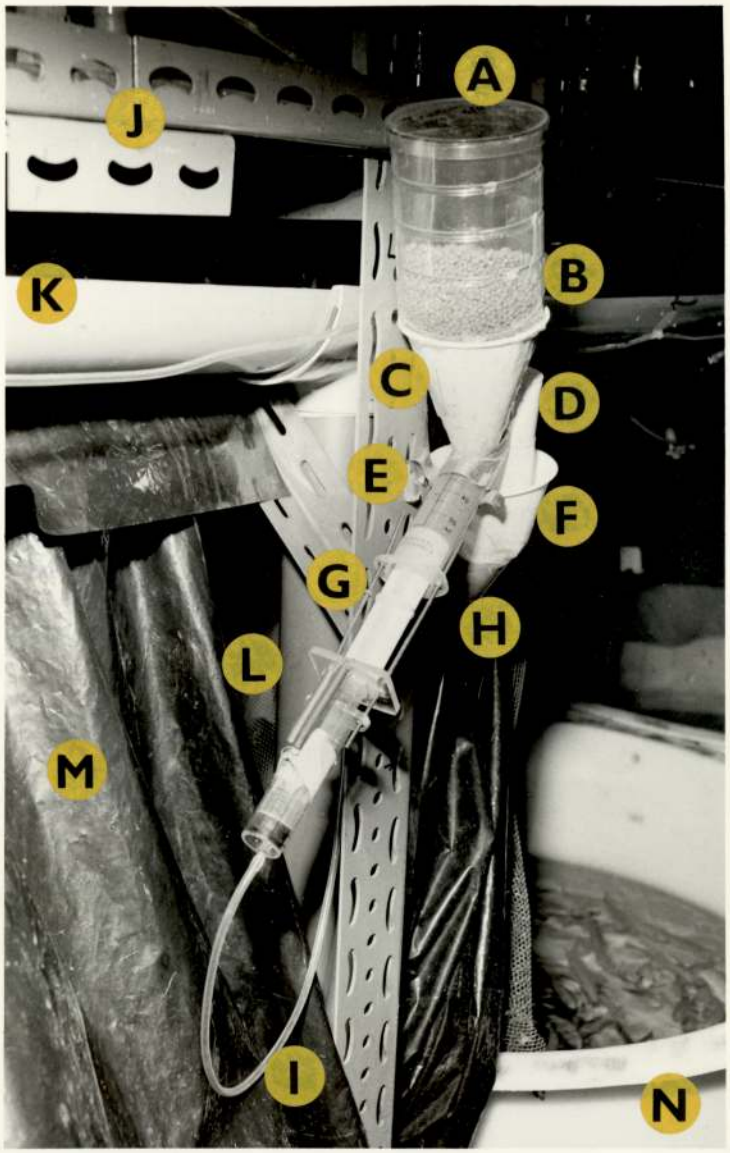
Figure 3.12 Plan of MTS feeder (approx. to scale)

Figure 3.13 LTS feeder

A Plastic hopper lid
B No.4 pellets in upper part of hopper
C Lower part of hopper
D Protective deflector
E Spring clip attachment to framework arch
F Receiver cup
G Calibration nut
H Delivery tube extending through blackout and shroud
I Compressed air supply tube
J STS trestle framework
K STS drainage gutter
L Drainage down-pipe
M Blackout curtain
N LT

Figure 3.14 Food delivery to an LT

A Delivery tube
B Food pellets
C Shroud attachment bolt
D Angled water inlet
E Inlet support arm
F Fish swimming against direction of flow
G Exit hole with simple mesh screen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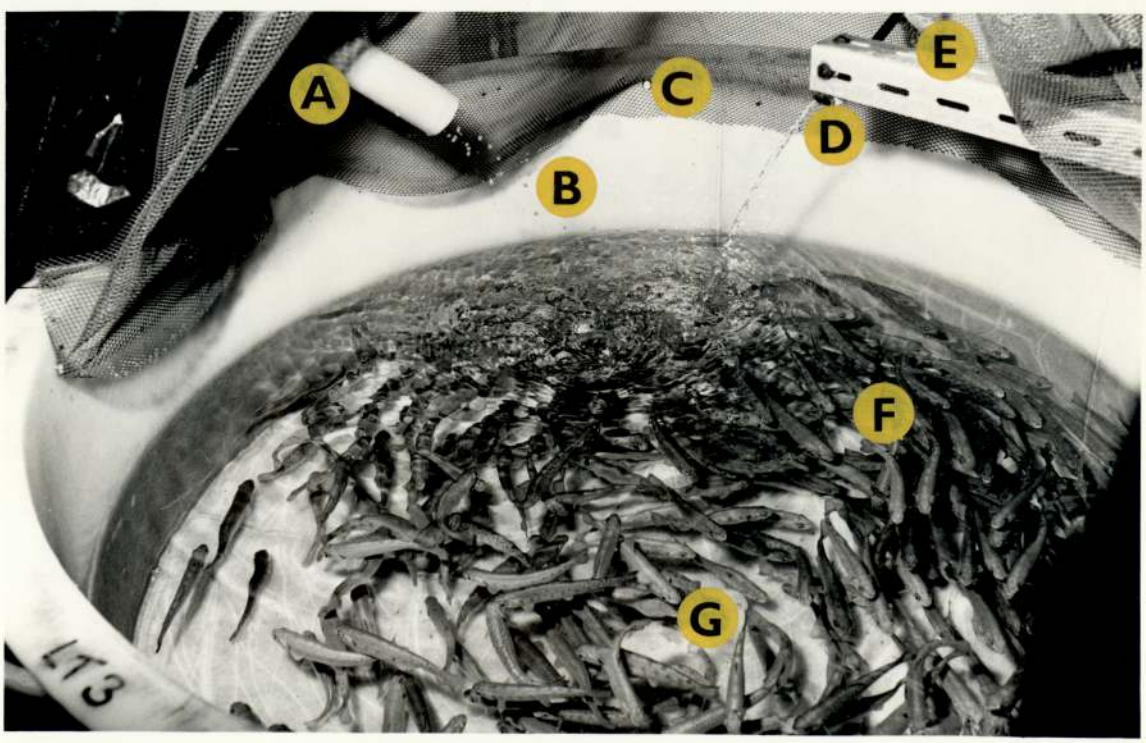
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operated five times by switching the remote override for about 6 to 8s each time, allowing for full piston return in between. The blind cups were then collected and their food content weighed. Any required calibration adjustment was made, and the process repeated several times until feeders were dispensing the correct amounts. Then the blind cups were removed, the hoppers filled with known weights of food, and the control system left to cause the first feed of the subsequent photoperiod automatically.

3.40 Feeders operated successfully with minimal maintenance over a period of 18 months, and were fundamental to the success of experiments (see Chapter 7). Receiver cups and delivery tubes required periodic cleaning as they collected dust from the food.

Blackout

3.41 An efficient blackout system was required and for simplicity and ease of access panels of heavy-duty black polythene sheet were chosen. These had the advantage over cloth curtains of being unaffected by water spray, but were rather less robust. They could be modified with ease, and were conveniently fixed in place to the walls, ceiling and framework, as required, by strips of double-sided adhesive tape. LTS panels were formed into curtains which could be drawn aside on curtain runners for tank access, but overlapped when closed. In addition, horizontal panels were placed below the MTS (and STS where necessary) to shut off the LTS from disturbance, and extra panels were positioned to supplement the STS cloth curtains, so that all three systems were light-isolated from each other and from the rest of the wet lab. The following facilities were outside the blackout and could be accessed without disturbance of the fish: feeders (except STS), volume indicators, overflow level controls, LTS U-tubes, inlet sample supplies. Access behind the blackout was required for tank inlet controls, STS

and MTS U-tubes and bleed-off tubes.

Measuring equipment

3.42 During the development of experimental systems, the following pieces of apparatus fundamental to measurement were procured:-

a) Sauter 10 kg automatic pan-loading balance (this replaced a Sartorius Model 707/10 manual pointer balance and a Gallenkamp manual balance)

b) Sartorius 1100 top-loading 200 g balance

c) Corning-EEL Model 12 Research pH meter (used with Corning or Activion rugged Combination electrode)(this replaced a Pye Dynacap pH meter).

3.43 At an early stage of development it was envisaged that an extra long electrode cable (about 6 m) would allow a reading to be obtained from each tank overflow level control. This proved impracticable due to problems of electrical conductivity. Instead, the pH meter was kept in the dry lab and samples brought to it.

Equipment sources

3.44 Manufacturers of important equipment are listed below.

Angle iron	-	Bartangle Ltd., Bilston
Glassware (to specification)	-	(J.A.R.&.M.K.Hill, Walsall (Glassblowers, Department of (Physics, University of Aston
Medium-size tanks (MT) (to specification)	-	Vortex (Fishery Equipment) Ltd., Meriden
PVC guttering	-	Hunter Plastics Industries Ltd., Woolwich
Process timers	-	Crouzet Ltd., Manchester
Light bulbs	-	Osram (GEC) Ltd., Wembley
24-hour timer	-	Venner Ltd., New Malden

Small electrical components	-	R.S. Components Ltd., London EC2.
Plastic mesh	-	Netlon Ltd., London WC2.
Large-size tank (LT) (to specification)	-	Cago Ltd., Birmingham

4. MEASUREMENT OF AMMONIA

INTRODUCTION AND LITERATURE REVIEW

4.1 The analysis of fish tank water for ammonia played a fundamental part in the work here described. Thus the methods used for ammonia analysis, and their validity and accuracy, are of prime importance in helping to assess the study of excretion and excretory tolerance.

4.2 It is important to note that in all following discussion, ammonia is quoted as ammonia-nitrogen. The importance of the ionisation state is discussed in Chapter 5; all methods used estimated the concentration of nitrogen due to total ammonia ($NH_3^o + NH_4^+$). Estimation of nitrogen due to unionised ammonia alone (NH_3^o) involves measurement of pH and temperature.

4.3 The most common standard method for ammonia analysis has for long been the use of Nessler's reagent (IWE 1960, APHA 1971). Nessler's reagent is an alkaline mercuric iodide solution which turns from red to yellow or brown, the colour intensity (due to a complex ion) being a measure of the ammonia present. Recommended levels of ammonia in the sample for good results are from 400 $\mu g\ \ell^{-1}$ to 5.0 $mg\ \ell^{-1}$ with a sensitivity to 200 $\mu g\ \ell^{-1}$ (APHA 1971), and the process when used directly is subject to interference (causing turbidity or greenish colouration) from calcium, iron, magnesium, sulphide and a wide range of organic molecules.

4.4 Another standard method is the phenol-hypochlorite reaction, which produces an intense blue colour with ammonia due to indophenol formation. First described by Berthelot (1859), it was applied to ammonia by Van Slyke and Hiller (1933), and has since been modified many times. Russell (1944) increased the sensitivity of the method and Riley (1953) applied it to seawater. Lubochinsky & Zalta (1954) introduced the use of nitroprusside catalyst. Since then the method has been used

extensively (e.g. Emmet 1969, Solorzano 1969, Nimura 1973). Cocking (1967) applied it to ammonia excreted by goldfish, and found that it allowed estimation of 25 $\mu g\ l^{-1}$; urea, creatine and creatinine did not interfere even at high concentrations. The method is recommended (APHA 1971) for values up to 500 $\mu g\ l^{-1}$. Croston (1969) quotes the method as more sensitive, stable and reproducible than Nesslerisation. With these points in mind it was decided that the phenol-hypochlorite method would be used for spectrophotometric determination.

4.5 The particular version used was that of Harwood & Kuhn (1970), which has the advantage of simplicity with a relatively small amount of preamble before photometric measurement.

Reagents:-

Buffer: 5% (weight/volume) Na_3PO_4 solution

Phenol stock: 500 g phenol dissolved in methanol, diluted to 800 ml with methanol, stored at $2^{o}C$.

27% NaOH: 270 g NaOH pellets dissolved in water, cooled, diluted to 1 l.

Reagent A: 15 ml phenol stock plus 0.02 g sodium nitroprusside, diluted to 100 ml with water.

Reagent B: 15 ml diluted commercial bleach (hypo) solution ($\pm$ 3% free Cl) plus 15 ml 27% NaOH, mixed, diluted to 50 ml with water.

Stock standard solution: 38.2071 g NH_4Cl dissolved in water, diluted to 1 l (gives 10 g l^{-1} ammonia-N); 1 ml of this diluted to 1 litre (gives 10 mg l^{-1} ammonia-N).

(All chemicals were of analytical reagent grade.)

4.6 In all cases ammonia-free water was required as the diluent. Following Harwood & Kuhn (1970), use was made of distilled water which had been deionised by passing it through a Permutit Mk.17 portable cartridge ion-exchange equipment. Such water should contain $<10\ \mu g\ l^{-1}$ ammonia-N (Beckett & Wilson 1974). Reagents A and B were stored in a refrigerator but

allowed to reach room temperature before use; they were made up weekly from the stock reagents when required. Stock standard solutions were made up fortnightly and similarly stored.

4.7 Procedure:-

To each 25 ml volumetric flask (one per sample or standard) was added (by pipette);

5 ml sample or standard,

2 ml buffer,

3 ml deionised water,

5 ml reagent A while swirling,

2.5 ml reagent B while swirling,

and deionised water to make up to 25 ml.

The flask was stoppered and mixed well, and left for at least 25 min. Two flasks were prepared for each sample or standard solution used (standards were made by appropriate dilutions from the stock standard); a blank using deionised water was also prepared. Since the developed colour was stable for 4 h, photometric measurement could be delayed for a short while if necessary.

4.8 Measures of the solution from the flask were pipetted into 1 cm glass cells and measured at 630 nm against the blank in a spectrophotometer. For pilot experiment P01 a Beckman DB was used, but for later work a Unicam SP 500 was used. The recorded measurement for each sample/standard was the average of the duplicates.

4.9 During P01 good calibration graphs were obtained for values between 0.1 and 1.0 mg ℓ^{-1}, reading the absorbance scale to give a straight line calibration. Unfortunately, most of the experimental values found in P01 were at or below 0.1 mg ℓ^{-1} and hence on the most uncertain part of the line. Although Harwood & Kuhn (1970) recommend the use of 4 cm cells for the range 0.1 to 1.0 mg ℓ^{-1}, the adequacy of the calibration lines between these values suggests that 1 cm cells are

satisfactory, although naturally somewhat less precise. On average, the correlation found between absorbance values and ammonia-N concentration was expressed by r = 0.9972. (Harwood & Kuhn (1970) found r = 0.9993 for 4 cm cells, and r = 0.9989 for 1 cm cells in the range 2 to 10 mg ℓ^{-1}.) Harwood & Kuhn also tested their ammonia measurements against those produced by an Auto-analyser method, finding very high similarities. When samples were presented for Auto-analyser analysis at Aston, it was necessary to use a range-expander with some loss of sensitivity and precision. The calibration graphs of transmission against concentration (plotted on semi-log graph paper) were of variable curve-form. For this reason, and also due to problems of machine access, the Auto-analyser was eliminated from consideration as a routine ammonia-measurement method.

4.10 The spectrophotometric method was satisfactory down to about 0.1 mg ℓ^{-1}, but its major drawback was the amount of manipulation needed to deal with large numbers of samples. In later stages of experimental work it was necessary to handle up to 40 samples per session. This made error or spillage extremely likely, and the time required would have interfered with other necessary operations during the day. As it was considered better to analyse samples as soon as possible rather than to store them, the procedure would become increasingly unwieldy with large numbers of samples.

4.11 An alternative speedy method of ammonia analysis was therefore sought, capable of handling large numbers of samples with minimum effort, and reliable over the ranges of values anticipated i.e. down to 100 $\mu g\ \ell^{-1}$. For this purpose an EIL laboratory ammonia probe (Model No.8002) was purchased in September 1972 and used for all subsequent experiments (from P02 onwards - see Chapter 6).

4.12 Up to the time of purchase, such instruments had been little used by other workers for ammonia analysis, although Barica (1971) had used a univalent cation glass electrode for determination of NH_4^+ ion. The probe, in contrast, operates by detecting the amount of free NH_3^o. More recently literature on the use of the probe has appeared, besides the information sheets issued by the major American manufacturer of selective-ion electrodes (Orion 1970 a & b, 1972). Barica (1973) described use of an Orion probe for fish tank water, and Midgley & Torrance (1972, 1973), Beckett & Wilson (1974) and Evans & Partridge (1974) tested the EIL probe in various other applications.

4.13 Barica (1973) evaluated an Orion model 95-10 for determination of total ammonia. He found that for values below 0.1 mg ℓ^{-1} it was unsuitable, and in the range 0.2 - 0.5 mg ℓ^{-1} it yielded results differing from those found by automated spectrophotometry by $\pm$ 17%. In the range 0.1 to 14.0 mg ℓ^{-1} he found that calibration curves were necessary, but above this range and up to 14 g ℓ^{-1} response was Nernstian (i.e. a plot of mV response against log ammonia concentration gave a straight line); below 14 mg ℓ^{-1}, mV response was rather less per (log) concentration unit.

4.14 Beckett & Wilson (1974) tested the EIL probe from 0.1 to 4.0 mg ℓ^{-1}. They found Nernstian response over this range (which roughly agrees with Evans & Partridge (1974) and the manufacturers' advice (EIL 1971)); but found that the calibration line slope may vary from time to time or between individual instruments. They found variability of results from about 10% to 3% as concentration increased from 0.1 to 4.0 mg ℓ^{-1}. This confirms the findings of Midgley & Torrance (1972). Beckett & Wilson found that 5 mg ℓ^{-1} of urea present in the water caused a comparable sized error to that due to diluent water, which they considered negligible. They estimated calibration time as 20 min, with 5-6 min necessary for probe

stabilisation per reading (agrees with Barica (1973)). Good agreement was found between the probe and other methods, and Beckett & Wilson recommended the probe for various applications, mentioning its potential for on-line water analysis as in Midgley & Torrance (1973). Midgley & Torrance (1972) preferred the probe to a phenol-hypochlorite method for power-station high-purity water (0.1 to 1.0 mg ℓ^{-1} ammonia-N).

PROBE METHOD FOR AMMONIA

Introduction

4.15 The EIL ammonia probe functions in a similar way to a selective-ion electrode but operates on a different principle. It consists of a transparent hard-plastic tube with a thin hydrophobic polymer membrane across one end, across which free ammonia (NH_3^o) can diffuse but ions cannot. Within the tube is an ammonium chloride solution, bathing the interior of the membrane and surrounding a glass pH electrode and a silver/silver chloride reference electrode. The pH electrode is pressed against the membrane, trapping a thin film of solution. Unionised ammonia will diffuse through from a sample, and since the NH_4Cl solution provides excess NH_4^+ ions, the important effect will be to cause an increase of OH^- ions, according to the amount of unionised ammonia, until the partial pressure of ammonia is equal on both sides of the membrane:-

$$NH_3^o + H_2O \longrightarrow NH_4^+ + OH^-$$

This changes the pH of the internal solution: a change which is sensed by the pH electrode, and can be displayed as an electric potential change measured in mV, where the potential change (E) depends on concentration of NH_3^o (C) thus:-

$$E = B - \left(\frac{2.303\ RT}{F}\right) \log C$$

(B = constant, R = gas constant, T = absolute temperature, F = the Faraday (96500 coulombs per equivalent))

4.16 Due to the temperature dependence, the probe is used for measurement at a constant temperature; thus standards and samples must be brought to a common temperature before measuring.

4.17 Initially the probe was used with a milli-voltmeter/pH meter (Pye Dynacap), but for most of the experiments it was used with an EIL Model 7030 equipped to function as pH meter, millivoltmeter or specific ion electrode meter; the latter function depends on a built-in ability to vary slope correction as necessary (for the slope of the graph of log C against E) and supplying readings on a logarithmic scale corresponding to direct concentration readings (e.g. mg ℓ^{-1}). Calibration graphs were thus unnecessary provided two standards were used, separated in value by a factor of 10 (e.g. 0.1 and 1.0 mg ℓ^{-1}). The slope correction control allowed standardisation of the log concentration scale against the standards, and hence direct readout for unknowns. Calibration was carried out before each day's measurements.

Procedure

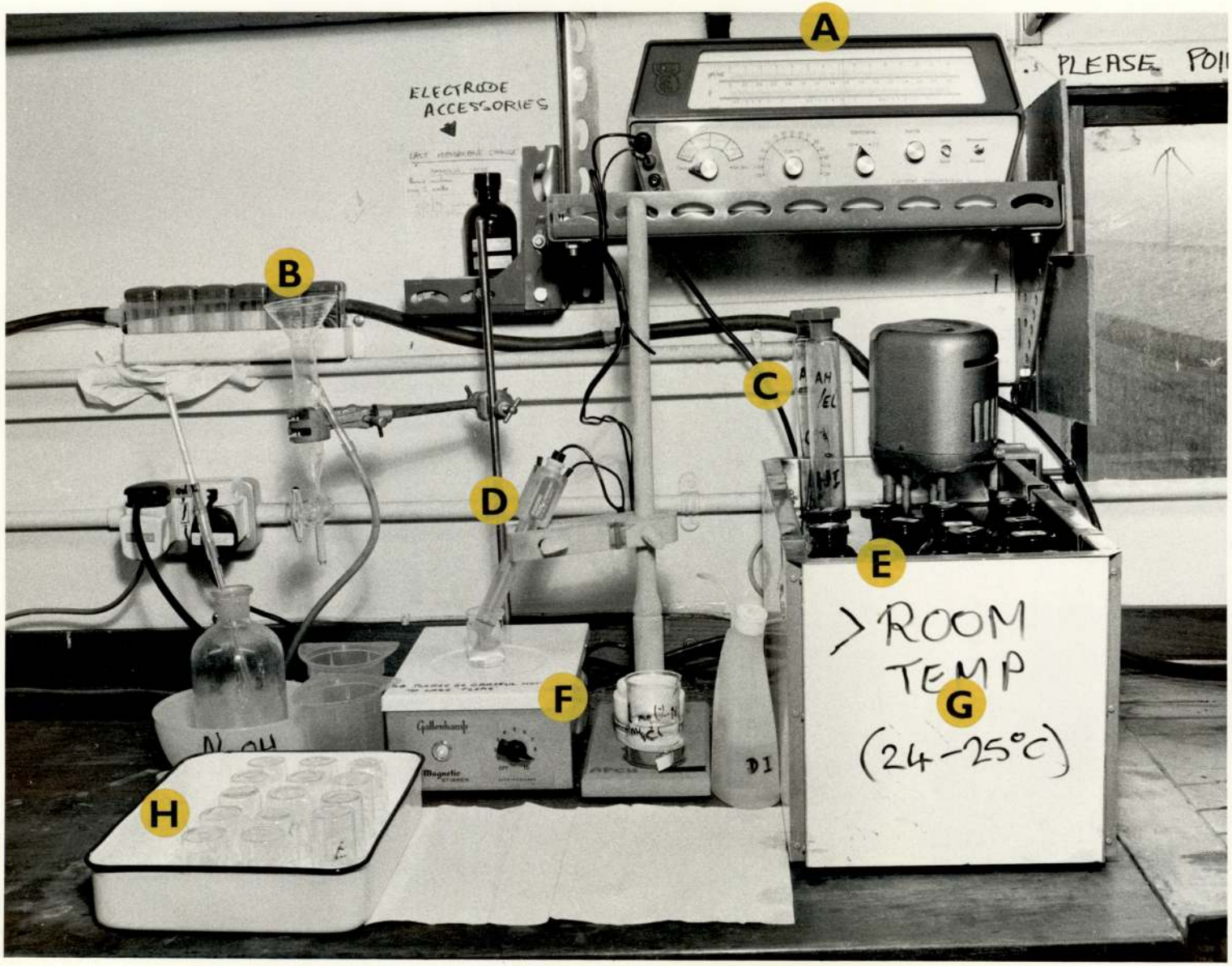
4.18 The measuring equipment is shown in Fig.4.1. Duplicate samples were taken from tank overflows (or inlet-sample lines) in 60 ml amber glass bottles with ground-glass stoppers. Each bottle was washed out twice in the water to be sampled before collection, then overfilled and stoppered without any air trapped inside. Sample bottles were transferred to a water bath at $\pm 25^{o}$C to reach measurement temperature.

4.19 Standards were prepared by diluting with deionised water the stock standard solution of NH_4Cl (10 mg ℓ^{-1}), and the standards (in 100 ml stoppered measuring cylinders) were also transferred to the water bath. A magnetic stirrer plate was set up, and a strong solution of NaOH (40 mg ℓ^{-1} or 1.0M) prepared.

4.20 Samples and standards were dispensed with the apparatus shown in Fig.4.1. This dispenser, made to specification by the University glass-blowers, was used because of its combination of speed and precision in delivering a fixed quantity ($\pm$ 22 ml), after being filled to the level controlled by the overflow arm with tap closed. The dispenser was first pre-

Figure 4.1 Ammonia measurement equipment

A EIL Model 7030 pH meter
B Sample dispenser
C Ammonia standard solutions
D EIL Model 8002 laboratory ammonia probe
E 60 ml amber sample bottles
F Magnetic stirrer plate
G Temperature-controlled water bath (25°C)
H 25 ml measurement beakers


A
B
C
D
E
F
G
H
ELECTRODE ACCESSORIES
PLEASE
> ROOM TEMP
(24-25°C)
Gallenkamp
Magnetic
DI

washed with the solution to be measured, and then filled to overflow. The delivered aliquot was collected in a 25 ml beaker containing a magnetic stirring-rod. 0.25 ml of NaOH solution was added to raise pH to $\pm$ 12.5, at which all ammonia present would be converted to the unionised form. The beaker was then placed on the stirrer plate (set to a stirring speed which would not cause bubbles or excessive vortex).

4.21 The probe, connected to the meter, required rinsing with deionised water and dabbing dry with soft medical tissue paper, in between each solution measured, in order to prevent carry-over. For measuring, the probe was lowered at an angle into the solution to prevent bubble formation and the formation of a "dead space" under the membrane, and was then allowed to settle completely before the reading was taken.

4.22 Calibration was carried out as follows. A measure of "low" standard (e.g. 0.1 mg ℓ^{-1}) was dispensed and its pH adjusted. The probe was introduced into the beaker, and time for stabilisation of reading allowed (about 5-6 min at 0.1 mg ℓ^{-1}). The calibration "BUFFER" control was then adjusted to set the reading to the lower calibration point on the middle (p) scale (Fig.4.1). A measure of "high" standard (e.g. 1.0 mg ℓ^{-1}) was then substituted for the "low", the probe introduced and allowed to stabilise (2-3 min at 1.0 mg ℓ^{-1}), and then the meter was set to the higher calibration point on the p scale, using the slope calibration control (second from left in Fig.4.1). This compensated for slope change between measurement sessions.

4.23 The process was then repeated, using both standards, a sufficient number of times (usually only once) to check the accuracy of the first attempt, and then the BUFFER control adjusted so that the standard used last registered its true value on the CONC scale (lowest of the three, Fig.4.1).

4.24 Each sample was then treated in an exactly similar way to the standards, and the reading, after sufficient stabilisation time was noted from the CONC scale.

4.25 All glassware used was rinsed in distilled and then deionised water after washing, and prewashed with deionised water before use to avoid ammonia contamination.

4.26 With each batch of readings taken, an inlet-sample (of water as it entered the fish tanks), and a "zero" sample (from the deionised water supply) were measured as checks. The values recorded were always similar, never exceeded 50 $\mu g\ l^{-1}$, and rarely exceeded 30 $\mu g\ l^{-1}$. Given the unreliability of the probe's measurement at such low concentrations, it is felt that neither of these sources would give rise to an error above 10 $\mu g\ l^{-1}$ for concentrations greater than 100 $\mu g\ l^{-1}$ (see Beckett & Wilson (1974), Evans & Partridge (1974)).

4.27 It was found, in agreement with Evans & Partridge (1974), that stabilisation time varied according to the concentration difference between the current and the previous sample under the probe. For a number of samples in ascending order of concentration, stabilisation time could be reduced below expectations, while widely differing concentrations required longer internal probe readjustments. Duplicate measurements were taken in all cases, and the mean of the two was accepted as the "actual" measurement.

4.28 On a few occasions measurement was not carried out immediately after sampling, and in these cases sample bottles were stored in a refrigerator at about 4°C, as mentioned by Beckett & Wilson (1974). Storage did not exceed 36 h, and samples were allowed to equilibrate in the water bath before measurement.

4.29 Occasional check readings with the probe suggested that whether stored at room temperature or in a refrigerator, samples containing about 1.0 mg ℓ^{-1} showed a maximum variability of about 5% in measured value over a period of about 8 h. (Kutty (1972) found no trend in change of ammonia content of samples for up to 4-5 h.) Whilst refrigerated samples maintained this record over about 24 h after sampling, those kept at room temperature changed by up to 25% over this time, hence samples were not kept unrefrigerated over periods longer than 1-2 h.

4.30 The Model 7030 meter could be read on the CONC scale to an accuracy of about 5%. Following Beckett & Wilson's advice (1974) that error due to diluent water can be ignored, and bearing in mind the noted similarity between diluent water and inlet samples, the quoted average error on probe readings of about 10% at 0.1 mg ℓ^{-1} (Beckett & Wilson 1974, Midgley & Torrance (1972)) seems to be a fair guide: the sum of the errors described above would agree with this, and a working value for error of 10% was accepted for the study. This is discussed in the context of the major excretory series of experiments in Chapter 7.

4.31 It was considered that of the various methods tried, none was completely satisfactory in accuracy of measurement of total ammonia below 100 μg ℓ^{-1}. The probe was both speedy and satisfactory above this level, and was used for all subsequent measurements from its date of purchase. Since 100 μg ℓ^{-1} was usually exceeded in major experiments, any values below this level were treated as unreliable.

PART 3

AMMONIA PRODUCTION

AND THE TANK ENVIRONMENT

5. INTRODUCTION TO AMMONIA PRODUCTION

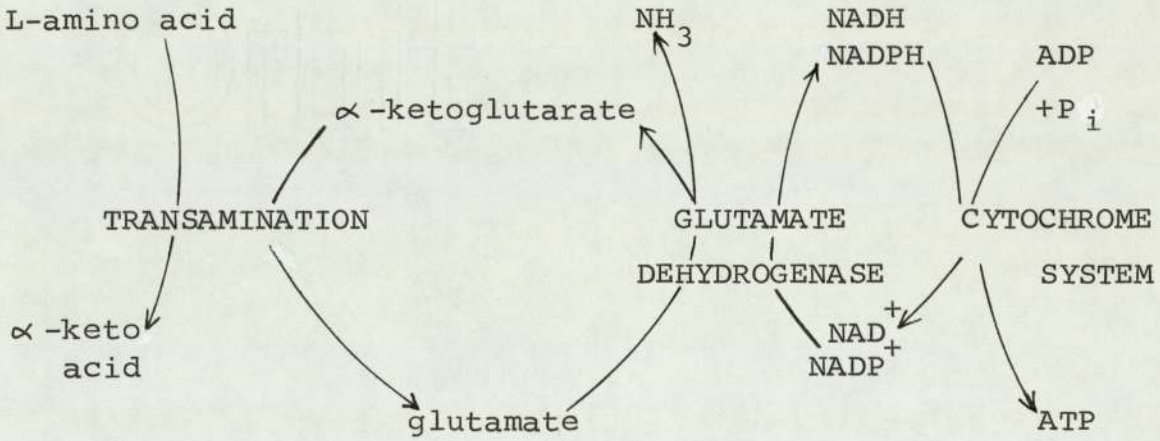
LITERATURE REVIEW

Ammonia as an excretory product

5.1 Excretion of fish is a topic widely surveyed in physiological literature, (e.g. Black 1957, Forster & Goldstein 1969, Goldstein & Forster 1970, Watts & Watts 1974), but rarely from precisely the viewpoint required in this study.

5.2 Much of the work derives from the experiments of Smith (1929) on the accumulation of excretory products in a static tank (see below). Such experiments revealed that the major compound excreted is ammonia.

5.3 Ammonia represents a physiologically "economic" excretory product which requires no energy expenditure in its formation. In fact some of the reactions involved can ultimately lead to ATP generation and the capture of free energy:-



(Forster & Goldstein 1969, Cohen & Brown 1960)

5.4 Ammonia can thus be thought of as a "low-energy" compound, compared with the other common animal excretory end-products, urea and uric acid. These latter products require an energy-consuming synthetic system (Cohen and Brown 1960) and can be thought of as being on a higher energy level; but where water conservation or excretory product storage are important, their less toxic nature makes them more suitable.

5.5 The dissociation of ammonia in solution is described by the relation:

$$NH_4^+ \rightleftharpoons NH_3^o + H^+$$

This equilibrium position is dependent on pH so that, according to the well-known Henderson-Hasselbalch equation:-

$$pH = pK_a + \log_{10} \frac{(NH_3^o)}{(NH_4^+)}$$

In acid solution the equilibrium is in favour of NH_4^+, whilst an alkaline solution will contain a majority of NH_3^o radicals. Below pH 7, the quantity of NH_3^o present is usually negligibly small; at slightly higher pH even the low proportion present may be important in its effects. Free or unionised ammonia NH_3^o readily diffuses into and out of cells because it is lipid-soluble, enabling rapid elimination without water loss. Such diffusion is dependent on the partial pressure (pNH_3) gradient across the membrane. pNH_3 is related to NH_3^o by the equation:

$$[NH_3^o] = \frac{\alpha}{22.1} pNH_3$$

- where α is the solubility co-efficient (see Maetz 1973). Although ammonia is predominantly ionised at body fluid pH (NH_4^+ : NH_3^o is about 100:1), the interconversion is instantaneous and hence would not limit the rate of excretion (Hoar & Randall 1969, Goldstein & Forster 1970).

5.6 Substantial discussion in the literature has been directed at attempting to identify the principal source of ammonia excreted at the gills. Originally Smith (1929) proposed extraction at the gills from ammonia already circulating in the blood, but later modified his opinion in favour of its production <u>in situ</u> at the gills. Later workers (e.g. Goldstein & Forster (1961), Goldstein et al (1964)) have argued in favour of both views and various combinations.

5.7 De Vooys (1969) points out that although mostly in the ionic form (at pH about 7.4), ammonia in fish blood would

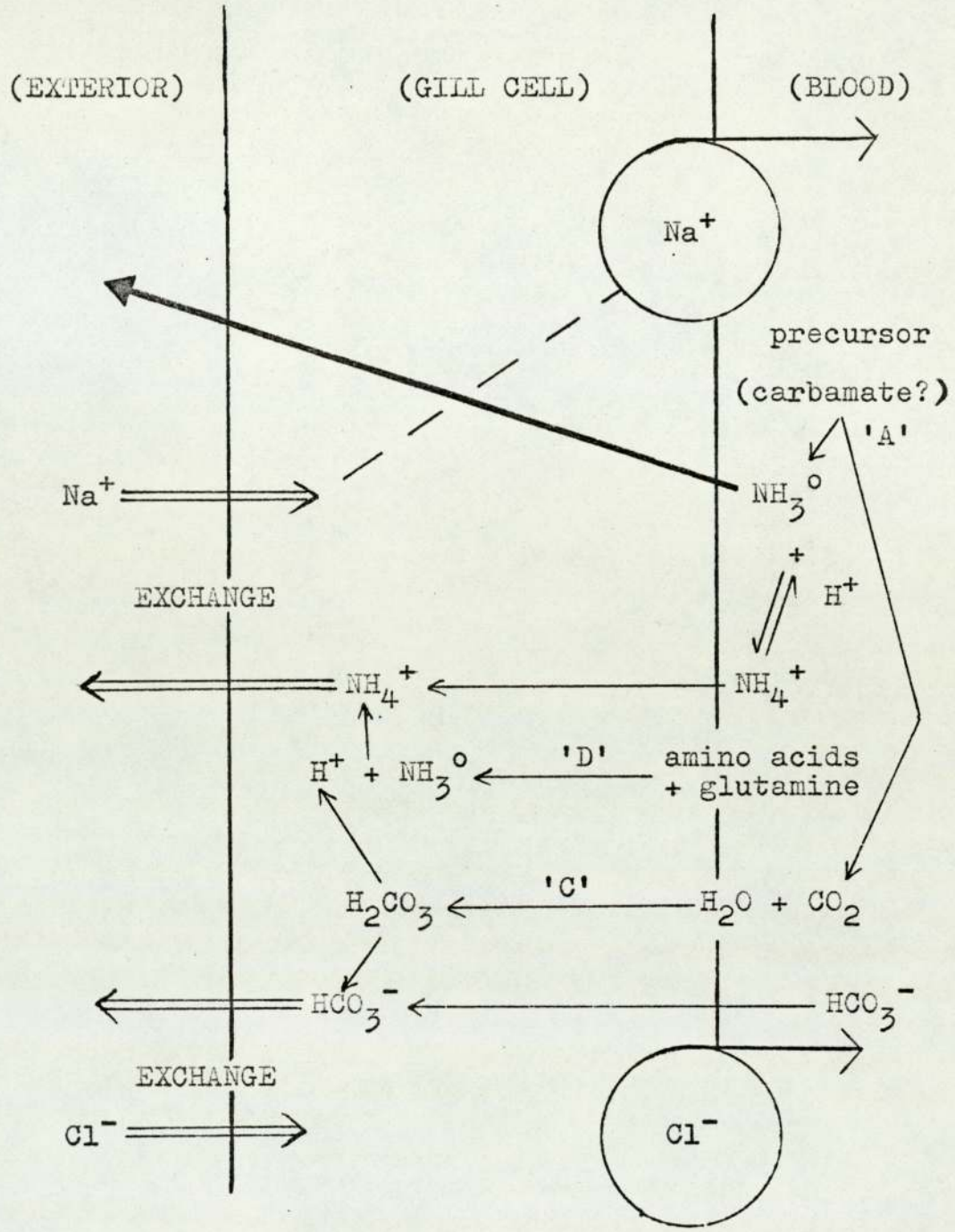
nevertheless continuously furnish NH_3^o molecules, which would act as a nerve poison. He suggests that ammonia occurs in the blood, is transported in a protein-bound carbamate complex and is released when CO_2 is liberated at the gills.

5.8 Thus both transport from other sites of formation (e.g. liver), and peripheral formation at the gills, seem to be possible routes of ammonia arrival at the gill surface. There is also controversy about the passage of ammonia across the gill membrane into the surrounding water; ionic ammonia could be immediately transformed to NH_3^o which could pass freely out, but another possibility also exists.

5.9 Maetz and Garcia Romeu (1964), in studies of ion transfer across the gills of the fasted goldfish Carassius auratus, postulated an exchange of NH_4^+ for Na^+ ions actively taken in. Romeu and Motais (1966) repeated this with the eel Anguilla anguilla. However, Kerstetter et al (1970) using Salmo gairdneri postulated a Na^+/H^+ exchange rather than exchange for ammonia, and De Vooys (1968) also failed to support a Na^+/NH_4^+ exchange, using carp.

5.10 More recently, Maetz (1972, 1973) has fully discussed the exchange across Carassius gills, and indicated that according to pH and available external sodium, ammonia can move either as NH_3^o or as NH_4^+, and that both H^+ and NH_4^+ can be excreted. Excretion is probably limited to NH_3^o only when there is no external sodium.

5.11 All of this work has necessarily been with individual experimentally fasted fish, and the importance of the relative occurrences of ion exchange and passive NH_3^o transfer are unknown for fish in a feeding culture situation. Under these conditions there would be a higher requirement for ammonia elimination, and hence it could be supposed that passive NH_3^o transfer would account for the greater proportion, much in excess of the amount of ion required for Na^+ exchange. Figure 5.1 summarises some possible mechanisms involved in gill



'A' = carbamate deaminase 'C' = carbonic anhydrase

'D' = deamidation/deamination enzymes

= active transport mechanism

Figure 5.1 Some possible ionic pathways in fish gills (modified from Maetz & Garcia Romeu 1964)

ammonia clearance.

Excretion in fish

5.12 Homer Smith's pioneering work (1929) on fresh-water fish in divided chambers established gill excretion as the major route of nitrogen loss, and that most of this was ammonia (60-90%), with a lower proportion of urea. Later studies have confirmed this (Wood 1958, Fromm 1963).

5.13 Kidney excretion via the urine was found to be relatively small (Denis 1913/1914, Smith 1929, Grollman 1929), and to consist of creatine, creatinine, uric acid and traces of other compounds including trimethylamine oxide (TMAO) and a very little ammonia.

5.14 Absolute values of nitrogenous excretory products seem at first sight to be relatively rare in the literature. The difficulty is clarified when the usual requirements of physiological work are taken into account:
a) to make valid samples over a period of time (usually requiring enclosure in a small tank for 12 or 24 hours);
b) to keep urinary and gill measurements separate (usually requiring anaesthesis, operation on the animal and catheterisation of the urinary papilla);
c) to exclude faecal contamination and variability due to feeding (usually requiring the use of fish which have been fasted for several days). Complying with these requirements is a pre-requisite for ionic balance work, but the penalty is paid in divorce of the experimental situation from "real life", and the necessary imposition of stresses which accompany experimental procedure. Such conditions are here designated "physiological".

5.15 Experiments have been performed under these kinds of conditions by several investigators (e.g. Smith 1929, Gerking 1955, Wood 1958, Goldstein & Forster 1961, Fromm 1963, Thornburn & Matty 1963, Fromm & Gillette 1968, Olson & Fromm 1971) in addition to those investigating ion transfer. Information

from these sources is included in Table 5.1 for comparison, although the more important data for this study are those tabulated from fish culture situations, especially those featuring salmonids. Table 5.1 also shows how often the important supportive information has not been recorded (e.g. nutritive status and temperature, see Fromm (1963) and Brockway (1950)). In the table specific excretory rate (SER) (mass of ammonia produced per kg per hour) is calculated from data from the authorities quoted.

5.16 Table 5.1 shows that under physiological (P) conditions of starvation, which lead to a fairly steady low excretion level, referred to here as the endogenous nitrogen excretion, ammonia output from fish is usually <10 mg kg^{-1} h^{-1} and frequently <5 mg kg^{-1} h^{-1}. (Endogenous excretion may be defined differently in nutritional studies (Gerking 1955)).

5.17 Culture situations (C) show a wider variety of values, usually higher. The data for salmonids are interesting in their diversity. Whilst Burrows (1964) quotes figures giving about 3-6 mg kg^{-1} h^{-1} for chinook salmon, presumably on production feeding schedules, Liao's (1970) survey of operating salmonid farms seems to indicate much higher values. Saeki (1958) and Shirahata (1964) roughly agree (between 10 and 20 mg kg^{-1} h^{-1} for rainbow trout), but Gigger and Speece (1970) found values about three times higher.

5.18 Clearly in the culture situation many factors can come into play, and temperature and feeding rates are likely to be particularly important. Liao's survey gives some indication of just how different feeding rates can be (see below), and it is not surprising that under these conditions the measured ammonia output is extremely variable.

5.19 Attention has been focussed on ammonia output, but it is necessary to examine briefly the role of the other excretory products, chiefly urea. The work of Olson and Fromm (1971)

Table 5.1 Ammonia excretion in freshwater fish

FISH	SIZE (g)	TEMP (°C)	NU. ST.	SER (mg $kg^{-1}h^{-1}$)	AUTHORITY	TYPE	COMMENTS
Cyprinus carpio (Carp)	420	-	S	1.36	Smith 1929	P	
	600	-	S	1.82			
	1500	-	S	1.15			
	1400	-	S	2.07			
	530	-	-	4.69			-starved?
	315	-	S	3.97			
	530	-	-	3.93			
	500	-	-	5.06			} -starved?
	500	18.5	-	4.05			
	368	18.5	-	5.46			
	1500	-	-	0.55			
	250	15	S	1.00	De Vooys 1968	P	10% of excreted N is urea
	-	19	-	14.5 T	Pora & Prekup 1960a	C	fed?
	30	16-25	F	4.17-8.33	Saeki 1958	C	
Carassius auratus (goldfish)	235	-	-	3.85	Smith 1929	P	-starved?
	255	-	S	1.61			
	220	-	-	3.84			} -starved?
	330	-	-	0.65			
	10-15	21	S	4.17 7.50 10.42 T	Thornburn & Matty 1963	P	
	80-330	18-23	S	1.05-7.00	Maetz & Garcia Romeu 1964	P	
	12	25	S	5.00	Cocking 1967	P	semi-starved (48 h)
	2	14.5	F	5.21	Saeki 1958	C	
	2	15.5	F	8.75			
	2	25	F	5.00			
	8-9	25	F	10.00			

Table 5.1 continued

FISH	SIZE (g)	TEMP (°C)	NU. ST.	SER (mg mg^{-1} h^{-1})	AUTHORITY	TYPE	COMMENTS
Lepomis Macrochirus (Bluegill sunfish)	34	20	S	9.15 T 10.55 T 8.37 T 9.42 T	Savitz 1973	P	after mild handling stress
	-	25	S	1.62 T	Gerking 1955 (quoted in Fromm 1963)	P	endogenous excretion of fish fed dextrose as energy source
	50	25	S	6.42 T	Gerking 1955	P	
	144	25	S	3.96 T			
Ameiurus nebulosus (Freshwater catfish)	-	-	-	1.05-4.90	Wolbach Heinenmann & Fishman 1959	P	starved?
Tilapia mossambica	8-30	30	S	upto 30.0	Kutty 1972	P	
average for several species	-	-	F	20.8 T	Saeki 1958	C	working figure for filter proposals
Salvelinus fontinalis	-	7.20	F	20.8 T	Saeki 1958	C	
(Brook or speckled trout)	16-30	10.50	S	4.79 1.29	Phillips Brockway et al 1954 (quoted in Fry & Norris 1962)	P	probably semi-starved
Oncorhynchus tschawytscha (Chinook salmon)	f	13	F	6.37	Burrows 1964	C	@loading (kg min^{-1}) 0.52
		11	F	4.97		C	0.61
		13	F	3.41-5.12		C	0.98

Table 5.1 continued

FISH	SIZE (g)	TEMP (°C)	NU.ST.	SER (mg kg^{-1} h^{-1})	AUTHORITY	TYPE	COMMENTS
Salmo gairdneri (Rainbow trout)	129	13	S	5.67 T 3.40	Fromm 1963	P	
	-	12-13	S	10.42 T 5.42	Fromm & Gillette 1968	P	controls for experiment
	150-350	13	S	1.68-4.20	Kerstetter et al 1970	P	
	50-100	13	S	6.67 T 3.54 1.25 U	Olson & Fromm 1971	P	at low ambient ammonia
	60	15	S	6.25 T	Phillips Brockway et al 1954 (quoted in Fry & Norris 1962)	P	
	-	20-25	F	12.50	Saeki 1958	C	
	4	13	F	7.92 18.75 T			
	f	-	F	17.0	Shirahata 1964	C	
	36	19	F	48.0	Gigger & Speece 1970	C	@stocking (g l^{-1}) 0.96
	36	18	F	31.5			2.39
	±150	11	F	14.90	Bozeman 1970	C	
salmon & trout generally	ALL	7-18	F	12.9-168.3 (mean 69.2)	Liao 1970	C	from questionnaire on hatchery practice

Notes:-

a) NU.ST. is nutritional status; S indicates starved to point of endogenous nitrogen excretion; F indicates fed

Table 5.1 continued

b) TYPE is the type of experiments providing data; P indicates physiological (similar to text description in para.5.14); C indicates culture (fish fed for growth)

c) SER values are for ammonia except where marked T (total nitrogen) or U (urea)

d) Sizes are by weight except where marked f (indicates fingerlings of unknown size) or ALL (indicates wide range of sizes up to maturity)

confirmed earlier suggestions (Fromm 1963, Fromm and Gillette 1968) that ammonia makes up 50-60% of the excreted nitrogen compounds under physiological conditions, while suggesting about 19% urea. However, it is important to note the difference between these conditions and those of the culture situation. Although Saeki (1958) also quotes about 50% as the proportion of ammonia, there is no evidence that this is based on measurements in the culture situation. There is no certainty that what may be true for starved fish is true for actively feeding and growing fish, although Fromm (1963) found ammonia at about 60% of total-nitrogen excreted after only two days of starvation.

5.20 In this context the evidence of Burrows (1964) is interesting, and suggests that, at least in chinook salmon, urea is a significant, even dominant, excretory product at low fish loading levels. Consideration of some measure of fish density in the water in connection with their excretion is a helpful concept. and in this study some of the various possible parameters are used as arbitrarily defined below:-

LOADING -- the fish mass per unit flow of water (measured in kg per unit flow rate; the resultant units are kg min ℓ^{-1})

STOCKING -- fish mass per unit volume of water (units: g ℓ^{-1}, kg ℓ^{-1})

DENSITY -- number of fish per unit volume of water (units: fish ℓ^{-1})

5.21 Burrows' results are shown in Fig.5.2. The ammonia levels are surprisingly low in the light of the work of Liao (1970), and Gigger and Speece (1970). Some question must be raised over the sampling technique used (from the effluents of large raceways) and the kind of feeding regime employed, which is not discussed. (It has since been shown that the ammonia profile varies in different parts of a stocked raceway and so effluent values may not be reliable excretion indicators (Bozeman 1970)).

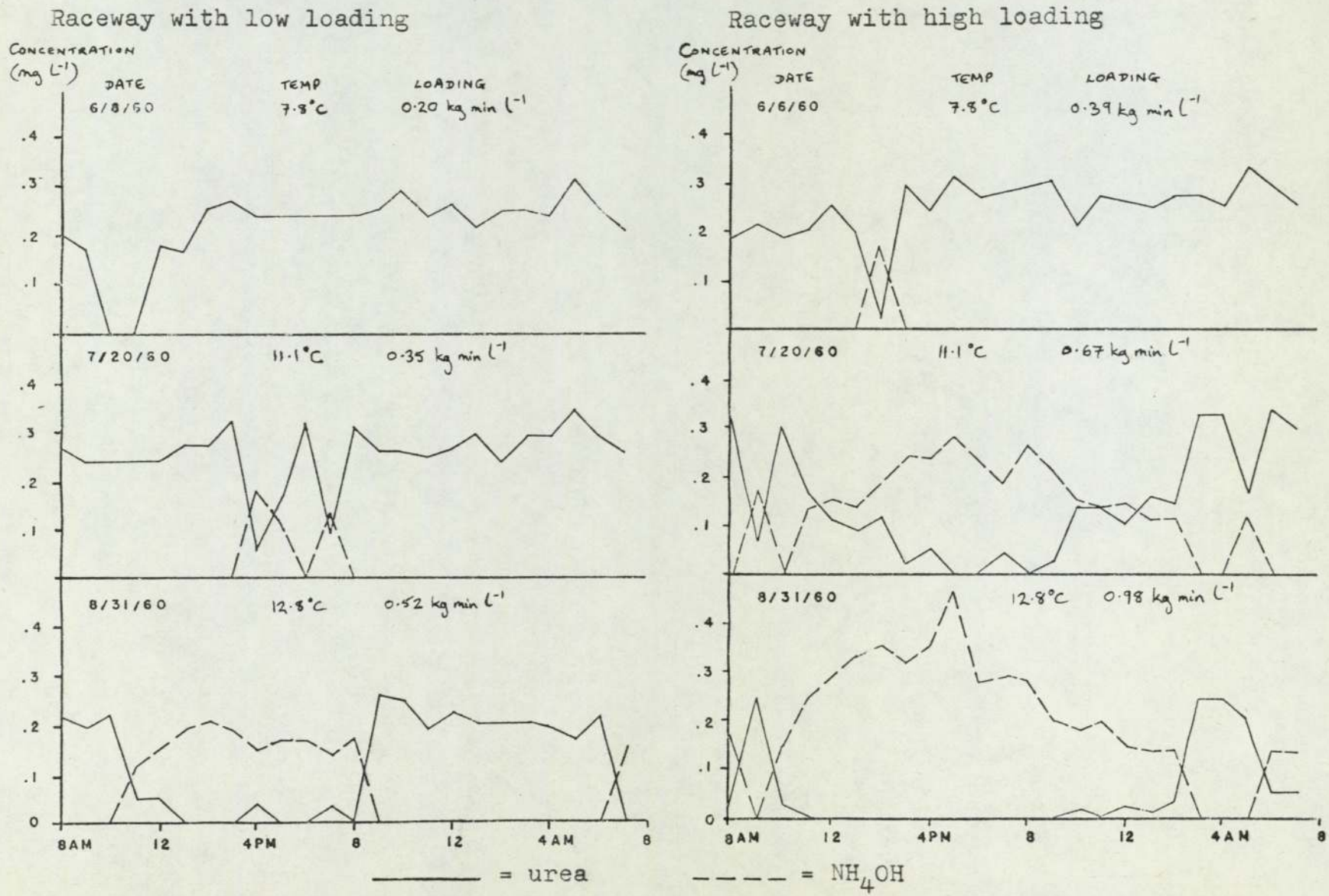


Figure 5.2 Patterns of chinook salmon excretion in raceways (modified from Burrows (1964))

5.22 Gigger and Speece observe that many bacteria can hydrolyse urea to form ammonia;

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

and appear to base their work on the assumption that this will happen in any biological filter employed, and that urea is consequently not important enough to measure. This seems illogical, because if a filter system is dealing with (among others) two reactions,

i.e. ammonia → nitrate

and urea ⟶ ammonia

- then the output ammonia level must depend on both the ammonia and the urea in the input. If, however, the urea proportion can be regarded as remaining approximately constant, and in particular if it is low, then a case can be made out for the dismissal of urea as a separate measurement variable, with minimal error caused thereby. Burrows' work suggests that at higher loading levels (more typical of intensive fish-farming situations) ammonia is greatly dominant, and so it was felt justified in the current work to assume for working purposes the relationship described above i.e. that urea is a very low and approximately constant proportion of excretory output.

5.23 Urea is a relatively unimportant teleost excretory product, compared to ammonia. Its production, and the evolutionary aspects of its occurrence, have been discussed by Cohen & Brown (1960, 1963), Prosser & Brown (1961), Huggins et al (1969), and Forster & Goldstein (1969). Although alternative pathways may also exist, the poor development of the common ornithine cycle route to urea formation in fish would support the contention that urea production is likely to be overshadowed by ammonia in a normal growth situation. Certainly Burrows' results do not seem to have been confirmed by later work. Bearing these things in mind, it has seemed reasonable to leave urea, and to concentrate solely on ammonia for the purposes of this study, recognising, however, that

urea ought also to be examined in the future.

5.24 Liao's (1970) work is particularly interesting in the ammonia production context. He sampled several salmonid hatcheries to measure pollutant production, especially in relation to feeding rates and stocking levels. His relationship-line derived from ammonia production data plotted against feed rates (the latter measured in % BWD) is somewhat unconvincing, and when examined statistically the graph (Fig.5.3) has a correlation coefficient of only 0.42 ($P>5\%$,NS). If the arrowed point is ignored, as the line invites, it can be seen that below 4.5%, %BWD seems to have a negligible effect on ammonia excretion, all values being below about 46 mg kg^{-1} h^{-1}. The graph suggests rather that above 4.5 %BWD (i.e. with very small fish below about 5g) the ammonia excretion rate is markedly higher, with almost no transition stage between the two zones.

5.25 Liao's data for ammonia output and stocking levels are plotted in Fig.5.4. The result is not similar to Liao's own figure, and the derivation of his plotted data is not given. Liao's contention is that the relationship between stocking and ammonia output is a downward curve. This would not give a satisfactory explanation of Fig.5.4, which does not lead to any definite statement.

5.26 Liao's work nevertheless introduces several very useful points. Emphasis is laid on the nutritive state and a measure of fish density in the water (in this case stocking), and the multi-variable conditions in hatchery practice are to some extent taken into account. Nevertheless, the approach is only the beginning of such a technique, and only treats a few of the variables. Size is not taken into account, nor temperature; and in the very varied feeding conditions likely to be involved, these factors are probably only roughly expressed through the feed rates (hence the inconclusive nature of Fig.5.3). It is clearly important and desirable to evaluate

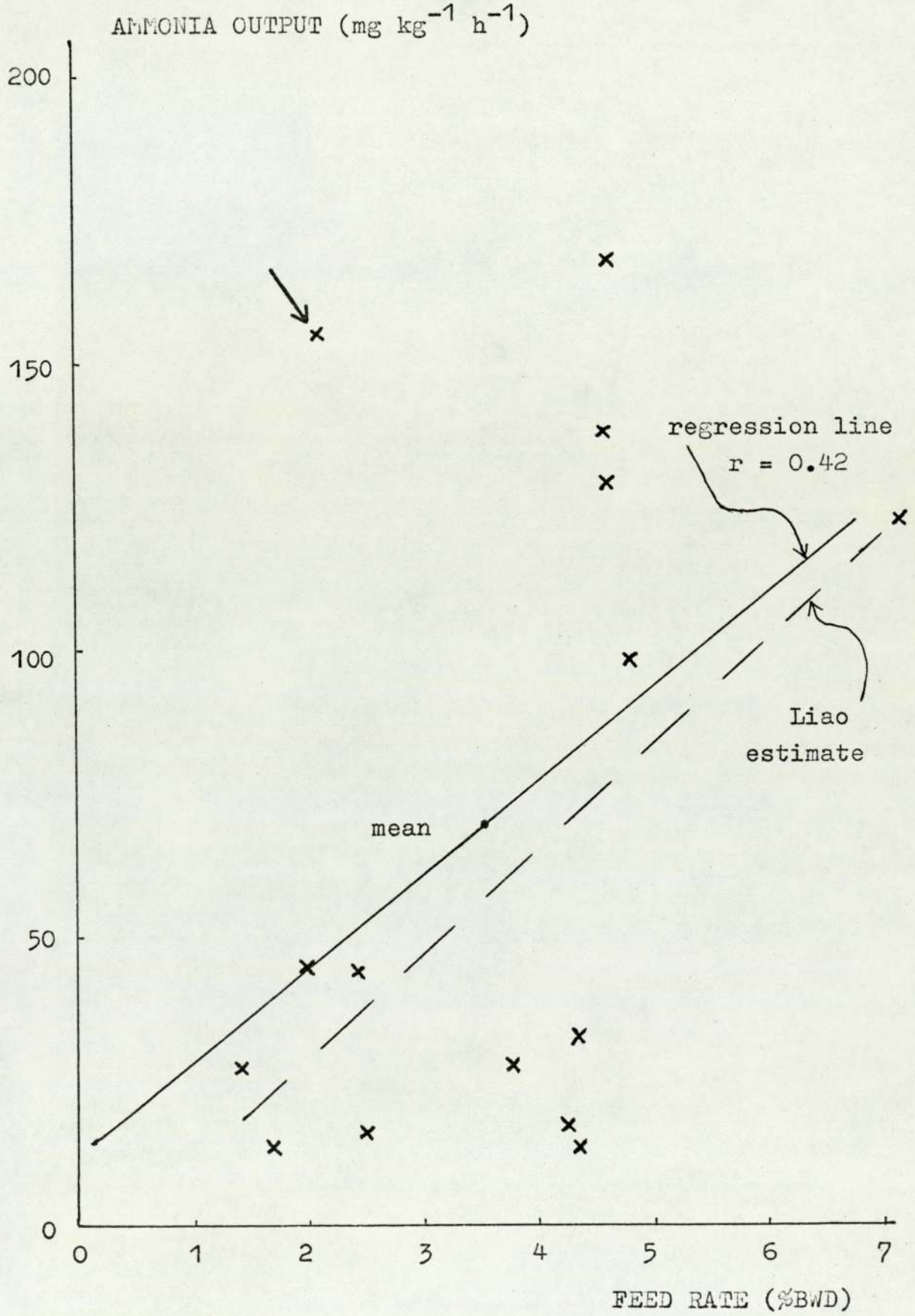


Figure 5.3 Ammonia production and feed rate

(from Liao 1970)

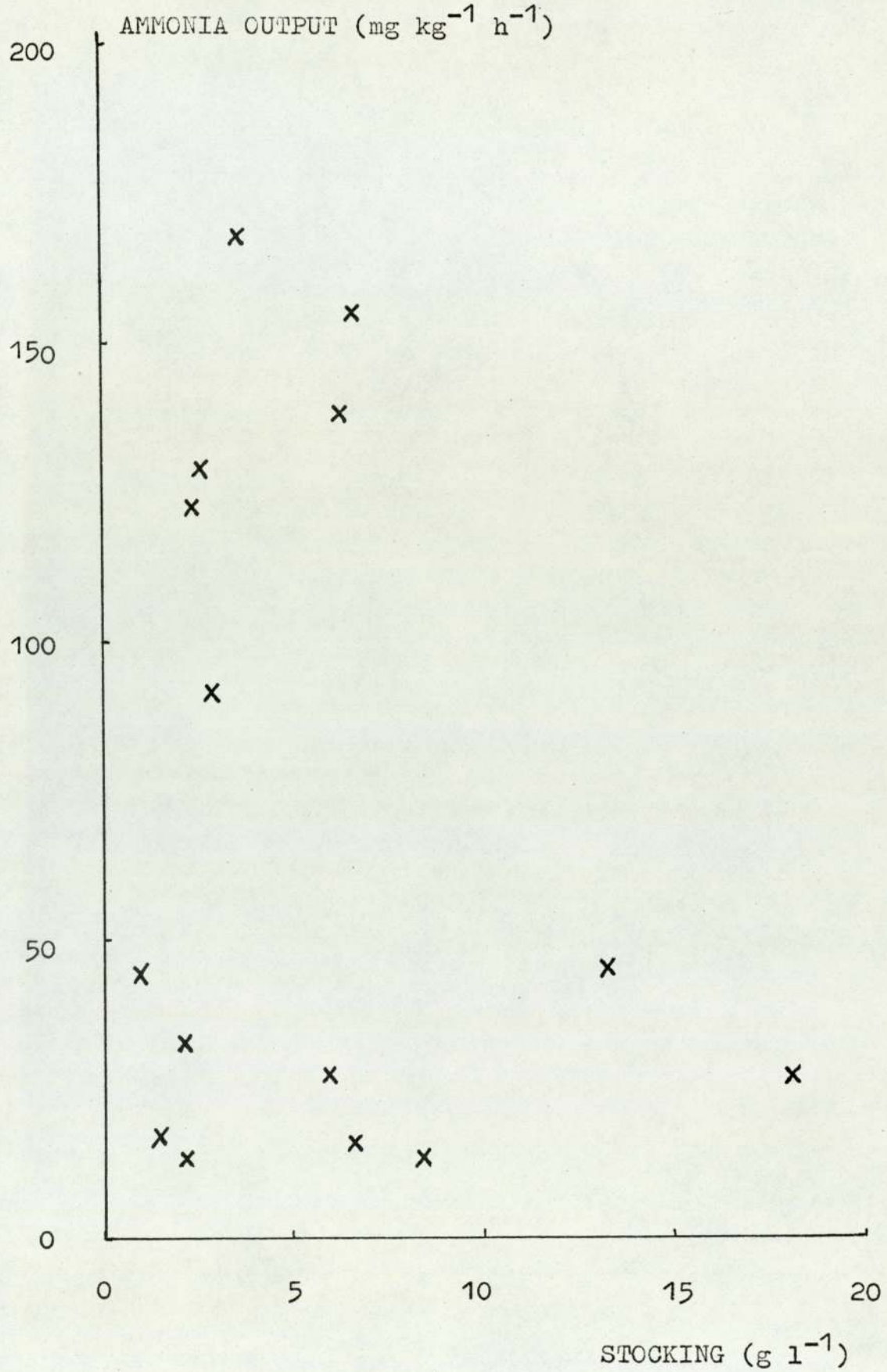


Figure 5.4 Ammonia production and stocking

(from Liao 1970)

the influence of environmental and culture factors on ammonia output.

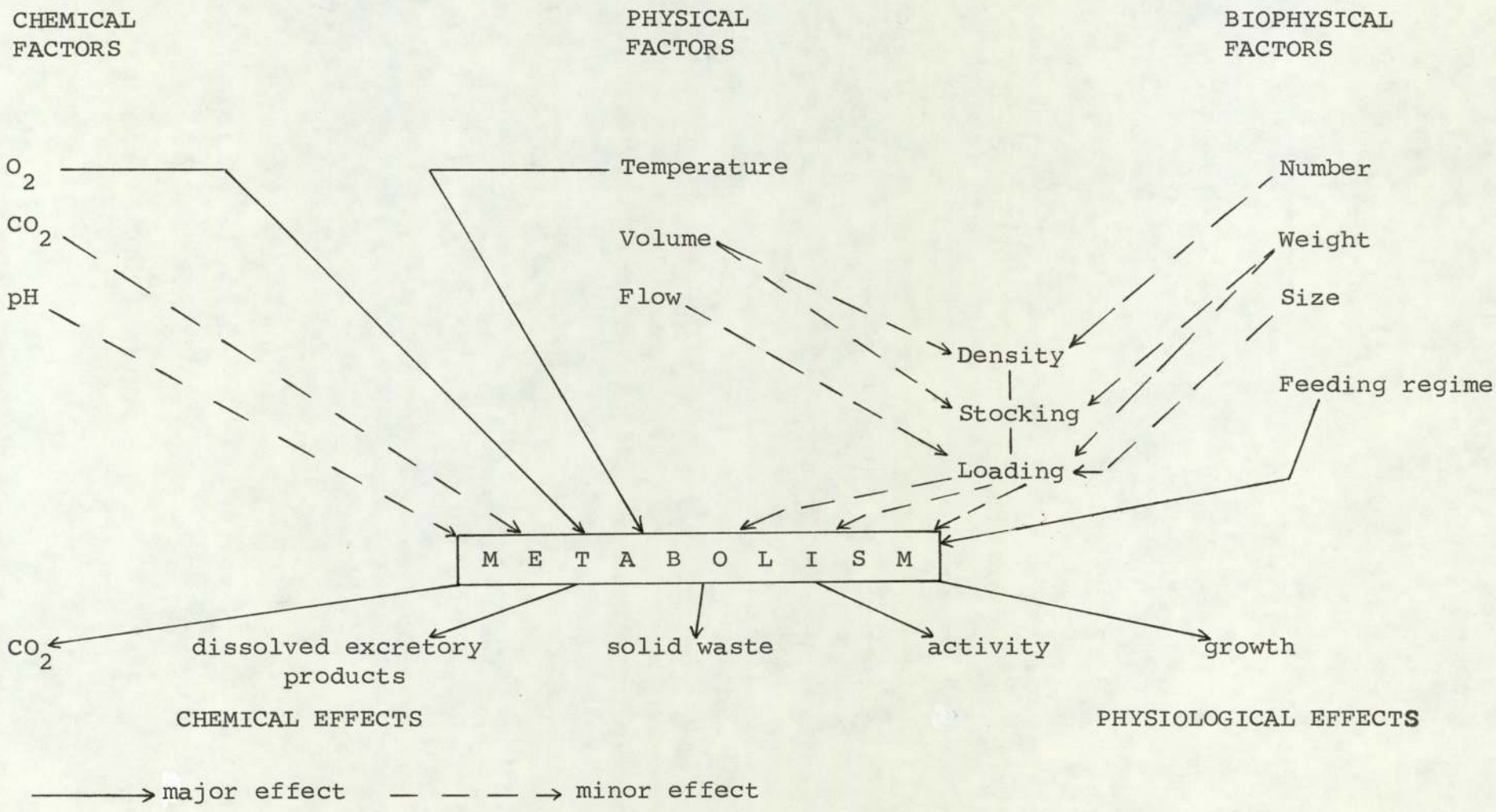
Influence of environmental factors on excretion

5.27 The excretory output of a fish can be considered, in a similar way to oxygen uptake, as a generalised measure of at least some aspects of the metabolism. Thus it is to be expected that factors which influence metabolic rate as determined by oxygen uptake, might also influence excretory, and in particular ammonia, production.

5.28 A much-simplified model of some of the conditions which could be involved in fish culture situations is given in Figure 5.5, where all internal changes, reactions and pathways are contained within the box labelled Metabolism. Metabolism is thus likened to a "black box", and is analogous to the position of Organism in simple stimulus-response behavioural theory.

5.29 Oxygen is clearly of extreme importance; this is a "supply" quantity which the fish draws upon constantly to service its energy requirements. Kutty (1972) reports experiments on Tilapia mossambica which indicate that when oxygen is plentiful (above a concentration of 5 mg ℓ^{-1}) the ammonia quotient (AQ) is about 0.23. (AQ is the ratio of volume of ammonia produced to volume of oxygen taken up). This value is quite close to 0.27, the theoretical value of AQ for 100% aerobic protein metabolism. At low oxygen, there is increased utilisation of protein as an anaerobic energy source, and the liberated ammonia is much higher in proportion to oxygen uptake. This anaerobic stage Kutty (1968) found at about 3 mg ℓ^{-1} oxygen in rainbow trout. As was necessary for his experiments, Kutty used starved fish, and hence the relationship of this work to cultured fish is not clear since energy in the culture situation will be provided by carbohydrate substrate. Whilst it is known that low dissolved oxygen can lessen growth

Figure 5.5 Simplified model of some environmental factors in culture



(Herrmann, Warren & Doudoroff 1962), the effect on ammonia excretion does not seem to be clear, and is probably influenced by the growth effect anyway. Clearly it would be advisable in ammonia production studies in culture situations to preserve growth and rule out complications by maintaining oxygen at non-limiting concentrations.

5.30 Temperature also has a fundamental effect on ammonia production. In early experiments Phillips et al (1947, 1949) detected rises in ammonia excretion with temperature increase in small-scale trials with brook trout. A ten-fold rise was associated with a temperature increase from 47 to 60°F (13 deg F; or 7.2 deg C from 8.3 to 15.5°C). These frequently quoted data (e.g. Brockway 1950, Coates 1962) give rise to a "Q_{10}" value of about 13.9, which is considerably higher than that deriving from Pora & Prekup (1960b) or Maetz (1972); 2.3 and 3.9 respectively. These authors were however using different fish (carp and goldfish) and those of Maetz were starved.

5.31 Nutritional effect has already been mentioned; it is important to note in this context that the feeding rate for salmonids in most culture situations is governed largely by temperature and size of fish (e.g. see Table 1.3 in Chapter 1). Thus effects said to be due to feeding rate might indirectly be ascribed to these quantities. Whilst Speece (1973) takes into account a rather different way of determining feeding rates (see Chapter 7), he also relates ammonia production by trout to the amount fed, quoting data from Bozeman. Ammonia is expressed as a proportion of the weight of food fed, and on this basis, with only two quoted data, Speece goes on to assume a definite positive relation between temperature and ammonia production, and further assumes it to be linear.

5.32 Despite repeated attempts, it has been impossible to obtain from Bozeman Center the original data on which these assumptions are made (Speece's reference does not contain

them); hence it must be assumed that only two points were originally defined. This must leave some doubt over the validity of Speece's argument, and his subsequent system of designing filter units to cope with the ammonia. More information is urgently needed on the effects of temperature and size, as expressed through feed rates.

5.33 Forster & Goldstein (1969) quote a pH effect on excretion, stating that acid or neutral water favours NH_3^o output as compared to alkaline water. Wolbach, Heinenmann and Fishman (1957) showed that internal pH changes could affect ammonia loss, and especially that internal acidosis decreased the gill concentration gradient for free ammonia diffusion to the outside. This would be equivalent to an external rise in pH; the external equilibrium would be shifted in favour of NH_3^o and the NH_3^o concentration gradient would be decreased, hence a decrease in ammonia output. However, it is likely that the pH changes involved in such comparisons are relatively large compared to the acidification caused by output of CO_2 by fish under culture; and since the latter, at high oxygen levels, will be dependent on some measure of body size or weight, it is unlikely that measurements of ammonia output under culture conditions need to involve a direct measure of pH effect on excretion, at least in a first analysis.

5.34 It is now necessary to examine the "biophysical" factors of fish biomass in culture situations as a series of factors which may affect excretion. Certainly stocking, loading and density (see para.5.20) will affect the ammonia concentration in the water, given a definite excretion rate, but it is also important to know whether they have feedback effects on the actual excretion rate itself. Early small-scale experiments by Phillips et al (1947) suggested that at constant flow, ammonia excretion fell with an increase in water volume in the container, and at constant volume ammonia excretion fell with an increase in flow. However the same group of workers (1948) later cast doubt over these experiments

and the difficulty of scaling up results for a raceway production situation. Pora & Prekup (1960a) quote a reduction in nitrogen excretion by carp when the stocking rate is increased, suggesting a feedback prevention leading to autointoxication, but a reasonable body of data is not found until the somewhat uncertain assertions of Liao as discussed in para.5.25.

5.35 The important topic of stress will be discussed in Chapter 8, but it should be pointed out that "feedback" effects such as those discussed above can be viewed as a kind of stress. Gigger & Speece (1970) have suggested that factors which increase activity (e.g. higher feed rates or mechanical stress in the water such as excessive fish-chasing while netting, causing over-excitement) can be viewed as major sources of increased excretion, whilst disease and presence of toxic materials would be negative in their effect on excretion by way of decreased activity.

5.36 Putting much of this past work into the context of fish culture, it is clear that there is a great shortage of reliable information on which to base prediction of excretory rates. Whilst many of the effective variables have been identified, their measurable effects have been only loosely quantified, and in a variety of ways, so that comparison between studies is difficult or confusing. With this in mind, a fuller investigation of ammonia excretion by rainbow trout in a circular-tank culture situation has been attempted.

INTRODUCTION TO E-SERIES EXPERIMENTS

Tank excretory theory

5.37 The relationship between flow, ammonia production and ammonia concentration in a constant volume of water can be described at a crude level in terms of a mathematical model governed by simple physical laws, if it is assumed in the first instance that fish produce ammonia at a constant rate

(Brookes 1971).

5.38 Let

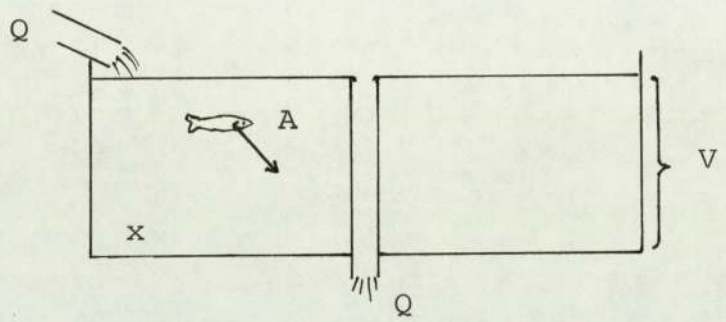
x = mass of ammonia in tank at time t

$x + \delta x$ = mass of ammonia in tank at time $t + \delta t$

V = volume of water (constant)

A = mass of ammonia produced per unit time (assumed constant)

Q = water flow rate



Then

mass ammonia produced in interval $\delta t = A\delta t$

Assuming inflow = outflow = Q

mass ammonia flowing out in interval $\delta t = \frac{Qx\delta t}{V}$

Since δx is the increased mass of ammonia in the tank,

$$\delta x = A\delta t - \frac{Qx\delta t}{V}$$

$$:- \quad \frac{dx}{dt} = A - \frac{Qx}{V}$$

$$:- \quad dt = \frac{dx}{A - \frac{Qx}{V}}$$

$$:- \quad t + \text{constant} = \int \frac{dx}{A - \frac{Qx}{V}}$$

$$= -\frac{V}{Q} \ln \left(A - \frac{Qx}{V}\right)$$

Now at time $t = 0$, mass ammonia = $x = 0$

$$:- \quad \text{constant} = -\frac{V}{Q} \ln A$$

$$:- \quad t - \frac{V}{Q} \ln A = -\frac{V}{Q} \ln\left(A - \frac{Qx}{V}\right)$$

$$:- \quad t = -\frac{V}{Q} \ln \left(1 - \frac{Qx}{AV}\right)$$

$$:- \quad 1 - \frac{Qx}{AV} = e^{-\frac{Qt}{V}}$$

$$:- \quad \frac{Qx}{AV} = 1 - e^{-\frac{Qt}{V}}$$

$$:- \quad x = \frac{AV}{Q}\left(1 - e^{-\frac{Qt}{V}}\right)$$

Under constant conditions, the bracketed function will increase to 1 as the exponential function disappears (at asymptotic value) (see Table 5.2) and so

$$\text{(maximum) } x = \frac{AV}{Q}$$

Now since the measured quantity (ammonia concentration)

$$= c = \frac{x}{V}$$

$$:- \quad c = \frac{A}{Q} \quad \text{or} \quad A = cQ$$

Thus the excretion can be calculated knowing ammonia concentration and water flow at equilibrium conditions, independent of volume.

5.39 In practical terms, the assumption that fish excrete at constant rate is unjustified, as previously discussed. Further, if the excretion rate is variable, then the bracketed function mentioned above may not be unity in all cases of measurement. This should always be borne in mind when calculating excretory rates from water concentrations. Nevertheless for the purposes of this study, the apparent specific excretory rate (SER) will be utilised, defined as the product of ammonia concentration and water flow per unit weight of fish.(Units employed: mg kg^{-1} h^{-1}.) The error involved in this approximation will depend on equilibration time, and the relative magnitudes of volume, flow and ammonia output. In circular tanks, mixing is usually good (Larmoyeux, Piper & Chenoweth 1973), so that creation of zones of different ammonia concentration is much less important than in large raceway production (Bozeman 1970) (see para.5.21); hence effluent sampling will be more reliable for excretion esti-

Table 5.2 Example equilibrium relationship between ammonia production and time

(Ref. para.5.38)

Let $A = 20$ mg h^{-1} (1 kg of fish excreting ammonia at 20 mg kg^{-1} h^{-1})

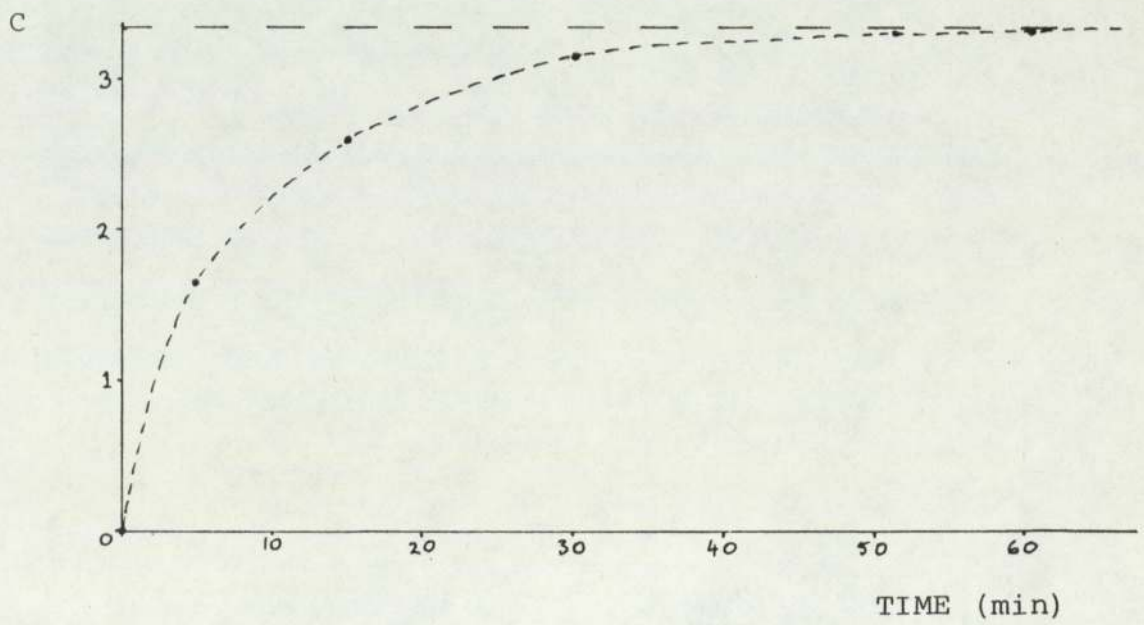
$V = 10\ell$

$Q = 1\ell\,min^{-1} = 60\ell\,h^{-1}$

and

$$C = \frac{A}{Q}\left(1 - e^{\frac{-Qt}{V}}\right)$$

t (min)	e FUNCTION	C (mg ℓ^{-1})
0	1.0000	0.0000
5	0.5000	0.1667
15	0.2231	0.2590
30	0.0498	0.3167
60	0.0025	0.3325
∞	0.0000	0.3333



mates. From the point of view of the operating experimental system, the ammonia concentration in the water remains the only measurable parameter for estimating ammonia output, and hence apparent SER is in practice a more useful quantity than the true excretory rate in a situation where the latter may be fluctuating. All subsequent references to SER indicate apparent SER, as defined above.

5.40 Burrows (1964) found evidence of diurnal fluctuations in levels of excretory products, and it is possible that the considerations of the preceding paragraph, under his particular raceway conditions, could account for the low values of SER which his data suggest. Liao (1970) took no account of such fluctuations in presenting his work, and more recent work at Bozeman Center (Smith 1972, Larmoyeux & Piper 1973) has been geared to ammonia concentration readings over very long intervals (two weeks). This necessarily does not take account of fluctuation between different days, although readings were standardised to "mid-afternoon".

5.41 If the diurnal fluctuation is accounted for by taking daily measurements at the same point in the fish cycle, then values ought to be reasonably comparable. It is then important to analyse the various factors which have contributed to the measured ammonia level. By reference to Figure 5.5, and with suitable additions, it seems that the inter-relationships involved in ammonia excretion can be summarised as in Figure 5.6. The ammonia SER will depend on various influences on metabolism, whereas total ammonia concentration or (TOA) and unionised ammonia concentration (or UIA) are more simply controlled (and hence simply calculated from measurements).

5.42 The design of the E-series experiments which are documented in succeeding chapters was such as to investigate the effects of most of these variables as fully as possible. (E-series designates those experiments on the effect of

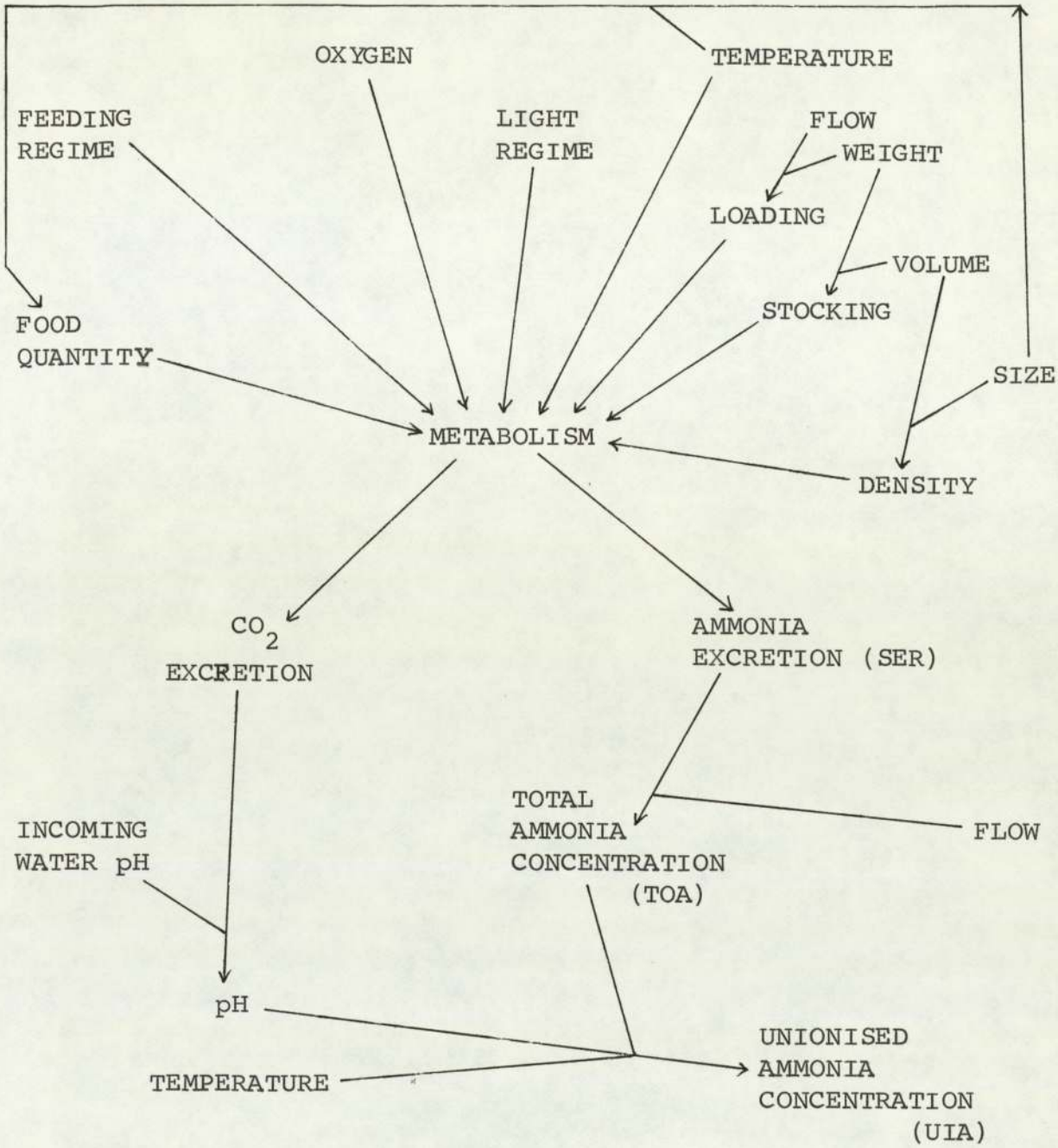


Figure 5.6 Relationships in ammonia excretion

environmental factors on ammonia excretion). However, with the considerations of para.5.29 in mind, all experiments were designed to operate in non-critical conditions of dissolved oxygen. Thus oxygen should be removed as an effective variable. Further design targets were to produce predictable and regular lighting and feeding regimes; so that under stipulated timing of light and food presentation, the major effects of temperature, loading, stocking and density could be studied. Food quantity is a major direct effect, but depends primarily on temperature and size; its treatment as a variable is complex, and requires ongoing explanation during the description of experiments.

5.43 Apart from initial pilot experiments (see below), the approach adopted was to allow a multivariable situation in which the varying factors and ammonia production were simultaneously measured in a series of similar experiments. This approach was used for two major reasons;

a) the apparatus and experimental conditions involved did not allow for temperature control, thus this important variable would have been able to fluctuate in any case;

b) a multivariate treatment, with suitable analysis, allows much more ground to be covered in a limited period of time than an experimental series where each trial has set controlled conditions, with just one variable. This latter situation is in any case extremely difficult to contrive in fish work, since attempting to keep all tanks the same except for one environmental variable is almost impossible. At the end of a single experiment under full control, the experimenter has a series of points to plot on one graph; these points are only reliable for those particular conditions, and to account for any new variables, the experiments required to gain a reasonable amount of information mount up in a geometrical fashion. If a full treatment of each variable is envisaged, the number of experiments involved rapidly becomes excessive. A multivariate approach gives up the precision of set levels of

independent variables in favour of allowing them to vary arbitrarily over their ranges, with simultaneous measurement of all of them. As all independent variables are free to vary, the number of experiments performed need only be enough to encompass a representative range of values for each one, together with enough replication to provide a reasonable number of points. The operational drawback is that all independent variables must be measured in every instance.

5.44 With such an experimental design it is important to prevent variables that cannot be measured from operating, if this is at all possible. A particular example is disturbance of the fish by people or other nearby movement; since the effect would be to increase activity, there could be an unmeasured effect on ammonia production. In this case the remedy is relatively simple; curtains surround the tanks and cut off the fish from disturbance or uncontrolled lighting.

5.45 In order to provide sufficient flow and to remove problems of filtration or ammonia accumulation in deriving data in the E-series experiments, an open-flow system was used, with all effluent water from the tanks run to waste. This system also ensures a continual supply of fresh aerated water to the fish, and conserves space in organising and building tank systems.

5.46 Although experience proved pH to be low enough to keep UIA to extremely low values, it is necessary to be aware of the probable changes in the nature of ammonia in a tank containing fish as compared to the influent water. Incoming water will contain a very low value of TOA (TOA_1) of which a small proportion is UIA (UIA_1). In the fish tank it could be postulated that this will undergo change as shown in Figure 5.7. The fish excrete ammonia, raising the TOA by ΔTOA to TOA_2. Since pH is somewhat lowered in the presence of the fish, the proportion of UIA due to influent falls to UIA_2, but added to

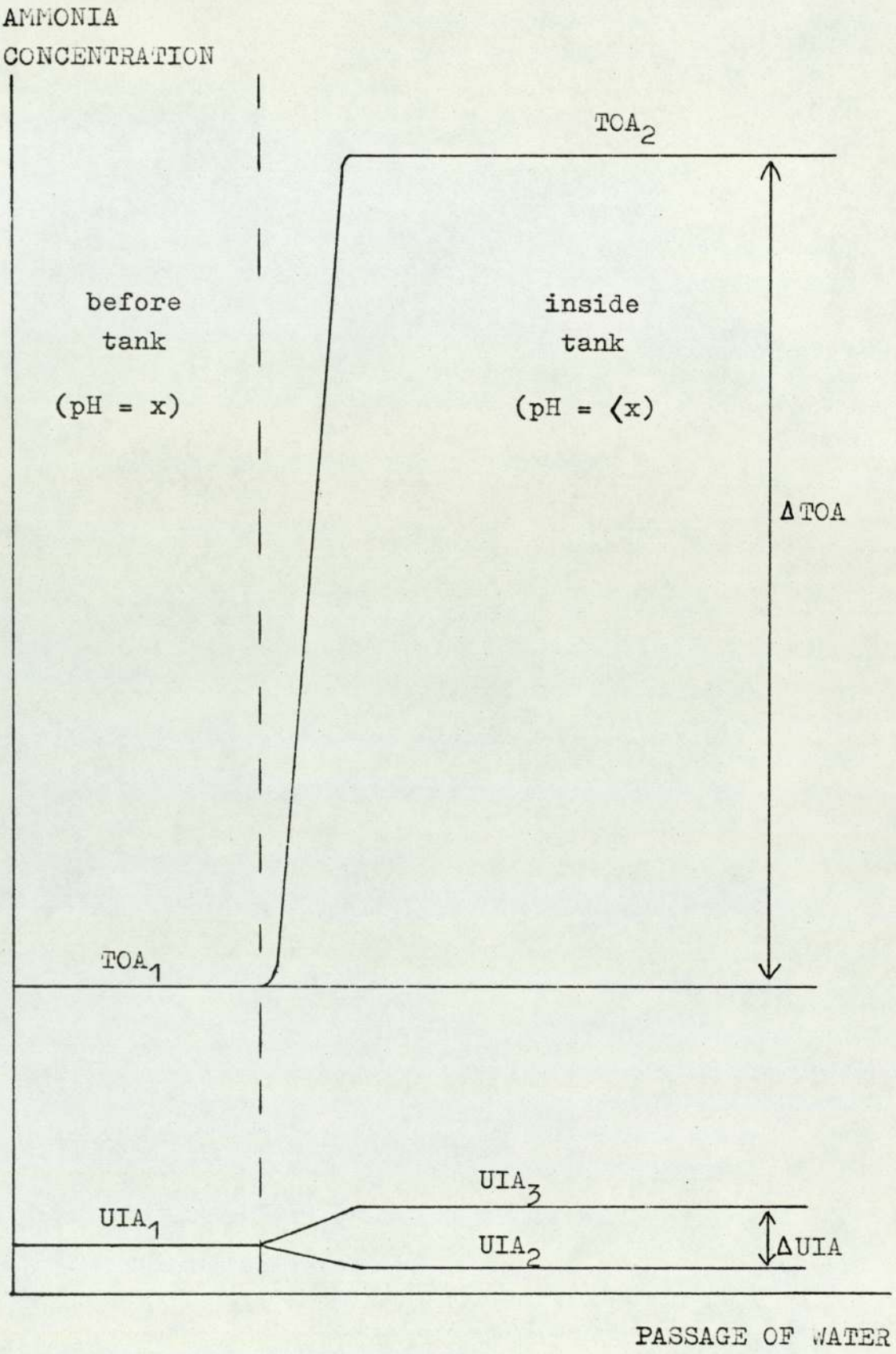


Figure 5.7 TOA and UIA in water passing through a fish tank

this is the UIA due to excretion, raising the overall UIA to UIA_3. By measuring ammonia in the water the experimenter observes the final TOA value (TOA_2, due to both excretion and influent), and by pH measurement and calculation arrives at UIA_3 (also due to both excretion and influent). In practice TOA_1 was found to be so low that the measured value of TOA_2 could be assumed to be equivalent to ΔTOA (due to excretion only), with a minimal error (see Chapter 4). Similarly UIA_3 would approximate ΔUIA. This model assumes complete mixing of water prior to measurement, a requirement which is normally fulfilled, if the tank has a reasonable flow and contains actively swimming fish, when dealing with circular tanks. Provided that pH is low enough (in practice below about pH7), the proportion of UIA present is so low that its concentration does not even approach 25 $\mu g\ l^{-1}$. This is the level at which Lloyd and Orr (1969) estimated UIA to have no diuretic effect on rainbow trout, and this is probably the most sensitive short-term indicator of adverse effect found to date (see Chapter 8). In all E-series experiments UIA was referred to this standard, and if below it (in nearly all cases) was ignored. Thus SER is envisaged in terms of TOA only.

Introduction to pilot E-series experiments

5.47 Before proceeding to experiments for a full multivariate analysis, it was decided to undertake several pilot experiments. These were necessary to formulate a reference framework for the later experiments both in terms of apparatus and experimental facilities, and in terms of methodology.

5.48 Using a pilot experimental system (see Chapter 2) three pilot experiments were carried out. The major objectives were as below:-

a) to test the feasibility of measuring ammonia concentrations under flowing fish culture conditions,

b) to evaluate methods and techniques required,

c) to evaluate apparatus and experimental system,

d) to check the behaviour and well-being of fish in the imposed conditions,

e) to obtain preliminary information on the levels of ammonia to be expected,

f) to obtain preliminary evidence on the effect on SER of some different stocking and loading conditions,

g) to briefly assess any diurnal rhythm in excretion and estimate optimum parts of such a cycle for later analysis.

5.49 For these pilot studies, the influence of "crowding" as a possible stressor was not assessed except in terms of stocking and loading. The concept of crowding is difficult to pin down in this kind of work. Fish might or might not react to any or all of the following quantities:-

a) stocking (weight per volume)

b) loading (weight per flow)

c) size (length)

d) combination of (b) and (c)

e) number of fish

f) combination of (c) and (e)

g) density (number per volume)

h) mean free path ($\sqrt[3]{\text{volume}/\text{number of fish}}$)

i) combination of volume and (b)

j) hierarchical effects due to size differences

k) pheromones

There are other possibilities also; for the purposes of the E-series in full, several of the likeliest quantities and combinations are tested in the multivariate analysis of data, but for the purposes of the pilot experiments, only stocking and loading are treated.

5.50 To set this in context, a list of stocking and loading values from various authors is given in Table 5.3, converted from the units used to those of this study.

Table 5.3 Salmonid stocking and loading from culture systems

AUTHOR(S)	YEAR	STOCKING ($g\ l^{-1}$)	LOADING (kg min l^{-1})	COMMENTS
Phillips et al	1948	3.2 - 47.8	-	quoted raceway usual values
		47.8 - 79.8	-	hatchery troughs
Tanizaki et al	1957	200.0	-	during transport in aerated container on truck (short term)
Saeki	1958	-	1.10	series of aquaria
De Witt & Salo	1960	-	0.21	combination of raceways and ponds
Burrows	1964	-	0.52	exptl. 4' x 40' raceways
		-	0.98	
Parisot	1967	0.3	0.10	4' diam. circular tanks
Burrows & Combs	1968	-	1.00	17' x 75' rectangular circulating ponds (projection)
Robinson & Vernesoni	1969	34.0	7.20	20' diam. circular pond
Bridges et al	1969	25.5	1.50	4') diam.
		25.0	3.33	6')- circular
		25.0	3.96	8') tanks
Liao	1970	(1.1 - 18.2 (mean 5.6	0.06 - 3.11) 0.51)	various US hatcheries
Bozeman	1970	55.9	1.69	6' x 60' exptl. raceway
Buss Graff & Miller	1970	505.0	1.40	jar) vertical
		125.4	1.80	drum)- unit
		136.9	1.66	silo) culture
FSC	1971	35.8	0.04	25' diam circular pond
Scott & Gillespie	1972	46.9	1.30	exptl. circular tank

Table 5.3 continued

AUTHOR(S)	YEAR	STOCKING ($g\ \ell^{-1}$)	LOADING ($kg\ min\ \ell^{-1}$)	COMMENTS
Bardach Ryther & McLarney	1972	-	2.20	market size finger-lings) US trout farm race-ways*
		-	1.50	
Prewitt	1972	-	11.98	series of cir-cular ponds
Michalek	1972	-	7.57	
JR/PS	1974	48.0	-	circular ponds on Scottish trout farm

* quoted for Snake River trout farm, world's largest trout producer

6. PILOT SYSTEM EXPERIMENTS

METHODS AND MATERIALS

Fish

6.1 Young rainbow trout for the three pilot experiments (PO1, PO2, PO3) were sampled from an original batch (Lot O1) of about 480 fish purchased as fish of less than 5 cm length (FCAT 1 see Chapter 1). Brought to Aston in May, 1972, they were fed progressively on Salmon No.2 and 3 diets and held in HCl conditions (see Chapter 1). For experiment PO1 40 fish were selected; for PO2: 39 fish; and for PO3: 84 fish; the only selection criteria being healthy appearance and required size (FCAT 4 for PO1, FCAT 6 for PO2 and PO3) to make up the desired fish weights for the experiments.

6.2 Since PO1 did not begin until early September 1972, all fish had at least a four-month history of growth and maintenance in Birmingham tap-water prior to experiments. Fish were changed to Trout No.4 floating pellet diet in November 1972, between PO1 and PO2. PO2 began in early January 1973 and PO3 in late January /early February.

Tanks

6.3 ST type 1 tanks were employed in PO1, ST type 2 in PO2 and PO3. In each case four tanks were used within the water bath. Flow through the tanks was maintained in the region of $1\,\ell\,min^{-1}$ for each tank in all experiments.

Feeding

6.4 During experiment, fish in the tanks were fed on a regime calculated to spread the physiological effects of feeding as much as possible over the photoperiod. This was done by feeding at approximately 2 h intervals. For the purposes of PO2, an 8 h photoperiod was used, in deference to the shorter light period experienced during the winter in the wild, but for a number of reasons (see Chapter 1) including the removal of one further

variable from consideration, all subsequent experiments were performed using a 12 h photoperiod.

6.5 Feed rates were calculated according to temperature from Table 1.3 and adjusted to the nearest 0.1 g each time food was weighed out (just prior to each feeding). Food was given by hand in one lot per tank at each feed. Table 6.1 summarises the conditions of feeding.

Stocking and loading (see Chapter 5)

6.6 Since one of the aims of the pilot experiments was to test measured ammonia concentrations at different fish loadings, the approach used in pilot studies was to apportion fish to tanks in such a way as to create particular stocking and loading conditions.

6.7 For PO1, an arbitrary exploratory range of values was chosen so that by combinations of two fish batch-weights and two tank volumes, four different sets of circumstances were set up. These approximated to the target values given in Table 6.2. As TOA levels proved to be fairly low in all these circumstances, greater batch-weights of fish were used in PO2 and PO3, generating higher stocking and loading (Table 6.2).

6.8 In PO1 weighing of fish prior to the experiment was only performed with the points of para.6.5 and 6.7 in mind, and an accurate weight determination was not made until the end of the experiment. In PO2 and PO3 accurate weights were taken both before and after the experiment.

6.9 Fish were weighed by a solution balance technique; a 5ℓ beaker half-full of water was placed on a support on the weigh-pan of a Gallenkamp BC/110 sliding-weight balance and the weight tared by counter-balancing on the other pan. Fish were added to the beaker until one tank batch was weighed to the nearest gram. A standard time of 15s was used as the drainage interval while fish were in the net between holding tank and weighing beaker.

EXPERIMENT	FCAT	TEMP (°C)	APPROX %BWD	FEED (g) L	FEED (g) H		TIME + 0730 0800 0830 0900 0930 1000 1030 1100 1130 1200 1230 1300 1330 1400 1430 1500 1530 1600 1630 1700 1730 1800 1830 1900 1930 2000
PO1	4	16-17	3.2	1.8	6.0	PHOTO-PERIOD:	← →
						FEED:	× × × × × ×
PO2	6	8-9	1.4	4.0	8.0	PHOTO PERIOD:	← →
						FEED:	× × × ×
PO3	6	8-	1.4	14.0	17.5	PHOTO PERIOD:	← →
						FEED:	× × × × × ×

L = low loading tanks H = high loading tanks + BST for PO1; GMT for PO2, PO3

Table 6.1 Feeding conditions in pilot experiments

EXPERIMENT	TANK (ST)	No.of FISH	TARGET FISH WEIGHT (g)	TARGET VOLUME (ℓ)	TARGET STOCKING (g ℓ^{-1})	TARGET LOADING* (kg min^{-1} ℓ^{-1})	ACTUAL INITIAL FISH WEIGHT (g)+
PO1	01	4	50	2	25	0.05	61
	02	5	50	8	6.25	0.05	57
	04	13	200	8	25	0.20	170
	06	18	200	2	100	0.20	211
PO2	09	7	300	4	75	0.30	311
	10	6	300	8	37.5	0.30	324
	11	13	600	8	75	0.60	618
	12	13	600	4	150	0.60	618
PO3	09	18	1000	6	166.67	1.00	998
	10	18	1000	8	125	1.00	1025
	11	24	1250	6	208.33	1.25	1241
	12	24	1250	8	156.25	1.25	1257

* assuming 1 ℓ min^{-1} flow

+ post-experiment values for PO1 (see para.6.8)

Table 6.2 Stocking and loading conditions in pilot experiments

Procedure

6.10 Table 6.3 outlines the procedure followed in the three pilot experiments. In PO1 and PO2 readings were obtained for background water levels of TOA prior to the introduction of fish, and subsequent sampling followed on every 4 h where possible; a full record of samples proved impossible to attain due to operator fatigue. In PO3 sampling was limited to 2 h intervals during the photoperiod.

6.11 Each sample was taken just prior to a feed, to prevent any disruption associated with feeding from affecting the water. Samples were partially immersed in a container holding water from the water-bath in order to conserve heat during transfer to the pH meter; temperature readings (by mercury-in-glass thermometer accurate to 0.1 deg C) were taken prior to pH measurement. During PO2 and PO3 the inlet water supply was also sampled each time from the by-pass tube.

6.12 A "dummy run" (without fish) was performed one week prior to PO1 in order to test timetabling, measurement speed, and coordination of activities. This was particularly important for PO1, where samples had to be treated in preparation for spectrophotometric measurement within a limited space of time.

6.13 Accurate volume measurements were taken at the end of each experiment by simultaneous closure of tank inlet and outlet (thus arresting water flow) and drainage of tank contents into suitable measuring cylinders. During experiments PO2 and PO3 volume was periodically checked by means of a previously calibrated dipstick, and if necessary adjusted back to target values. Flow measurements were periodically taken; overflow water was collected in a wide-necked 500 ml volumetric flask, being timed to "full" with a stopwatch, for each tank. After each flow measurement session, adjustment was made if required toward the target value of 1 $\ell\,min^{-1}$ in each tank.

EXPT	PHASE	DATES	TIMES* AND EVENTS (unless otherwise stated, feeds as in Table 6.1)
PO1	1	4/9/72	PRE-FISH SAMPLE:0925/INAUGURATION:1400+/POST-FISH SAMPLE:1725/FEEDS:1800 2000
		5/9	SAMPLES:1200 1600
	2	6-8/9	SAMPLES:10000 1400 1800 2200 (-) 0600 1000 1400 1800 2200 (-) 0600 1000/TERMINATION:1100+
PO2	1	9/1/73	PRE-FISH SAMPLE:1000/INAUGURATION:1100+/FEED:1500/SAMPLE:2200
	2	10-15/1	SAMPLES:0200 0600 1000 1400 1800 (-) (-) (-) 1000 1400 1800 2200
			0200 0600 1000 1400 (-) (-) (-) (-) 1000 1400 1800 (-)
			(-) (-) 1000 1400 (-) 2200 0200 0600 1000/TERMINATION:1100+
PO3	1	2-3/2/73	(2/2) INAUGURATION (NO SAMPLES) (3/2) NO SAMPLES
	2	4-6/2	SAMPLES:1000 1200 1400 1600 EACH DAY
		7/2	(NO SAMPLES) TERMINATION

*BST for PO1; GMT for PO2, PO3 (-) indicates samples missed from 4 h regime

Table 6.3 Pilot experiment procedures

6.14 In between taking samples and measurements, periodic checks were made of experimental conditions e.g. apparent variations in volume or flow; correct functioning of lights, tank drainage and aeration systems; behaviour of fish in case of apparent stress.

6.15 During PO1 and PO2 it was assumed that all food offered to the fish was being consumed. The results of PO2 indicated that this might not be so, and so for PO3, an attempt was made to quantify the food consumed by netting off and counting any uneaten pellets. By relating this "net index" to the number and weight of pellets offered, the amount of food taken could be estimated.

RESULTS

6.16 For a full tabulation of results see Appendix A1.

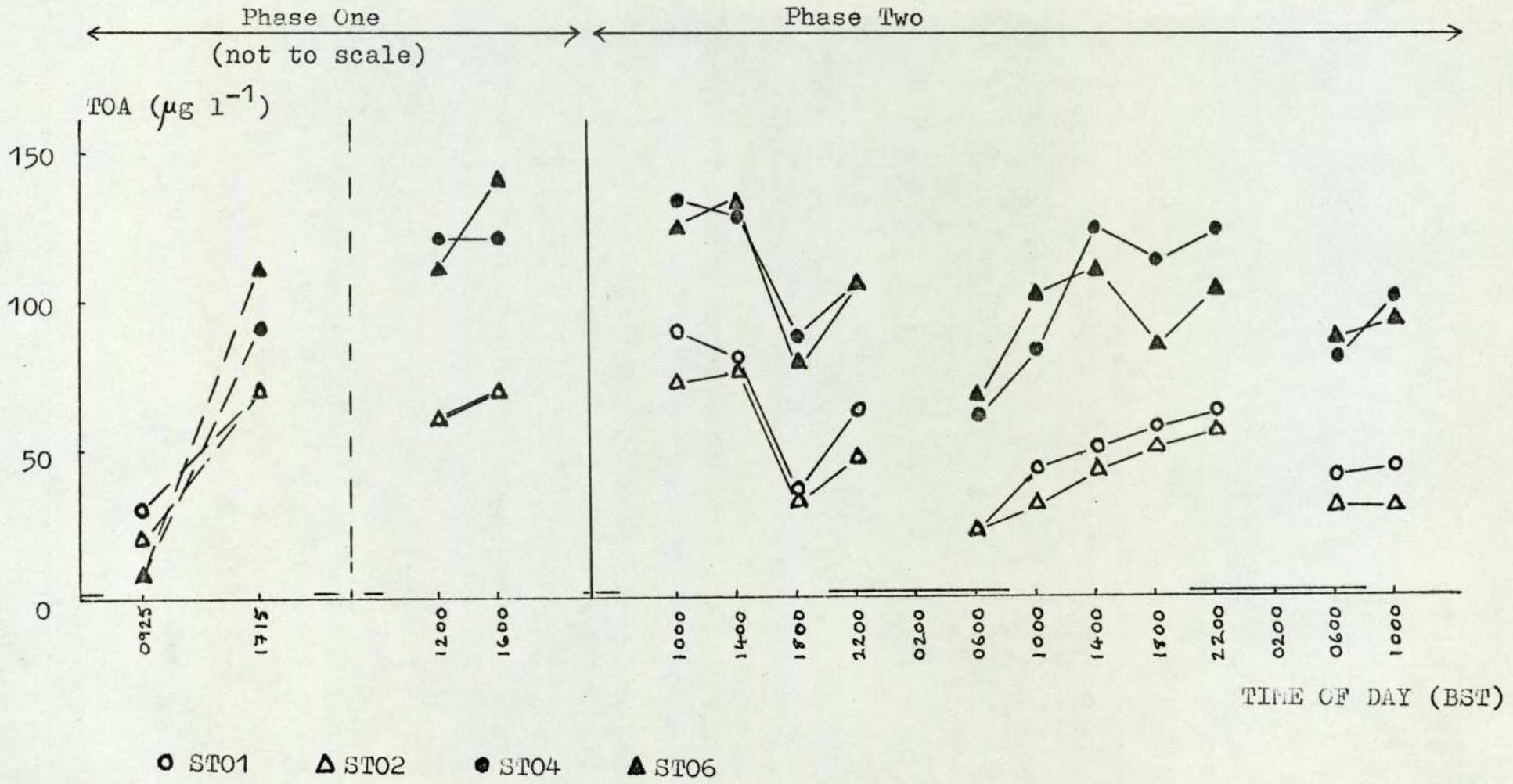
6.17 TOA results for PO1 are plotted against time on Graph 6.1 and show several points of interest:

i) a clear rise in TOA upon the introduction of fish;

ii) a clear separation of TOA effects between higher and lower loading levels, irrespective of stocking;

iii) some indication of a diurnal rhythm in TOA effects, with (after two days of settling down after inauguration) a fall during the dark period and a rise during the photoperiod (i.e. wavelength of 24 h).

6.18 As indicated in Chapter 4, TOA measurements below 100 $\mu g\ l^{-1}$ are subject to doubt, and as the majority of PO1 values fell in this category, it would be unwise to make further deductions from the data obtained. However, it should be mentioned that by calculation from TOA, pH and temperature readings, the UIA present would at all times have been below 1.0 $\mu g\ l^{-1}$ (even allowing for the error in TOA). Comparing this to the diuresis criterion of 25 $\mu g\ l^{-1}$ reported by Lloyd and Orr (1969) (see Chapter 8), it is probably that UIA effects were negligible.



Graph 6.1 P01 TOA results

6.19 The fish used in PO1 were previously held in conditions where average TOA was approximately 450 $\mu g\ l^{-1}$ (estimated by twice-daily measurements for several days preceding PO1). At prevailing pH values the UIA in the holding tanks would be no more than 0.2 - 0.3 $\mu g\ l^{-1}$.

6.20 In experiment PO2 stocking and loading were higher but measured TOA was in the same range as in PO1 (Graph 6.2). Similar results were obtained, with a rise in TOA on fish introduction, eventual separation of TOA values in high and low loading tanks, and more evidence for a daily rhythm (rising values during photoperiod and low values during the dark period).

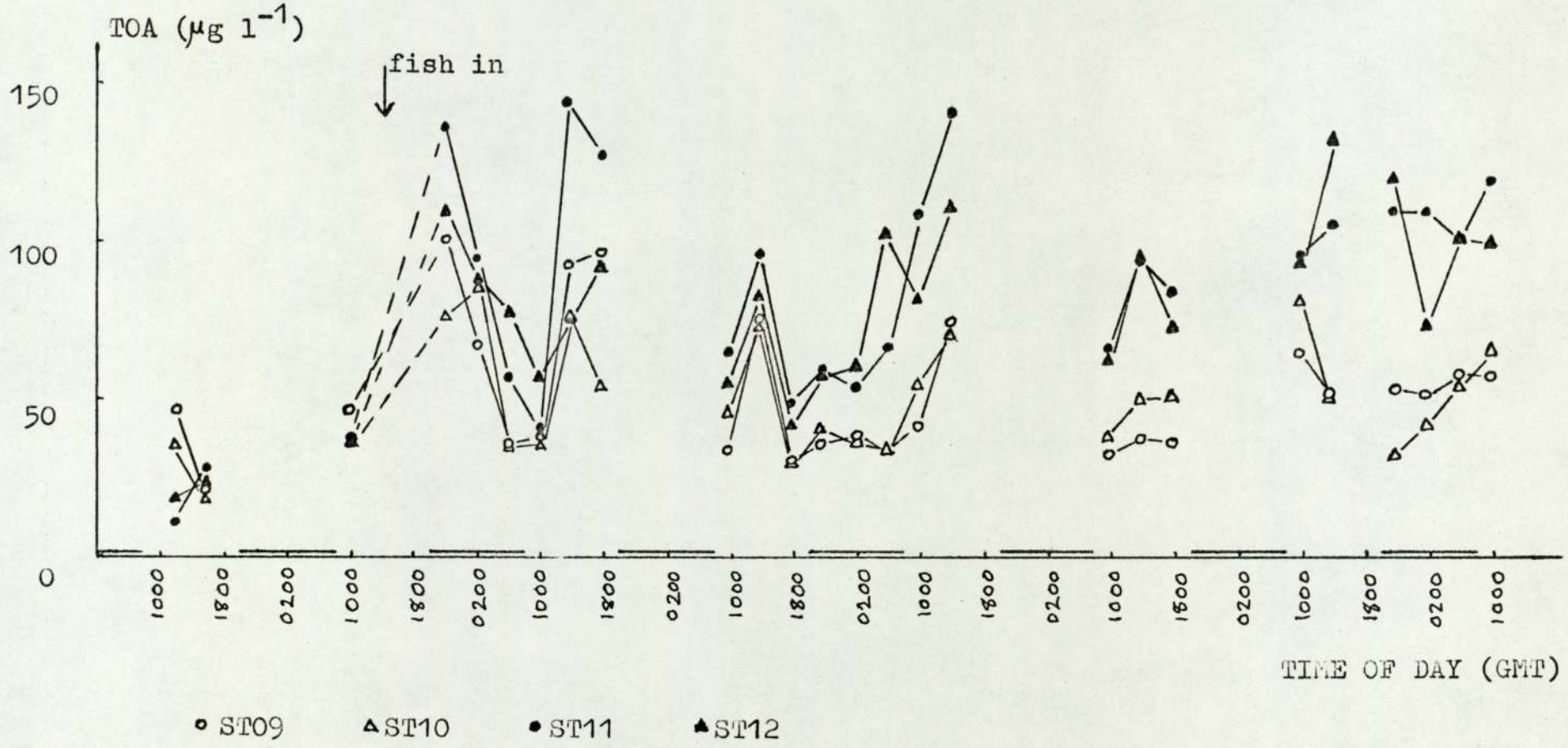
6.21 The results for PO2 also show a fall in pH associated with fish loading; this is more marked than in PO1, with an average fall of 0.4 - 0.5 pH unit in the low loading tanks and about 0.6 unit in the higher loading tanks. The pH pattern reflects that of TOA in following a diurnal rhythm (Graph 6.3).

6.22 PO2 is similar to PO1 in that calculated UIA is probably negligible, at 0.1 $\mu g\ l^{-1}$ or less.

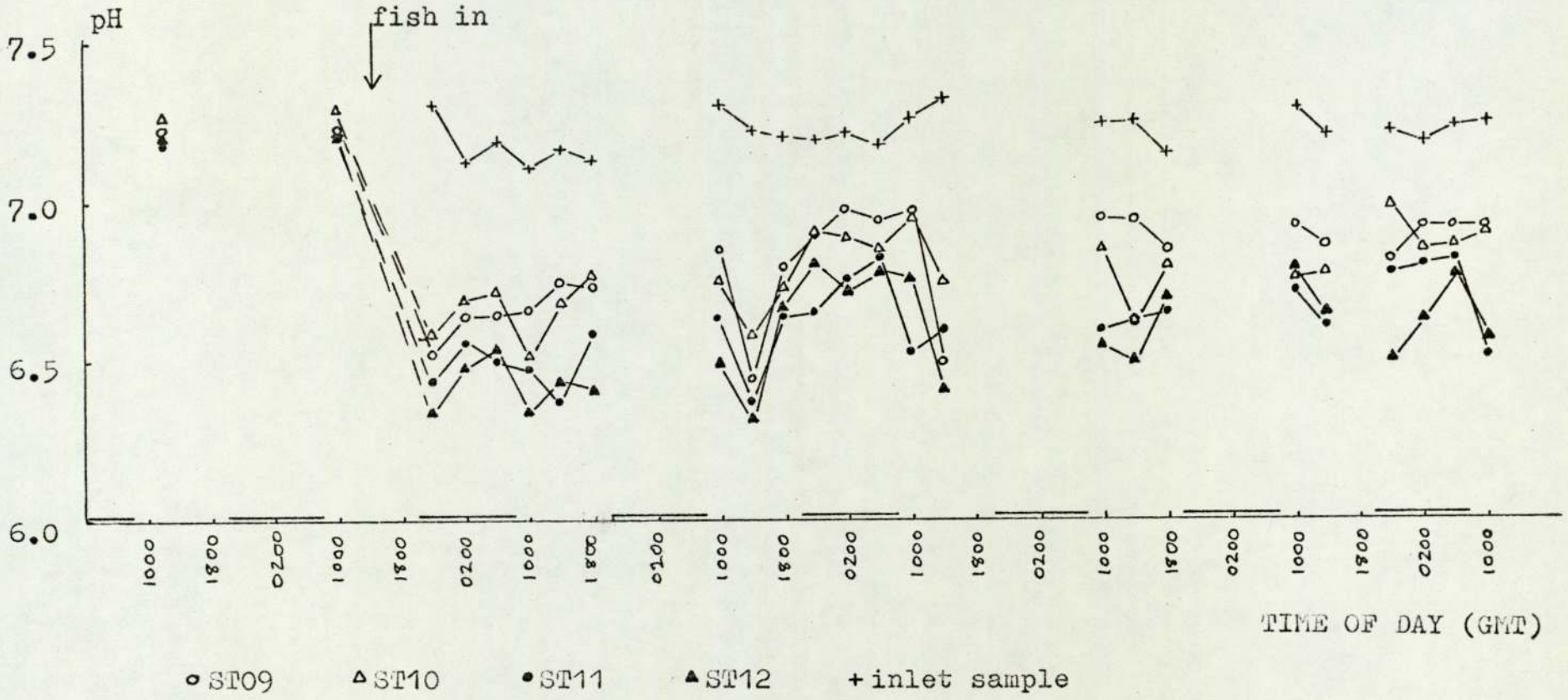
6.23 In most cases, the results for TOA in PO2 show a peak at 1400 during the day, with lower values at 1800. Bearing in mind that the photoperiod ended at 1530, the highest values of TOA are associated with the latter hours of the photoperiod (feeding period).

6.24 Regarding the weight results for PO2, (Table 6.4), a fall in weight was noted for tanks 09, 11 and 12. This lack of growth over a 6-day period has an important bearing on the results (see Discussion section).

6.25 Fish used in PO2 and PO3 were held prior to each experiment in approximately the same holding conditions; the average TOA was estimated to be in the region of 730 $\mu g\ l^{-1}$ (corresponding to about 0.4 $\mu g\ l^{-1}$ UIA).



Graph 6.2 P02 TOA results



Graph 6.3 P02 pH results

EXPERIMENT	TANK (ST)	No. of FISH	WEIGHT (g)				
			TANK INITIAL	TANK FINAL	TANK CHANGE	FISH MEAN CHANGE	TANK MEAN
P01	01	4	-	61	-	-	61
	02	5	-	57	-	-	57
	04	13	-	170	-	-	170
	06	18	-	211	-	-	211*
P02	09	7	311	301	- 10	- 1.4	306
	10	6	324	327	3	0.5	325
	11	13	618	601	- 17	- 1.3	609
	12	13	618	564	- 54	- 4.1	591
P03	09	18	998	931	- 67	- 3.7	965*
	10	18	1025	1045	20	1.1	1035
	11	24	1241	1121	-120	- 5.0	1181
	12	24	1257	1216	- 41	- 1.7	1237

* see Appendix A1 re these data

Table 6.4 Weight results from pilot experiments

6.26 In experiment PO3, TOA values were measured only during the photoperiod. Graph 6.4 shows how values gradually rose over the four days of measurement, and the clear rise over the course of the photoperiod during each day, as suggested in PO1 and PO2. Three days after inauguration, the separation between high and low loading tanks is apparent, but subsequently the effect is altered, and higher TOA is found in tanks with lower stocking rather than higher loading.

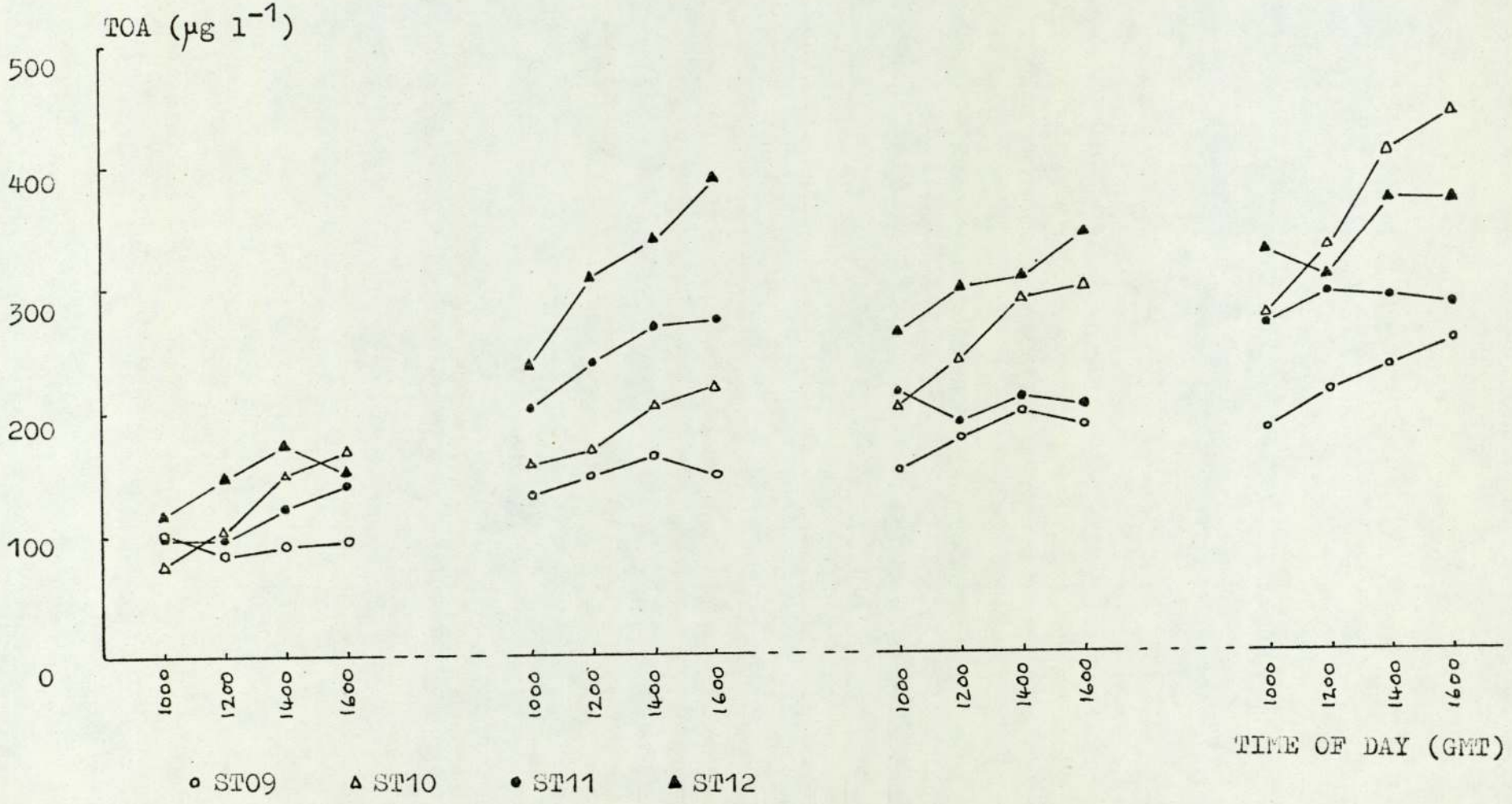
6.27 Most TOA values in PO3 were above 100 $\mu g\ l^{-1}$ and so their values are more reliable than those of PO1 and PO2, but due to other difficulties with PO3 (see Discussion) further inferences are not made directly from the results.

6.28 pH records for PO3 indicate a fairly steady difference of about 1.0 pH unit below the inlet water supply value with little indication of a diurnal pattern. Temperature records indicate a similarly steady difference of about 0.5 to 1.0 deg C rise within the tanks, an effect also present in PO2.

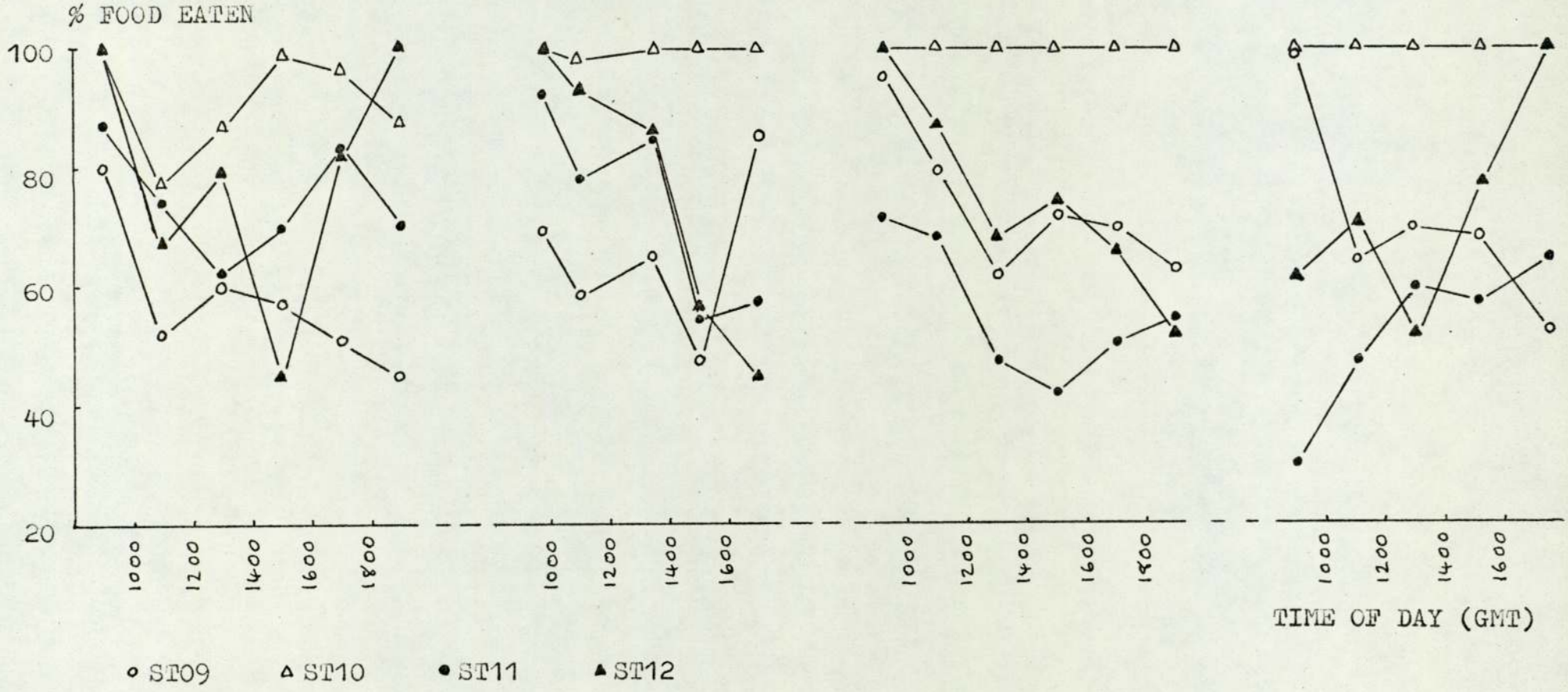
6.29 The stocking and loading in PO3 were the highest in the pilot series of experiments, and were associated with rather higher TOA than those found in PO1 and PO2. Values of UIA were around 0.2 $\mu g\ l^{-1}$ and below.

6.30 In most cases, TOA in PO3 continued to increase throughout the photoperiod. However, in several cases measurements taken at 1600 (the last reading) showed either very little rise, no rise, or a fall compared to TOA values at 1400. In the case of ST 10 (see below), the steepest rise each day occurred between 1200 and 1400.

6.31 In examining PO3 weight results a loss in weight is again noted in tanks 09, 11 and 12, with a little growth in tank 10. Reference to Graph 6.5 shows that ST 10 was the only tank with a consistently high feeding record as measured by the net index of uneaten food. By the last day of experiment, ST 11 had the



Graph 6.4 P03 TOA results



Graph 6.5 P03 food consumption

worst feeding record (even early in the photoperiod), and this corresponds to the greatest loss in weight over the experimental period. On the second day after inauguration (the first day plotted on Graph 6.5), the lowest feeding levels shown by ST 10 are associated with the lowest TOA levels for that tank in Graph 6.4.

6.32 Fish behaviour in PO1 was active (good feeding, some jumping) but not indicative of stress. In PO2 some sign of bullying by the largest fish was seen, with otherwise quiet behaviour and some reluctance in feeding. These signals of stress were magnified in PO3, especially in terms of very poor feeding behaviour; fish became dark and inactive apart from sudden darts frequently associated with bullying.

DISCUSSION

6.33 The three pilot experiments, besides yielding valuable information on method, gave a preliminary outline of the micro-environment situation inside the tank.

6.34 Despite doubt surrounding absolute values of TOA in PO1 and PO2, and a developing stress situation in PO3 (see below), clear indications were obtained that TOA rises during the photoperiod and falls during the dark period, and that when food consumption is at or near the maximum (limited by the amount offered), high loading, as opposed to stocking, is associated with high TOA (together with a drop in pH and a slight raising of temperature).

6.35 Statistical procedures were not employed for the data (bearing in mind the doubts about absolute values), but calculated mean values of TOA support the above contention (when diurnal rhythm effects are roughly balanced out).

6.36 It would seem fair to conclude that TOA in the water is

directly affected by the food consumption of the fish during the course of the photoperiod, although the exact "process time" (the time taken for the effects of one meal to be measurable in terms of TOA) is not clear.

6.37 In all conditions of the three experiments UIA never exceeded 1 $\mu g\ l^{-1}$ and was thus considered to be negligible.

6.38 Fish used in the experiments were previously subject to higher TOA (in holding tank) than encountered in the experiments; to define a level to which they were acclimated would however be difficult due to the probable diurnal fluctuation of TOA in the holding tank. Two or three days seemed to be necessary for fish under experiment to surmount the stresses associated with experiment inauguration, settle in experimental conditions, and reach maximum feeding; this period was characterised by TOA measurements which did not conform to the final pattern. As all fish were from the same batch and holding tank, acclimatory effects should have been similar for both high and low loading.

6.39 Values of TOA, fish weight and flow taken from Table 6.4 and Appendix A1, are used in Table 6.5 to calculate approximate mean values and ranges for stocking, loading and specific excretory rate (SER). It can be seen that SER is roughly similar in all tanks within the confines of each experiment and the limits of experimental error. Overall mean values of SER are 36.99 mg $kg^{-1}\ h^{-1}$ for PO1 and 7.84 mg $kg^{-1}\ h^{-1}$ for PO2, whilst the value of 8.51 mg $kg^{-1}\ h^{-1}$ for ST 10 in PO3 is probably the most reliable datum for that experiment.

6.40 It thus seems probable that the specific excretory rate (which determines TOA in the tank) is inversely related to loading (Graph 6.6) but it is important to recognise the differences between experiments PO1 and PO2, the most important being temperature. Thus the hypothesis lines on Graph 6.6 would be one possible way of interpreting the situation in the light of the

Table 6.5 Pilot experiments: stocking, loading, SER

EXPT	TANK (ST)	MEAN STOCKING (g ℓ^{-1})	FLOW (ℓ min $^{-1}$) MEAN		LOADING (kg min ℓ^{-1}) MEAN		TOA (μg ℓ^{-1}) MEAN		SER (mg kg^{-1} h^{-1}) MEAN	
			MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
P01	01	26.52	0.896		0.068		53		46.70	
			0.882	0.909	0.067	0.069	22	89	19.08	79.58
	02	6.95	0.913		0.062		44		42.31	
			0.909	0.923	0.063	0.062	22	76	21.05	73.79
	04	19.77	0.917		0.185		102		33.01	
			0.896	0.937	0.181	0.190	60	132	18.98	43.66
	06	81.15	0.912		0.231		100		25.93	
			0.896	0.923	0.229	0.236	67	132	17.07	34.65
P02	09	85.00	0.819		0.374		54		8.67	
			0.805	0.833	0.367	0.380	31	100	4.89	16.34
	10	41.67	0.827		0.393		53		8.09	
			0.816	0.845	0.385	0.398	30	84	4.52	13.10
	11	79.09	0.821		0.742		92		7.44	
			0.811	0.833	0.731	0.751	40	145	3.20	11.90
	12	155.53	0.828		0.714		85		7.14	
			0.805	0.851	0.695	0.734	42	133	3.43	11.49
P03	09	160.83	0.638		1.513		137		5.43	
			0.622	0.667	1.447	1.551	83	196	3.21	8.13
	10	136.18	0.638		1.622		230		8.51	
			0.504	0.706	1.466	2.054	74	435	2.16	17.80
	11	207.19	0.642		1.840		200		6.52	
			0.622	0.674	1.752	1.899	93	292	2.94	10.00
	12	154.62	0.622		1.989		275		8.30	
			0.594	0.649	1.906	2.083	115	385	3.31	12.12

Table 6.5 continued

Notes:-

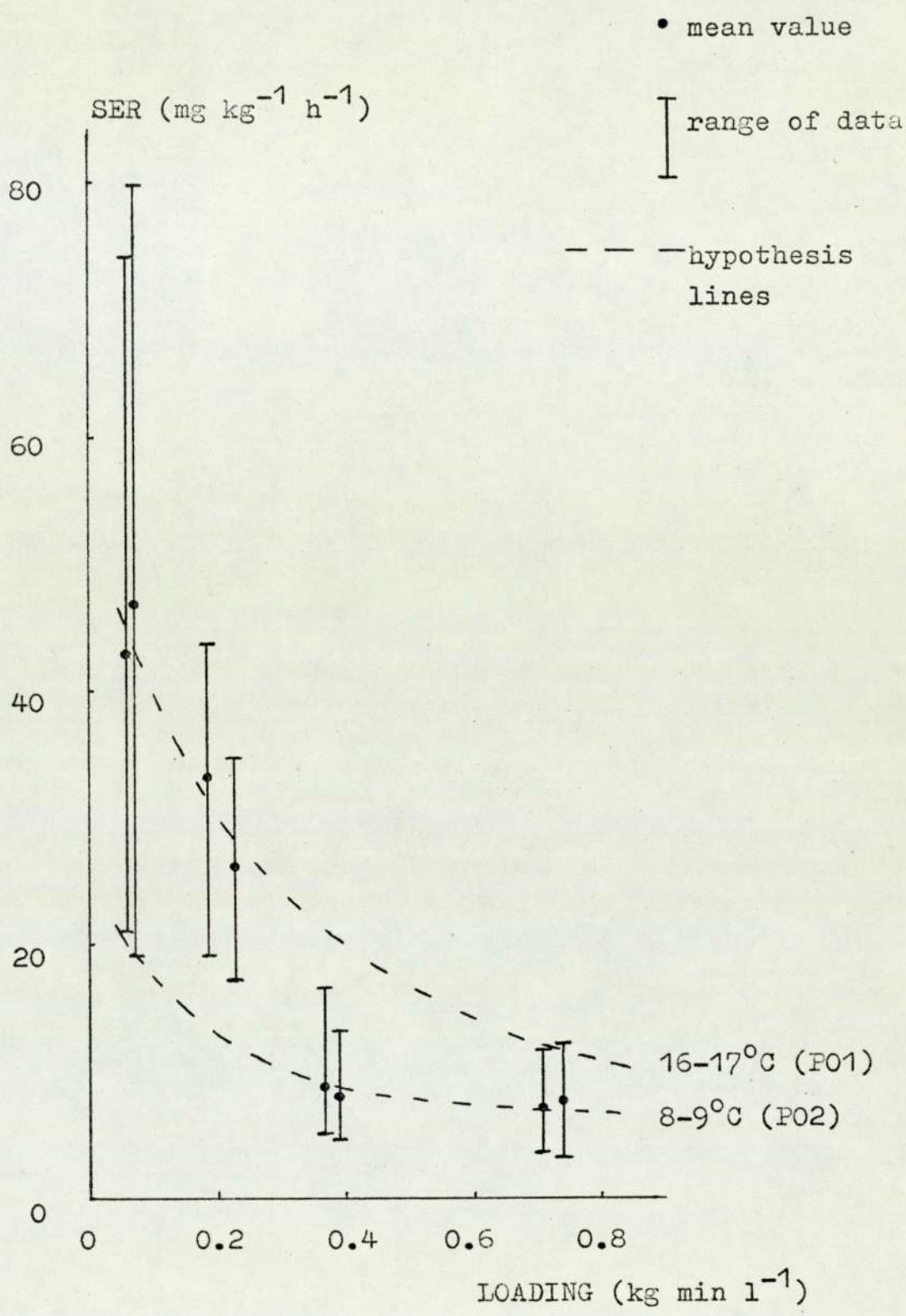
a) MIN = minimum value, MAX = maximum value
b) Stocking is only expressed by the mean, as only one volume determination was made in each case.
c) SER is calculated thus:-

$$SER_{MEAN} = \frac{0.06 \times TOA_{MEAN}}{LOADING_{MEAN}}$$

$$SER_{MIN} = \frac{0.06 \times TOA_{MIN}}{LOADING_{MAX}}$$

$$SER_{MAX} = \frac{0.06 \times TOA_{MAX}}{LOADING_{MIN}}$$

(0.06 is the conversion factor for units)



Graph 6.6 Hypothetical relationship between loading and excretory rate

temperature difference.

6.41 Graph 6.7 shows a similar plot for stocking levels; here the possible relationship is much less clear.

6.42 A further speculation not incompatible with the data is that SER could be related to loading whilst stocking is low (e.g. below 100 g l^{-1}), but that at higher stocking SER is depressed even at lower loading.

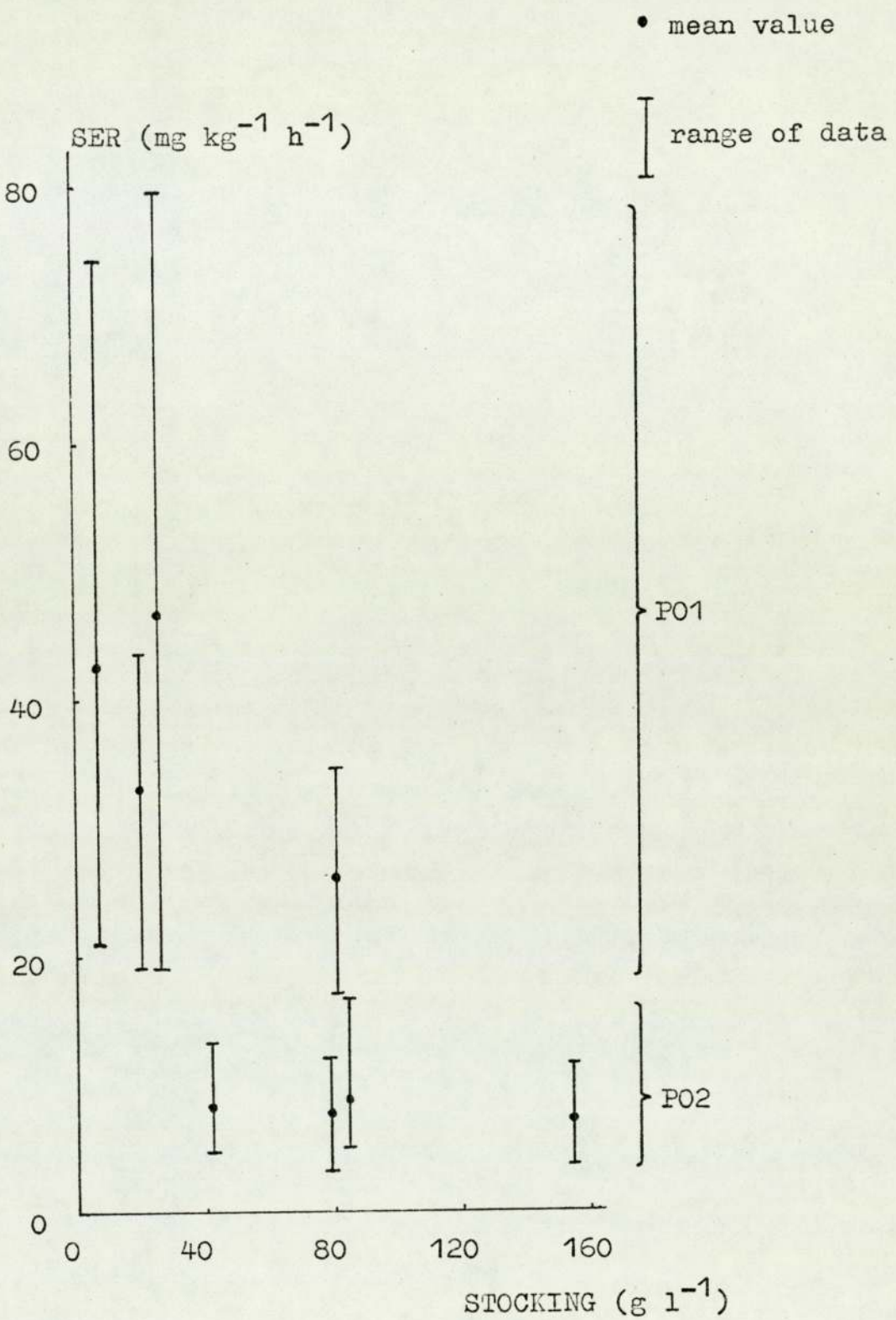
6.43 Testing of such hypotheses arising from the pilot experiments clearly depends on accumulation of a greater body of data, especially in regard to different levels of stocking, loading and temperature, and subsequent experiments were designed to provide such data.

6.44 However, several other aspects of the pilot experiments were relevant to the design of subsequent experiments, in particular the state of feeding, the estimation of weight, and the choice of representative TOA values.

6.45 It is clear that fish were not feeding properly in PO3. This led to lack of growth and casts all other results of this experiment into doubt. It would seem from observation of fish behaviour in PO2 that similar conditions may have occurred there to a lesser extent (para.6.32). A general explanation for this effect would be to assign it to "stress", but it is necessary to define more closely which aspects of the situation were involved in creating a stress whose symptom was reduced feeding.

6.46 By inspection, it is likely that the following factors were involved in creating such a stress:-

a) Inauguration routine. The method of weighing prior to placing fish in tanks was naturally stressful in requiring handling of fish, but this was enhanced by the small size of the weighing container (see para.6.9) and the time that the fish spent in it while balance was achieved. Fish were close to over-



Graph 6.7 Stocking and excretory rate in pilot experiments

turning from lack of oxygen in the case of PO3, though they were subsequently revived in the experimental tanks by extra aeration.

b) Photoperiod change. In PO2 the length of photoperiod was not drastically changed from the holding condition, but it was moved to about half an hour earlier. In PO3, the photoperiod length was 12 h (extra light all occurring at the end of the day), as compared to around 9.5 h under holding conditions. This sudden acceleration and relative displacement could be involved in the fish stress response.

c) "Crowding". Probably the most likely effects would be those due to the relative sizes of fish and tanks and the number of fish used, in experiment PO3. The difficulties involved in assessing these factors are mentioned in Chapter 5, but it is not surprising that 24 fish averaging 16 cm length enclosed in a tank of about 28 cm diameter holding less than 6ℓ of water (the case for ST 11 in PO3) should produce evidence of stress. Replacement of ST type 1 tanks (used in PO1) for tanks of larger diameter was probably still insufficient for fish of the size employed in PO3.

6.47 From the experience of PO3, it was clear that for future experiments, fish of this size would require larger tanks, and that experiments in tanks near to ST size (about 10ℓ) could only employ small fish, for example up to FCAT 4 (as used in PO1). It was also clear that inauguration stress should be reduced to a minimum by means of a better weighing method, and that photoperiod shock should be avoided.

6.48 A longer period of measurement was also indicated, to be sure of getting clear of any early confusion due to inauguration effects (para.6.38). This, however, would mean magnification of an error already built-in to pilot experiments: that of growth effects over the period of the experiment. In pilot experiments a simple mean was taken from the starting and finishing weights.

For measurements over a longer period, a method would be required to ascertain weight at different times in the experiment; it would also be desirable to measure flow and volume frequently,

6.49 The clearest result of the pilot experiments, in terms of TOA, was the extreme variability of the values measured. Postulating a diurnal cycle in TOA (i.e. wavelength 24 h), the amplitude of the oscillation seemed to depend on loading in PO1 and PO2, being greater in higher-loading tanks. The evidence of PO2 and PO3 points to a maximum value at the end of the photo/feed period, and a minimum at the end of the dark/fasting period. (The actual positions of the extremes may be at the beginning of the subsequent period rather than within the ones indicated. It also seems likely from PO3 that the quickest change in value on the 12 h photoperiod regime is between 1200 and 1400, with lesser changes both before and after (see para.6.30 and Graph 6.4). Thus an asymmetrical, slightly "saw-tooth" type of waveform is suggested. Figure 6.1 shows the possible form of such an oscillation, with maximum TOA rise from 1200-1400, and the assumption made (in the absence of any definite evidence) that TOA fall during the dark period is the reverse of the photoperiod pattern (with peaks and troughs at the light-changes); a smooth curve has been drawn to connect hypothetical points plotted in this fashion.

6.50 With these points borne in mind, it becomes necessary to select a time reference for subsequent experiments so that inter-experiment TOA comparisons are valid and minimise error due to position on the waveform.

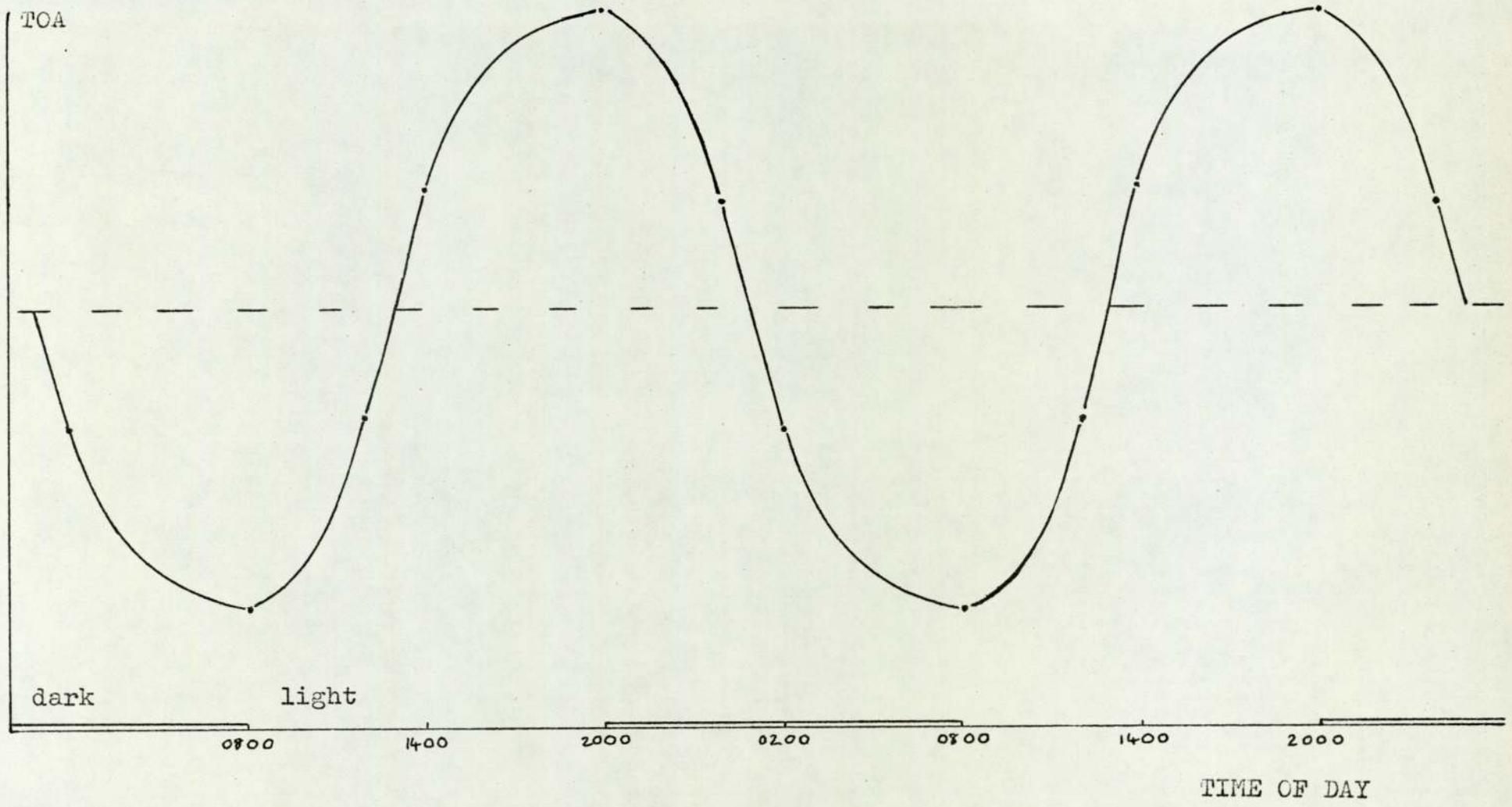


Figure 6.1 Hypothetical oscillation of TOA under 12 h regime

7. FULL SYSTEM EXPERIMENTS

PRELIMINARY WORK

Background monitoring program

7.1 From early October until early December 1973 Lots 01 and 02 (pooled) were employed in refining a weight-estimation technique and in testing experimental procedure for the following E-series, so that fish were treated as if on experiment, except for the measurement of ammonia. This invaluable experience was termed the background monitoring program (BMP); larger fish were used than could be accomodated in the actual E-series; these were fed equal amounts five times per day, and the total fed was adjusted according to temperature difference or fish mortalities.

7.2 The BMP was divided into four 2-weekly periods beginning on a Wednesday, so that each alternate succeeding Wednesday was a grading day. On the grading day, fish were weighed individually after anaesthesia, their lengths (to nearest 0.5 cm) noted, regraded according to FCAT attained by growth in the previous two weeks, and then returned to tanks (LTS) for the next two weeks. Full records were kept of weights, lengths, food fed, temperature, mortalities, and tank flow and volume.

7.3 Since BMP was a design and testing exercise, the results are relatively unimportant; they are given in Appendix A2. Graph 7.1 shows that over the spread of fish size (halfway through BMP) the fish population was fairly homogeneous; the condition factor (K) (relating length and weight) at this point averaged 1.47 (values below 1.00 indicate emaciated unhealthy fish), and had risen from 1.44 two weeks previously, indicating healthy growth.*

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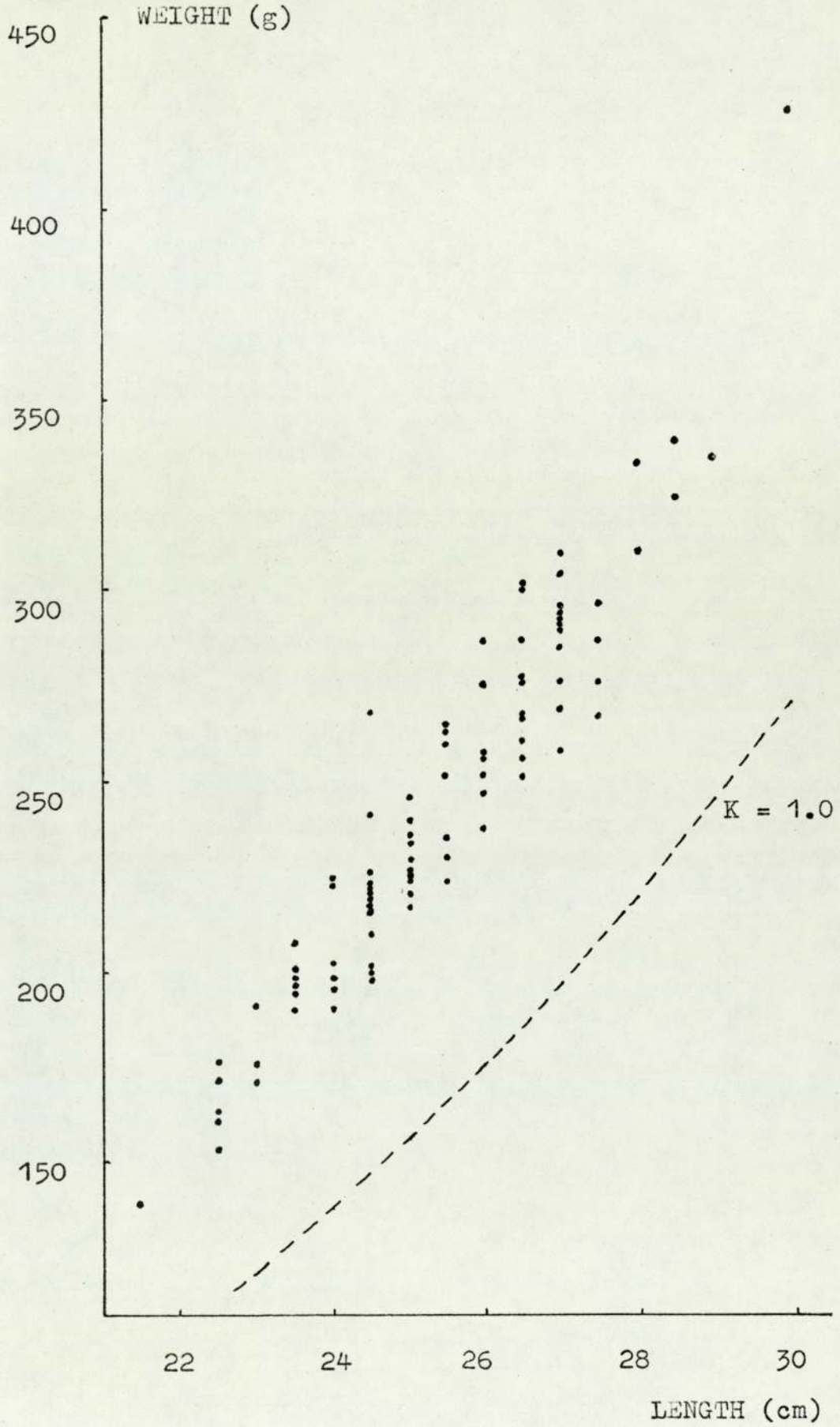
* (condition factor is given by: $K = \frac{\text{weight} \times 100}{\text{length}^3}$ (Brown 1957)



Graph 7.1 Length and weight of fish during BMP

Units in this case would be g cm^{-3}, but:

a) this differs from density in that the cube of length is a different quantity from fish volume;

b) units are not usually quoted in literature references to condition factor; when other units are used instead of cm and g, the resulting factor is related to this one by a constant coefficient.

7.4 Weight estimation data treatment is explained later, under DATA ANALYSIS.

Pilot experiment P04

7.5 Although out of chronological order, it is appropriate to mention at this point the fourth pilot experiment P04 which was undertaken between 30/8/74 and 13/9/74, and thus performed under the E-series routine of method. Only one tank was used, to test the feasibility of experimenting on large fish (FCAT 6) in the MTS, with temperatures of about 14 to 16°C. Although some growth was achieved, conversion (see METHODS section) was poor, fish showed clear signs of stress including dark colouration, and bullying and cannibalisation were severe enough to cause 37% mortality, thus invalidating the intermediate weight estimation technique.

7.6 P04 results are given in Appendix A2. They indicate that at high temperatures fish of FCAT6 were unsuitable for MTS experimentation, and subsequently only one MTS E-series experiment featured fish as large as this, at a lower temperature. (FCAT 6 was used successfully in LTS E-series experiments.)

Rehearsal experiment E00

7.7 Just prior to the E-series full experiments, a one-week rehearsal trial was undertaken with two MT from 5/3/74 to 12/3/74. Each tank contained 30 fish from Lot 03 of FCAT 3 and 4. The full E-series routine was used except that volume was not monitored due to a delay in equipment delivery. Ammonia readings were taken.

7.8 Temperature at this time was at the lowest of the year (see Chapter 1) hence excretion rates were expected to be very low. This is reflected in the low TOA measured.

7.9 Appendix A2 gives a summary of EOO results. These were disqualified from further consideration on four counts:-

a) the experiment only lasted half the standard period,
b) this short period magnifies the error due to mortalities in weight estimation (see DATA ANALYSIS section),
c) a fall in condition factor in MT8 showed poor feeding and growth,
d) the measured TOA tended to be low, and in MT8 was below 100 $\mu g\ l^{-1}$ (see Chapter 4).

EOO was nevertheless a successful rehearsal in terms of time-tabling, procedure and feasibility, and was followed almost immediately by the first of the full E-series experiments, EO1.

METHODS AND MATERIALS

Fish

7.10 Fish Lots O3 and O4 were used for E-series experiments. Lot O3, originally 1350 fish of FCAT 1, arrived at Aston in early December 1973 and was maintained under HC2. This Lot was sampled for experiments EOO to EO5 inclusive and for PO4; EOO began in early March 1974. Lot O4, 1500 fish of FCAT 2, arrived in late August 1974 and was held separately under HC2 until early November; Lots O3 and O4 were then pooled and the combined population sampled for experiments EO6 to EO9.

7.11 Selection criteria for experiments were, simply, required FCAT and healthy appearance. Fish were sampled by netting out of their holding tank into buckets; in most cases the majority of fish from one holding tank would be used as several experimental tanks would be involved. Each experimental tank was allotted a population by number, no attempt being made to attain target weights of fish.

Tanks

7.12 All MT were employed in one experiment or another, although all eight were rarely in operation together, due to cleaning, repairs or limitation on fish stocks of required size. On two occasions the LTS was employed, allowing larger fish and different stocking and loading conditions to be used.

Feeding

7.13 Both Lots 03 and 04 progressed during holding from Salmon No.2 diet through No.3 to Trout No.4. All experiments were conducted using Trout No.4 floating pellets.

7.14 Feeding routine during experiments was automatically controlled (see Chapter 3) with food dispensed five times per day. Photoperiod lasted from 0800 to 2000 (GMT or BST according to time of year), with feeds at 1000, 1200, 1400, 1600 and 1800. 1400 marked the "mid-day" point. This regime was calculated, as in pilot experiments, to spread physiological effects as much as possible over the photoperiod.

7.15 Feed rates were calculated rather differently from pilot experiments, in an attempt to feed by a predictive method worked out by American fishery scientists over a number of years (Haskell 1959, Freeman et al 1967, Buterbaugh & Willoughby 1967). The method attempts to prevent food wastage, and is based on a theory of growth and temperature interaction originally formulated by Haskell (1959) and since refined.

7.16 The background for this method (and the CNP feeding chart which derives from similar origins) rests on the use of hatchery records to define terms of reference for individual trout farmers, and whilst this is an excellent pragmatic approach in the field situation, it lacks the feeling of ability to derive from first principles which ought to characterise a truly scientific method. Nevertheless, in any experiment which is designed to reflect the field situation to a reasonable extent, this kind of approach to feeding, a basic factor in the complex, must be accounted for. Hence the use of this method as explained below.

7.17 Haskell (1959), from studies of US trout hatchery records, proposed that fish growth under production conditions could be described thus:-

$$\text{daily \% weight gain} = \frac{3 \times \Delta L \times 100}{L}$$

where L = fish length and ΔL = daily gain in length.
Since both L and ΔL could be estimated from individual hatcheries' records (or L calculated from weight records by means of a generalised relationship), then daily weight gain could be easily related to daily amount to feed by means of a suitable feed conversion.

7.18 The conversion referred to here is that known to nutritional literature as the gross conversion ratio, defined thus:-

$$\text{gross conversion} = \frac{\text{weight of food fed over a certain period}}{\text{weight of animal flesh produced over that period}}$$

Both weights measured are "raw" or "wet" weights of the material, as opposed to dry weights which are the results of oven treatment to remove all water. In the case of fish, the weights are of fish food (pellets) from the supply bag, and of fish immediately as harvested. Commercially, the lower this conversion is, the more fish the farmer obtains per quantity of food required, and hence per cost incurred.

7.19 Haskell's relationship can thus be rephrased:-

$$\text{\% body weight to feed daily (\%BWD)} = \frac{\text{conversion} \times 3 \times \Delta L \times 100}{L}$$

Determination of conversion seems to depend on one of the following:-

a) appraisal of past conversion achieved and its assumption for the future,
b) choice of a target conversion which seems economically desirable,
c) calculation of a reasonable conversion from estimates of trout requirements and feed contents. It is clear that these considerations may not all give the same answer, and it is not

always obvious in any given situation whether one can choose a desired conversion or can only accept the conversion which experience dictates. The room for manoeuvre between these two states will depend greatly on feeding practice and general husbandry. In the present situation, with no records on which to rely, a target conversion was assumed for purposes of feeding. This assumption followed Phillips (1970) in presuming an average trout production requirement of about 16590 kJ per kg of fish produced, and in presuming that a modern pellet diet contains about 11060 kJ per kg of feed. Thus the conversion ratio implied is 16590/11060 or 1.5.

7.20 Assuming that water temperature stays constant, Buterbaugh and Willoughby (1967) consolidated the derived formula to:-

$$\%BWD = \frac{H}{L}$$

- where H = conversion x 3 x ΔL x 100 and is termed the hatchery constant.

7.21 However, for situations where water temperature varies, it is necessary to estimate ΔL by using Haskell's temperature unit theory of growth. This maintains that a definite rate of growth can be predicted for any temperature between 38.6^{o} and 60^{o}F, and the temperature unit (TU) is defined as the average Fahrenheit temperature, for the period over which it is wished to calculate (usually one month), minus 38.6. In converting this process to metric units, one may take note of Speece's recent statement (1973), quoting Bowen (1971), that 32^{o}F can be substituted for 38.6^{o}F. This means that TU in metric units is numerically equal to the temperature in ^{o}C. Having derived TU for the required predictive period from hatchery records, these records must further be consulted for the TU usually required per unit length increase, in which case:-

$$\Delta L = \left[\frac{\text{TU expected in next month}}{\text{TU required per unit length growth}}\right] \div 30$$

Thus ΔL can be derived for the future month, H can be calculated (assuming a conversion) and%BWD finally estimated.

7.22 In the current work there were no suitable records on which to rely for source information, with the exception of water temperature records maintained since the beginning of fish holding (however change of HC rendered these of only limited help). Under these conditions, several assumptions were made:-

a) conversion would be 1.5;

b) TU required per cm of length increase would be 7.9 (calculated from Speece (1973) as a reasonable usual value);

c) that the process was equally applicable over a shorter period i.e. 14 days;

d) that the assumed (expected) temperature for the 14 days (FPT)* would be reasonably estimated by reference to previous records and the current temperature, and arbitrarily selecting a likely value. *Forward projected temperature.

7.23 It follows that, in order to estimate the food required for the duration of an experiment (14 days) it was necessary to assume the FPT and to measure the total weight and average length of the fish. Thus feed calculation could not be performed until fish had been measured and sorted to experimental tanks. Use was made for feed calculation of Piper's (1970) slide rule technique, suitably modified.

7.24 After feed calculation, feeders were individually calibrated (ensuring no feed reached the fish) as in Chapter 3 to dispense one-fifth of the daily food requirement per stroke of the feeder piston.

7.25 No food was delivered during the afternoon of sorting, since usually the full afternoon was required, and feed calculation could not be performed until sorting was completed. This time, followed by the dark period, allowed fish to settle down before the first feed the following morning. As fish were weighed with empty guts during the initial sorting period (they were not fed during the preceding morning), it was necessary to

maintain comparability, to starve them on the last morning (14th day) prior to weighing and sorting on the final afternoon.

7.26 Since any mortality during an experiment would affect the total fish weight in the tank, it was necessary to re-calculate the daily feed in the case of mortality, having taken into account the change in total weight. As the weight and length of the dead fish were also necessary data for the process of intermediate weight estimation, dead fish were always immediately weighed and measured for length when removed from the experimental tank.

7.27 Feeder calibration (and re-calibration after mortality) was, as explained in Chapter 3, a process of "zeroing-in" on the target value. After sorting and feed calculation on the first day, each feeder would be calibrated by means of several non-delivery trials to dispense the correct amount of food. After the first full day's feeding, the amount dispensed would be compared to the target value, and the calibration nut slightly adjusted in respect of any excess or deficiency. Over-compensation would be corrected for on the following day, and so on. There also occurred, at times, gradual slippage of the calibration nut over 24 h, which would necessitate re-adjustment on the following day. Thus the overall effect was for the actual amount of feed dispensed to vary around the target value. This introduced a certain variability into the feed data supplied for analysis, expressed as "noise" over the target amounts. It is not unreasonable to suppose that similar considerations might apply in a full-scale fish production situation.

7.28 Using this system of feeding, it was found in the great majority of cases that food consumption was virtually total. It was never necessary to net out uneaten food, and although a very small proportion of food may have been swept out of the tanks before fish could consume it, the situation never reached the acute conditions of PO3. Thus in general it was safe to

assume total food consumption; any tank failing to achieve this would furnish poor growth indication data (see DATA ANALYSIS section) at the end of the two-week period. This would disqualify data from further treatment.

Sorting

7.29 Unlike the pilot experiments, the E-series experiments were not designed around target values of stocking and loading. Instead, a reasonable number of fish of the desired FCAT were accommodated in each experimental tank so that the tank was well populated but not overcrowded (by eye appreciation) bearing in mind the volume and flow available. Since crowding is such a difficult phenomenon (see Chapter 5), it was felt that this was an acceptable method of setting up. With the variations of volume and flow which occurred, a fairly wide range of loading and stocking values were obtained for the multivariate analysis used.

7.30 Initial conditions of all experiments are given in Table 7.1. Experiments were performed under the 12 h photoperiod, five-feed regime previously discussed; in these respects there was no abrupt change between holding conditions and experiment.

7.31 The sorting procedure was as follows. Fish were starved in holding tanks during the morning before sorting to enable weighing with empty guts. Commencing at about 1400, the experimental tanks, having been cleaned, prepared and put under flow, fish of the desired sizes were sampled from the holding tanks. For each experimental tank required, fish were netted out in successive batches into a 10ℓ bucket containing water from the holding tank with accessory aeration. Fish were then transferred a few at a time into a second bucket containing aerated anaesthetic (MS.222) at a strength of approximately 50 mg ℓ^{-1}. After 2-3 minutes fish would be sufficiently quiescent for individual length measurement (just at the overturning point).

Table 7.1 Initial conditions of E-series experiments

EXPT	STARTING DATE	FPT (°C)	TANK	FCAT	No.of FISH	%BWD	INITIAL MEAN LENGTH (cm)	INITIAL TOTAL WEIGHT (g)	INITIAL STOCKING ($g\ l^{-1}$)
E01	14/3/74	6.5	MT1	3	30	1.37	9.00	292	(24.3)
			2	4	20	1.09	11.37	382	(31.8)
			4	3	30	1.35	9.12	309	(25.8)
			5	4	20	1.10	11.27	361	(30.1)
			7	3	30	1.36	9.05	297	(24.8)
			8	4	20	1.08	11.47	406	(33.8)
E02	30/4/74	10.0	MT1	3	30	2.10	9.05	283	32.2
			2	4	20	1.75	10.87	321	33.8
			3	3	30	2.12	8.97	280	29.8
			4	4	20	1.68	11.35	386	41.5
			5	3	30	2.15	8.83	267	28.7
			6	4	20	1.68	11.32	355	35.9
			7	3	30	2.16	8.77	257	25.7
			8	4	20	1.65	11.50	390	36.8
E03	28/6/74	16.5	MT1	2	27	4.31	7.28	121	13.7
			2	3	40	3.32	9.44	404	40.0
			3	4	30	2.94	10.65	428	43.2
			4	3	40	3.32	9.44	405	42.6
			5	4	30	2.94	10.65	422	38.4
			6	3	40	3.28	9.55	407	34.5
			7	4	30	2.61	12.02	578	55.0
			8	5	20	2.35	13.35	547	47.2
E04	13/8/74	17.0	MT4	3	30	3.39	9.53	321	29.2
			5	4	20	3.05	10.58	276	22.6
			6	3	30	3.39	9.53	316	30.4
			7	4	20	3.07	10.53	273	22.2
			8	4	20	2.88	11.20	324	26.3
E05	23/9/74	12.5	MT6	5	31	1.71	13.87	866	61.0
			7	5	30	1.72	13.85	878	68.6
			8	6	26	1.40	16.96	1585	115.7
E06	12/11/74	8.0	MT5	4	30	1.32	11.55	530	42.4
			6	4	30	1.28	11.85	573	62.3
			7	4	30	1.30	11.72	571	51.0
			8	4	30	1.31	11.60	547	48.8
E07	14/11/74	8.0	LT2	6	63	0.95	16.14	3226	38.4
			4	5	212	1.09	14.05	6824	70.4
			5	4	147	1.28	11.83	2948	35.1
			6	3	350	1.75	8.72	2932	53.3

Table 7.1 continued

EXPT	STARTING DATE	FPT	TANK	FCAT	No.of FISH	%BWD	INITIAL MEAN LENGTH	INITIAL TOTAL WEIGHT	INITIAL STOCKING
EO8	29/11/74	6.5	MT5	4	40	1.05	11.72	816	65.3
			6	4	40	1.03	11.95	889	90.7
			7	4	40	1.06	11.59	781	67.9
			8	4	40	1.04	11.86	835	73.2
EO9	6/12/74	6.5	LT2	6	93	0.76	16.17	4728	53.1
			4	5	235	0.89	13.95	7671	69.7

Notes:-

a) Stocking values for EO1 are estimates, based on a tank volume of 12 ℓ. Volume measurement equipment was not available and so stocking figures are not reliable.

b) In EO3, MT1 was stocked with fish of FCAT 2, fed on Salmon No. 3 diet, for comparison with fish of larger FCAT. Because of these differences, MT1 is not included in any further analysis of EO3 data.

For this, each fish was placed against a wetted length guide (see Figure 7.1) and length (from snout to tail-fork) read off to the nearest 0.5 cm and noted. The fish was then transferred to an appropriate aerated recovery bucket according to FCAT. Working at speed, the length measurement process (including anaesthesia) could be reduced to an average of about 3.5 min for each fish (the times overlapping as fish were continuously dealt with), and thus the stress of the procedure was minimised. When the desired number of fish for a batch had accumulated in a recovery bucket, that batch was weighed.

7.32 For weighing, a weigh-bucket containing between 5 and 8 ℓ of aerated water was placed on the pan of the 10 kg balance and its weight tared off. The fish batch was poured from the recovery bucket into a suitably-sized net and held steady for a standard draining-time of 15 s. The batch was then gently transferred into the weigh bucket and the batch weight read off to the nearest 1g from the direct-read scale, and noted (Fig. 7.1). Fish were then immediately transferred to a further aerated bucket to settle down, and then by small hand-net to their experimental tank. Weighing of a batch could be accomplished in about 1 min from pouring to settling-bucket and hence stress was minimised (most fish were still slightly under the anaesthetic influence in any case). Fish batch size varied according to fish size and water temperature. Maximum values were; FCAT 3:50; FCAT 4:40; FCAT 5:30; FCAT 6:20. It was estimated from repeated trials that the maximum error of the process would be about 1% to account for variations in drainage, and a further 1% in the accuracy of the balance. Sorting at the end of the experiment followed a similar process.

7.33 After the required number of batches had been sorted in successive buckets, the fish superfluous to experimental requirements were restored to their holding tank. In all cases, fish in both holding and experimental tanks were found to consume food as normal when presented with their first feed after sorting.

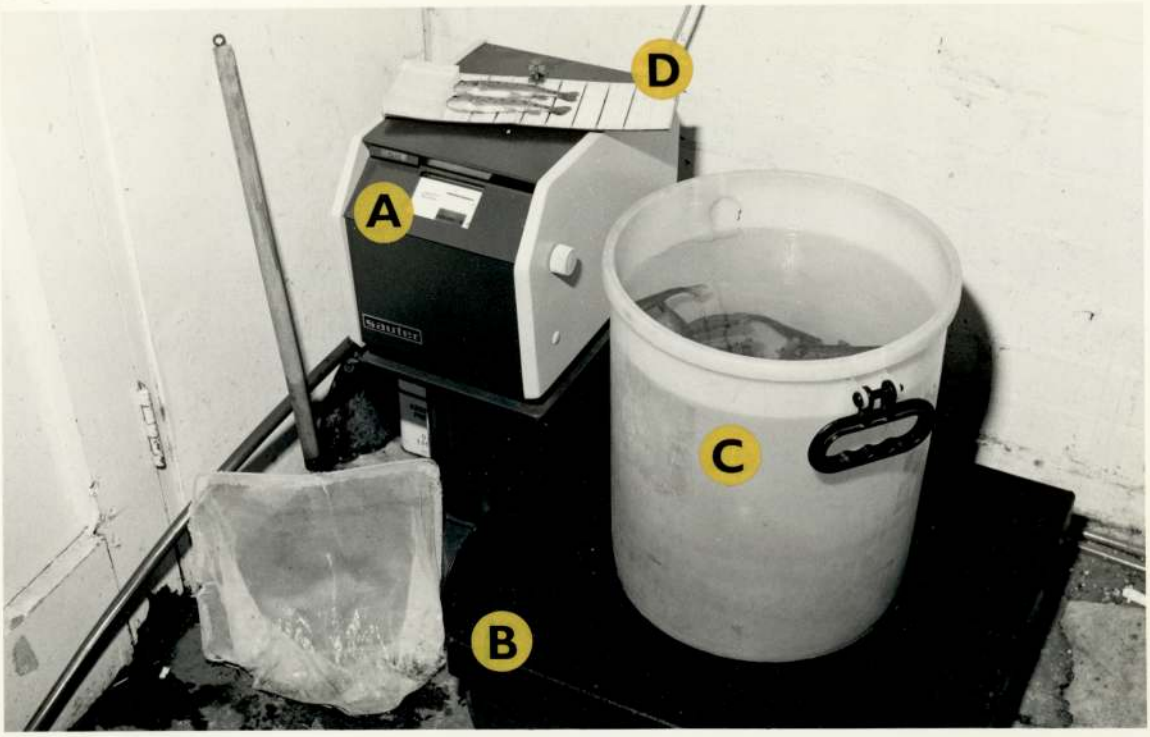


Figure 7.1 Sorting equipment

A Direct-read scale

B Balance platform

C Weigh-bucket with fish

D Length measure

This indicates that the low handling stress involved, followed by a dark period for recovery, was quite adequate to condition the fish for feeding.

7.34 Several authors have commented on the influence of various stresses in setting up physiological experiments for fish. Two main areas of effect are involved, anaesthetic and handling. In the case of this work, the MS.222 concentration used, 50 mg ℓ^{-1}, is within the "useful" range recommended by Bell (1964) and is the same as quoted by Larsen and Snieszko (1961) in their efforts to avoid partial asphyxiation (which may occur at higher concentrations) and hence prevent disturbance in blood parameters such as haematocrit, and possibly deeper-seated effects. 50 mg ℓ^{-1} is also below the value (80 mg ℓ^{-1}) which Wedemeyer (1970) found to be increasingly effective, after up to 12 min exposure, in disturbing ACTH production (indicating hormonal stress) in soft water. His recommended remedy, of preventing pH falling to about pH 4.0 by buffering, was unnecessary in the present work, since exposure was so short (3 min) and the concentration lower. Hunn and Wilford (1970) reported a requirement of 24 h for the diuretic effect of anaesthesis and catheterisation to clear; in the present study stress would be much less severe since no catheterisation was involved. Effects of handling stress have been commented on by several authors, usually at rather higher levels of handling activity than in this study. Wedemeyer (1972) reported effects on sugar, chloride and calcium levels in steelhead trout blood when, using water chemically similar to that of this study, he netted fish into a bucket and "transferred them 25 metres". He estimated that 24 h was necessary for normalisation of blood parameters; it is possible that steelheads react differently from rainbow trout, but this seems a good guide. Savitz (1973) reported no effect on the nitrogen excretion of starved bluegill sunfish over four days following rather heavier handling stress involving hand-catching from a

bucket; and McKim (1966) found that even severe handling stress on individual fish only lasted in its effect on corticosteroid production for a few days. The stress associated with batch drainage in the weighing procedure is unknown, but Eisler (1957) used the same drainage time without reporting lasting effects, and in the present study some degree of anaesthesia was frequently still present.

7.35 It is concluded that handling and anaesthetic stresses were almost minimal, certainly much less than during pilot experiments. In all cases, fish were left to settle overnight before feeding (which was always successful) and usually 72 h were allowed to elapse before ammonia measurements were begun. This should have allowed ample time for general metabolism to settle down, going by the guidelines of Wedemeyer (1972).

Measurements

7.36 Details of all systems and apparatus used are given in Chapter 3. Bearing in mind the comments of para.6.50 on the necessity of selecting a time-reference for comparability between experiments, and the findings of pilot experiments that the midpoint of the 12 h photoperiod (1400) seemed a suitable sampling time (since it occurred after the steepest rise in TOA in the diurnal pattern), it was decided to standardise on an approximately "mid-day" time for TOA sampling during experiments. Since there was a feed at 1400, the time chosen was 1330. This allowed all tanks to be sampled, and temperatures taken, (also pH readings when required) in good time before the 1400 feed. As 1½ h had elapsed since the previous feed (1200), all disturbance effects associated with feeding would have subsided well in advance of the sampling time. Sampling at lunchtime allowed TOA measurement to take place in the afternoon, and time was available during the morning for making ready sampling bottles and measuring equipment.

7.37 Flow and volume of the tanks were measured prior to

sampling. Since these quantities would affect the resultant TOA at 1330, they were measured well before (while "in operation"), usually at about 1230 to 1245. Flow was measured by timing with a stopwatch, how long it took to fill a 500 ml or 1000 ml volumetric flask from the overflow; choice of flask was dictated by speed of flow, and measurements were taken to the nearest quarter-second. Volume (except in E01, see Table 7.1) was measured with the calibrated indicator tubes for MTS, or by measuring water height for LTS (see Chapter 3). Volume was read off calibration graphs to the nearest 0.1 ℓ for MTS, and to the nearest 1.0 ℓ for LTS.

7.38 The 60 ml bottles for TOA sampling (see Chapter 4) were rinsed out twice each in water from the appropriate tank overflows, before being overfilled. They were then removed to the water-bath prior to TOA measurement. It was important to avoid faecal contamination (a possible source of excess ammonia) and so after flow measurement at 1230-1245, any accumulated debris in the tank U-tube was removed. The effects of this process on flow and volume were only momentary, and negligible.

7.39 The other major measurement was of food fed. Each morning, before the first feed, at 1000, the feed hoppers were emptied into weighing cups and the food weighed to the nearest 0.1 g. By difference between successive days' measurements, with topping up when necessary, the amount delivered over five feeds per day could be measured.

7.40 Thus for each day of the experiment, and each tank, readings were collected for: TOA, flow, volume, temperature, and food consumed over the previous 24 h. For the full 14-day period, readings were collected at beginning and end of the period for number, total weight, and individual lengths of fish.

7.41 Periodically, check-readings were taken of dissolved oxygen (DO) content of the tank water (both inflow and outflow) and pH of the outflowing water. In nearly all cases DO was

close to saturation values in inflowing water and only 20 to 30% depleted in outflow, while pH rarely rose above 7.0 or fell below 6.3. While these values were maintained, no further checks were carried out and these parameters were ignored. Values never departed substantially from the ranges quoted except for some oxygen levels in one of the high-temperature experiments during the middle of summer. Data from tanks affected were disqualified from further treatment. Oxygen was measured from water samples either by a modified Winkler's method (Spotte 1970) or by oxygen electrode and meter (Simac Model 65). pH was measured from water samples from tank overflows. At the pH levels recorded, UIA would not have been of any importance.

Experimental timetable

7.42 Due to the requirement for TOA, etc. readings at 1330, the food consumption of interest was that for the preceding 24 h i.e. since 1330 the previous day. Thus the experiment, although taking place under the normal photoperiod regime of 0800 to 2000, was divided for data treatment purposes into 14 "experimental days", each commencing at 1400 on one date and finishing at 1400 on the next. Thus readings were taken at the end of each "experimental day", which, as thus defined is referred to as DAY in order to avoid confusion. Table 7.2 gives a summary of the events from DAY 1 to DAY 14.

7.43 Between other activities detailed in Table 7.2, and in particular at the beginning of each photoperiod, systematic checks were made of experimental conditions, including water supply, lighting system, tank drainage, waste gutter and pipe maintenance, feeding system operation, feeding behaviour, and general fish behaviour.

DATA ANALYSIS

Basic data

7.44 There are three main sources of data in the E-series experiments. The first is the information gathered at the

	1400	1500	1600	1700	1800	1900	2000	DARK PERIOD	0800	0900	1000	1100	1200	1300
Morning prior to DAY 1									Fish not fed in holding tanks Cleaning and preparation of exptl tanks Establishing of required fish number Flow initiation in tanks Equipment preparation					
DAY 1	Sorting: weight and length measurement and allotting to tanks Feed rate calculation/Acclimatisation								Feed preparation		1st feed		2nd feed	
DAY 2	3rd feed		4th feed		5th feed				Feed measurement	Feeding regime continues as explained in text				
DAY 3 to DAY 14	(Feed regime continues) Ammonia measurement for previous DAY								Feed measurement Preparation for sampling	(Feed regime continues)			A	B
DAY 14	(Feed regime continues) Ammonia measurement for previous DAY								Feed measurement	No further feeding				
Afternoon following DAY 14	Final sorting: weight and length measurement and fish returned to holding tanks													

Table 7.2 E-series experimental timetable

Table 7.2 continued

Notes:-

a) During DAY 3 to DAY 14, box labelled 'A' refers to measurement of temperature, water flow and volume (1230).

b) Similarly, box labelled 'B' refers to sampling of tank overflows for TOA measurement, accompanied by pH measurement when carried out (1330).

c) Checks of dissolved oxygen were normally carried out during the morning period, after the 1000 feed.

beginning and end of the experiment (14-day data), which comprises weight, length and number of fish. The second source is the data gathered on each day of TOA measurement (1-day data), comprising TOA, temperature, flow, volume and food. The third source is any data due to mortalities; in each case, the number, weight, and length of the fish concerned, together with the day of death, were recorded.

7.45 The major omission from the 1-day data is the weight and length of the fish corresponding to each set of 1-day data. Since the only measurements of weight and length were taken as 14-day data, it was necessary to estimate intermediate values. Over the BMP period, an intermediate weight estimation process was evolved in preparation for the E-series.

Intermediate weight estimation

7.46 A simple case is considered first in order to clarify the method, and various problems are then accounted for. For a hypothetical tank with no mortality and exactly the same amount of food fed for each DAY, it could be reasonably assumed that growth in weight would be linear when measured on a daily basis over a short period such as 14 DAYs (Fig.7.2). Although in practice the proportions of food used for maintenance and growth will gradually increase and diminish respectively as the fish grow, this change in proportion is unlikely to be of great importance when considering the overall effect on a whole population in a tank over such a period. In order to estimate intermediate weights, it is necessary first to determine the overall conversion ratio (CR). This is given by:-

$$CR = \frac{F_t}{(W_{14} - W_o)}$$

- where F_t = total weight of food fed,
  W_o = initial total fish weight
  W_{14} = final total fish weight

Since food fed for DAY 1 is known (F_1) the weight increase for this DAY (W') is given by:-

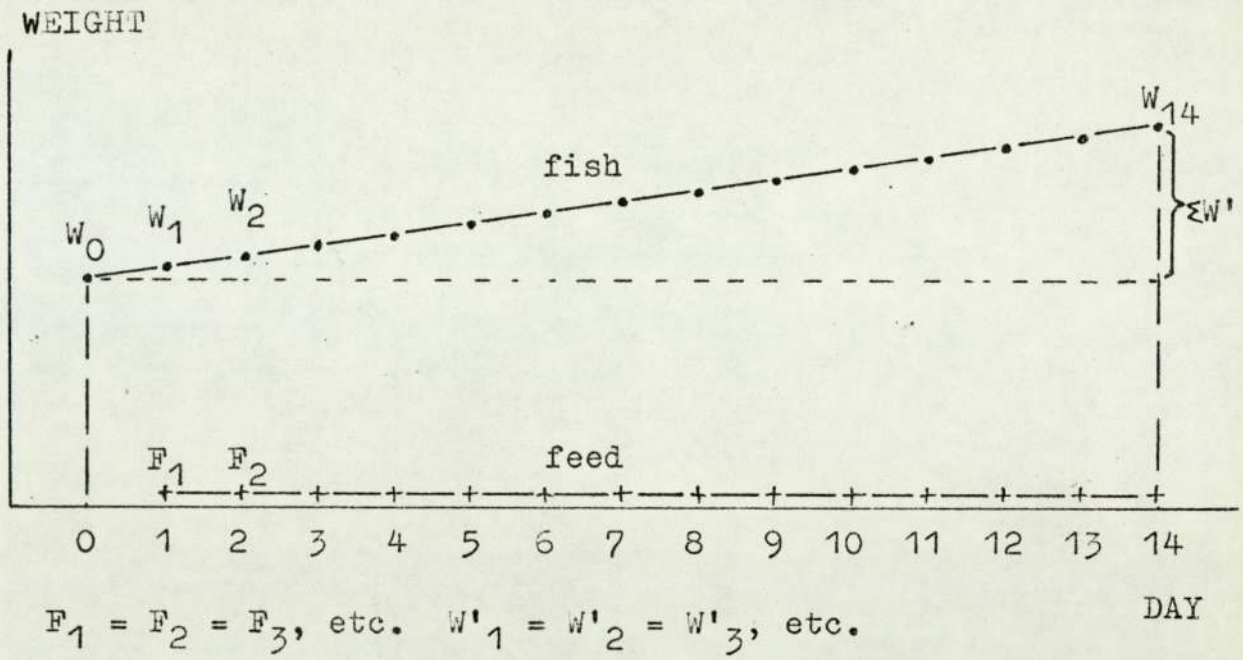


Figure 7.2 Intermediate fish weights (constant feed rate)

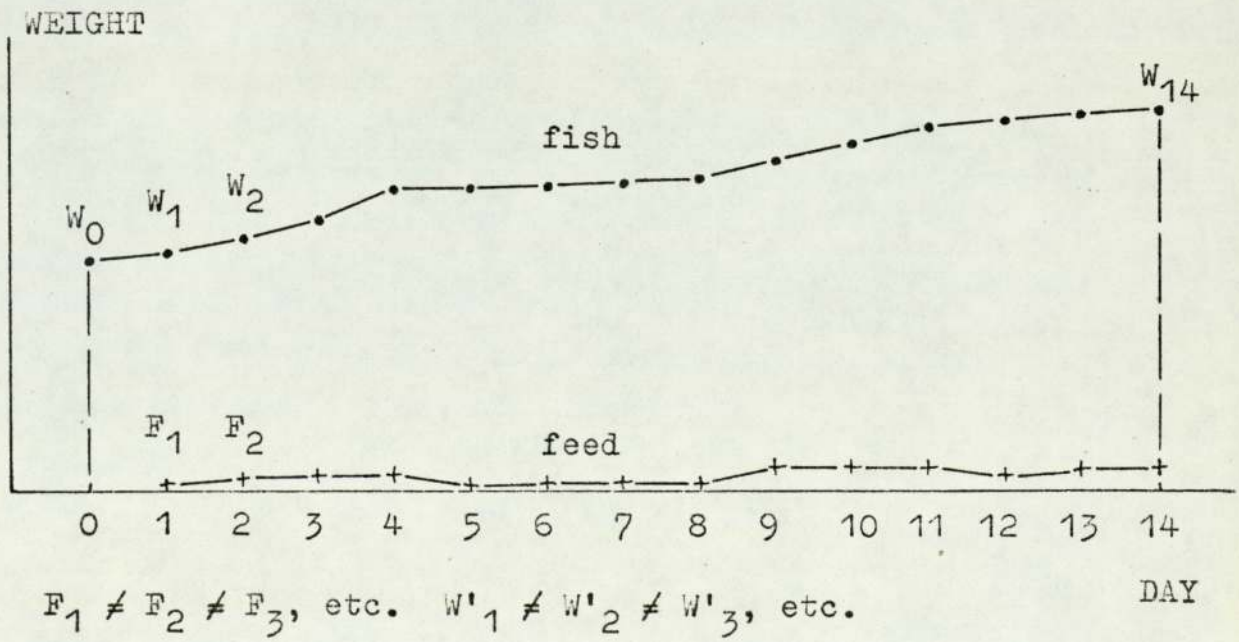


Figure 7.3 Intermediate fish weights (variable feed rate)

$$W' = \frac{F_1}{CR}$$

Hence the current total fish weight at the end of DAY 1 will be $(W_o + W') = W_1$. In the present case, W' remains constant for each day hence $W_2 = W_1 + W'$, $W_3 = W_2 + W'$, etc.

(Hence

$$W' = \frac{W_{14} - W_o}{14} \text{ and } W_{14} = W_o + \Sigma W'$$

- since there are 14 DAYs in the experiment)

7.47 If, as is usual, the food fed for each DAY is slightly different, it is assumed that growth in weight will vary in proportion (Fig.7.3). Estimation of each DAY's increase in weight must therefore be independently carried out, thus:-

$W'_1 = \frac{F_1}{CR}$, $W'_2 = \frac{F_2}{CR}$, etc.

Then:-

$W_1 = W_o + W'_1$, $W_2 = W_1 + W'_2$, $W_3 = W_2 + W'_3$, etc.

In the cases referred to so far, estimation of W_{14} by calculation will provide the same answer as the originally measured W_{14}. However, if any mortalities occurred in the tank, this would not be so, and a method was therefore required to account for mortalities.

7.48 To explain this a second hypothetical tank can be considered, in which one fish dies at DAY 7 (Fig 7.4). For simplicity, the food fed per DAY will be assumed constant, since the difference due to variability in this quantity has been explained above. Let the weight of the dead fish on removal be w. Then in order to estimate a conversion for DAY 1 to DAY 7, it is necessary to estimate what the value of W_{14} would have been had the death not occurred (W_F). The best estimate of W_F is the sum of the final mean fish weight and the actual final total weight, i.e.

$$W_F = W_{14} + \frac{W_{14}}{N_F}$$

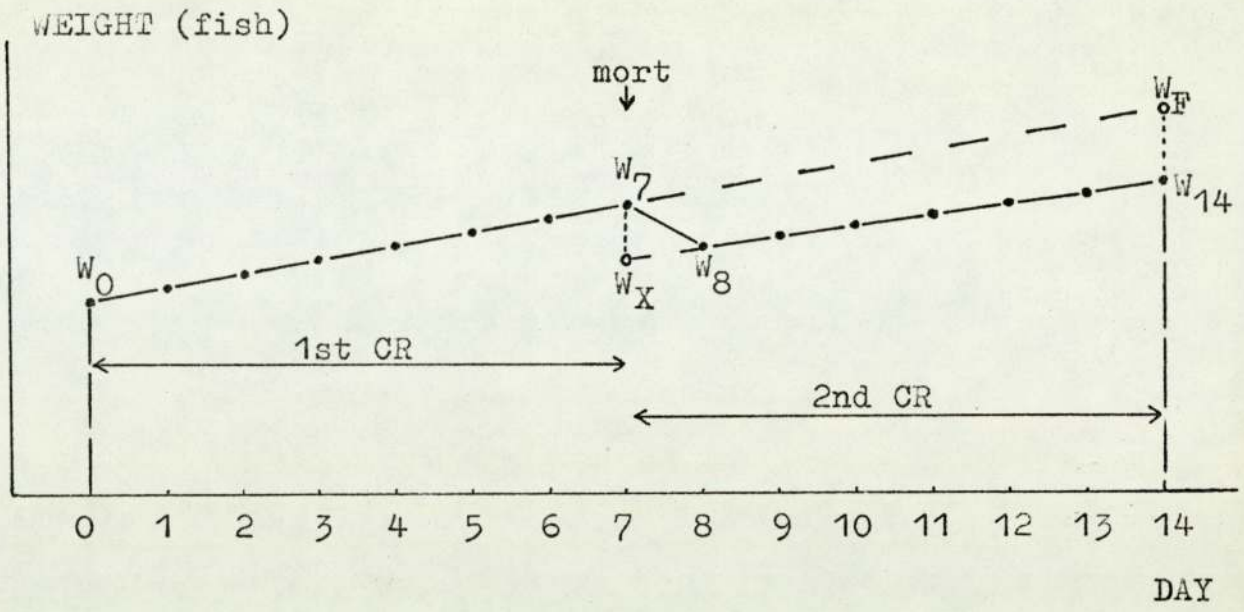


Figure 7.4 Intermediate fish weights (one mortality)

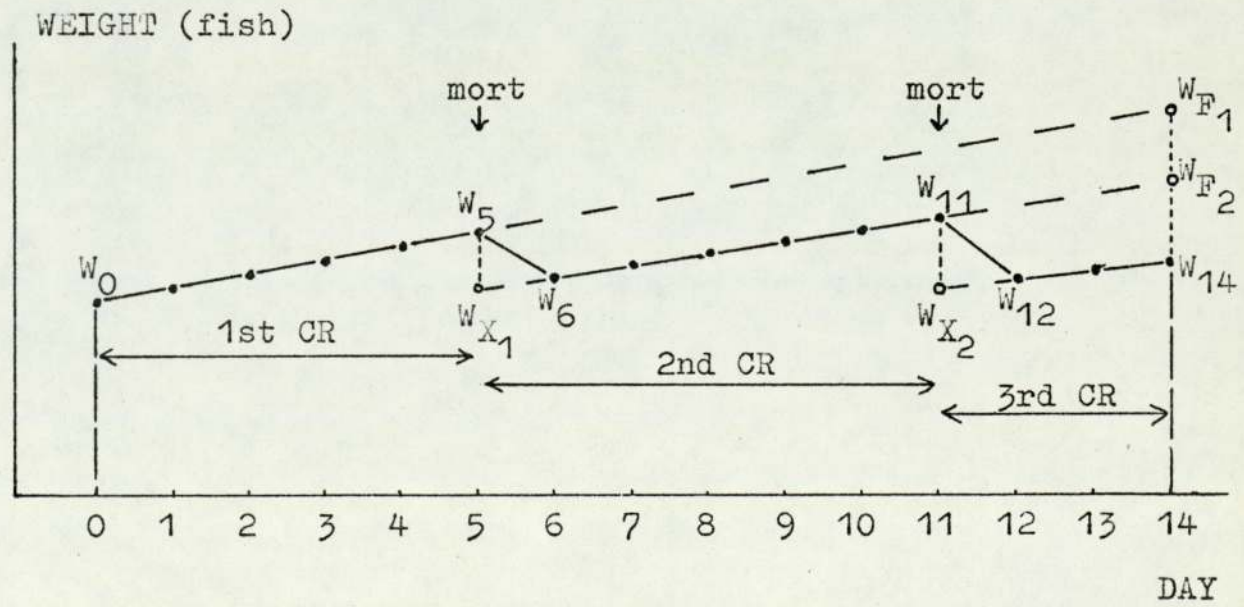


Figure 7.5 Intermediate fish weights (two mortalities)

- where N_F is the final number of fish in the tank. Using W_F in place of W_{14}, CR can be calculated and values for W_1 to W_7 calculated in the normal way. The mortality weight is then brought into use, to estimate a second CR covering the time from DAY 8 to DAY 14. In this case it is necessary to estimate W_X, the DAY 7 total weight less mortality weight, i.e.

$W_X = W_7 - w$; then

$$\text{second CR} = \frac{\Sigma(F_8 \ldots\ldots F_{14})}{(W_{14} - W_X)}$$

Using the second CR, values for W_8 to W_{14} can then be calculated.

7.49 When there is more than one instance of mortality, the process of para.7.48 is repeated each time, and more values of W_F and W_X are required (see Fig. 7.5). With each mortality accounted for, the errors involved (see below) have greater significance.

7.50 A process similar to that used for weight can be followed to estimate intermediate length data. The process corresponds to the weight process in dealing with "total" or cumulative length (i.e. sum of the lengths of all the fish in the tank), and involving a length conversion i.e. ratio of food fed to cumulative length increase. The mean fish length is then simply calculated by division of cumulative length by the number of fish in the tank at the time.

7.51 The processes used for intermediate weight and length estimation are liable to the following sources of error.

a) If feeding is incomplete, then the assumption that growth is directly proportional to feed is probably invalid, unless the same proportion of the food delivered is consumed throughout, which seems improbable. However, feeding was in general excellent, and this error is thus unlikely to be important. In cases where feeding was poor, the growth indication data (see below) would reflect the situation.

In the case of mortalities, several sources of error occur:-

b) The effect of sickening. A fish lost at a certain time (e.g. DAY 7 in Fig.7.3) would almost certainly have been sickening for some time previously, and hence not feeding. Thus the weight loss on its death would probably be underestimated by taking the weight of the dead fish (w). This would make W_X artifically high, affecting the subsequent CR and values of weight.

c) It is quite likely that the fish which die are not of average size, as is implied in the calculation of W_F. In the case of death by bullying, the victim would probably be one of the smallest in the tank (hence W_F would be overestimated); or in the case of death from crowding or oxygen stress, the victim would probably be above average size (hence W_F would be underestimated).

d) The spread of weights for large FCAT fish was greater than for small FCAT; thus errors in averaging (estimation of W_F) would be larger.

e) The first CR calculation involves use of F_t; this is the total food fed in practice. If no mortality had occurred, the fish would have consumed the same total amount but received less each; thus after mortality with more food to go round, growth is probably better. This consideration leads to underestimate of the first CR.

f) The proportions of consumed food which go to maintenance and growth (see para.7.46) may be extensively altered by a number of mortalities especially where the fish which die are particularly larger or smaller than the mean (see below).

7.52 Mortalities are notoriously difficult to deal with in any study involving fish growth (Brown 1957); one possible practice in nutrition work is to ignore the dead fish's effect from the beginning of the experiment. In the case of this study such an approach was not possible, since a fish lost at one point in the experiment could have been affecting its environment, and particularly TOA, quite substantially at an earlier point. So long as the number of fish involved was fairly high, the loss of one

fish would not introduce serious error e.g. for a batch of 20 fish of total initial weight 300 g loss of one fish, would probably have a maximum error of about 0.7%. This is below the accuracy of weighing techniques. However, with a mounting number of mortalities, the error in calculation of W_F becomes larger, especially in the calculation of the first (highest) value, which will depend on the proximity to mean weight of the dead fish. For 5 deaths out of 20 fish, with each one being larger than the final mean weight, the best estimate of W_F is

$$\frac{5W_{14}}{15} + W_{14}$$

$$= \left(\frac{W_{14}}{3}\right) + W_{14}$$

If, however, all 5 lost fish are 2 g heavier in final effect than the final average, then true W_F

$$= 5\left(\frac{W_{14}}{15} + 2\right) + W_{14}$$

$$= \frac{W_{14}}{3} + 10 + W_{14}$$

If the error of 10 g in W_F is related to a starting weight of 300 g, with 5 losses estimated at 15 g each, the error is over 4%; this still seems modest, but the calculation has so far been applied to starting weight only. In practice, the errors of individual occurrences of mortality affect each other, causing error in an increasing proportion over all estimates of weight for which W_F values have been employed. This kind of cumulative effect can lead, after several mortalities, to such a large discrepancy between theoretical and actual values of W_{14}, that the only theoretical solution is to assume no growth, or even loss in weight, after the last mortality. In the actual situation, such a case will be well signposted by the growth indication data after computation (see para.7.58); the loss in growth may well be true, but whether it is or not, such data are unreliable.

7.53 One possible way of avoiding such accumulation errors and non-average mortality effect, would be to have an individual

fish identification and ranking system, since fish often maintain their hierarchical (size) rank position. Unfortunately, in the case of these experiments, the time involved to weigh each fish individually would have been prohibitive, and also profoundly stressful. Stress would also have been involved in any individual marking system, especially with fish of smaller FCAT. The built-in danger signals of error in the process (growth indication data) were felt to be sufficiently good as a guide to selecting reliable data.

Calculated data

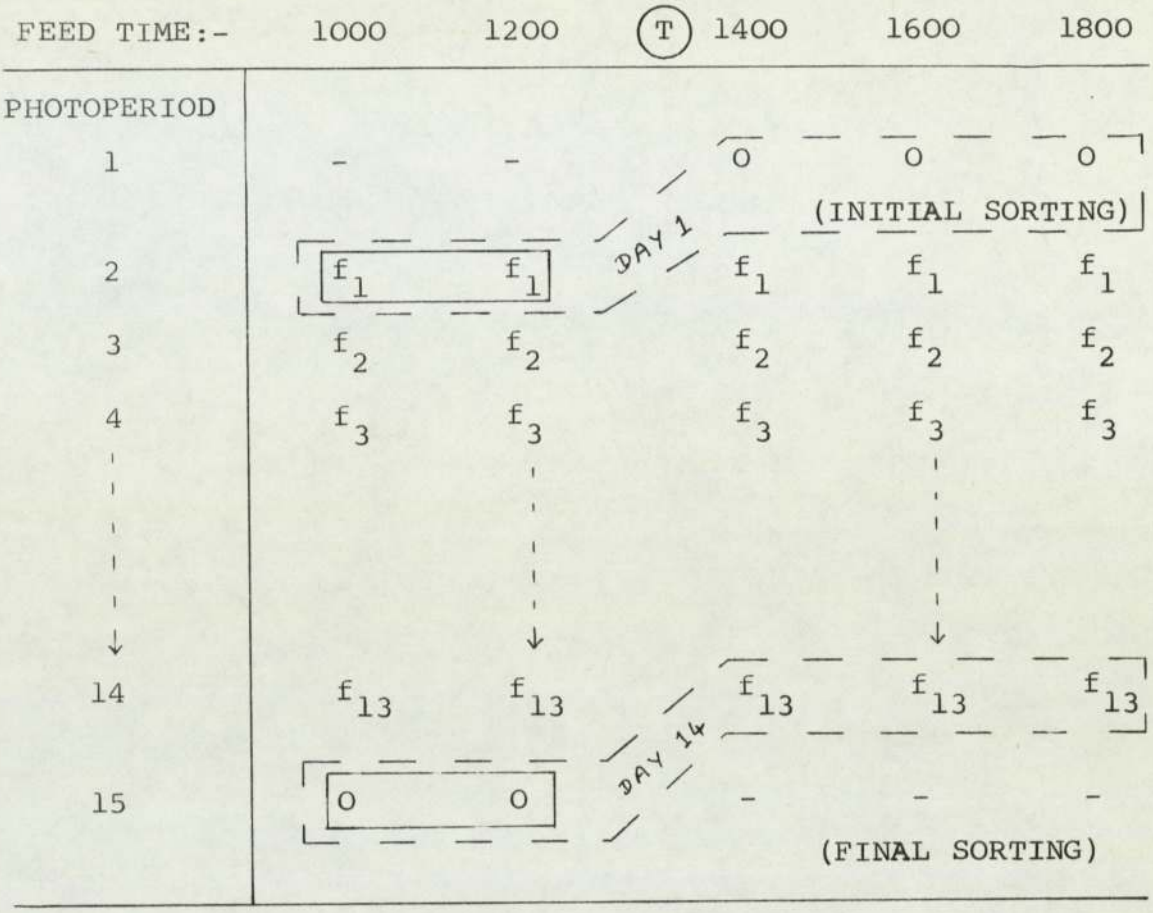
7.54 After the collection of basic data (14-day, 1-day and mortality sets), two stages of computation were involved to generate a second class of data, the "calculated data". Stage 1 was the estimation of intermediate weight and length, as above; stage 2 was calculation of SER, loading, stocking, density and food fed for each set of basic data supplied. Thus for any one tank on any one DAY:-

BASIC DATA:	CALCULATED DATA:
TOA	total weight
flow	mean length
volume	SER
temperature	loading
food fed	stocking
	density
	food fed

It will be noted that food occurs twice. This is because the collected (basic) food data required manipulation before it could be set up as corresponding to the other data.

7.55 Manipulation of food data was due to the system of measurement, which was conveniently performed between 0900 and 1000 (see Table 7.2). Thus the actual measurement was of food fed over 5 feeds during the course of a natural day (photoperiod), and not during an experimental DAY. To calculate the food fed per DAY, it was necessary to divide each food measurement by 5 and group the individual feed amounts as shown in Figure 7.6.

Figure 7.6 Grouping of food data



Notes:-

a) Food calibration / measurement is at beginning of photoperiod (e.g. for photoperiod 2, 5 x f_1).

b) For DAY period, total food fed (F) = last three feeds of one photoperiod + first two of next photoperiod
e.g. $F_1 = 0 + 0 + 0 + f_1 + f_1$, $F_2 = f_1 + f_1 + f_1 + f_2 + f_2$ (dotted box).

c) For DAY period, last 2 feeds (G) = first two of photoperiod
e.g. $G_1 = f_1 + f_1$, $G_2 = f_2 + f_2$, $G_{14} = 0 + 0$ (solid-line box).

d) (T) indicates time of TOA measurement.

For later treatment of data, two quantities were calculated; the food fed in the previous 24 h before a TOA measurement (i.e. food fed per DAY), designated as F; and the food fed over the last two feeds (the morning feeds at 1000 and 1200) prior to TOA measurement, designated as G. Thus G = 2F/5.

7.56 In summary, the following calculations were performed on each basic data set:-

a) estimation of total weight (W) and mean length (L);
b) manipulation of food data to give F and G;
c) loading = W/flow;
d) stocking = W/volume;
e) SER = TOA/loading;
f) density = number/volume

(Each calculation embodied suitable conversion factors in order to attain appropriate units.)

7.57 All basic and calculated data are computer-listed in Appendix A3. Data from P01 to P04, and E00, are included for comparison, although some values are unreliable estimates only.

Data selection

7.58 Before further treatment, it was necessary to screen data to remove unreliable sets. All sets were reviewed, and rejected if they were unsatisfactory in terms of any of the following criteria:-

a) More than 2 mortalities (para.7.52).
b) Fall in condition factor (sign of poor growth).
c) Weight conversion ratio which is negative or in excess of 3.0; these indicate loss of weight or poor feeding.
d) Lack of, or negative, growth in mean weight or length.
e) Errors in sorting (e.g. miscalculation of fish number).
f) Irregular setting-up conditions (e.g. as in E03, MT1; see Table 7.1); this includes pilot experiments and E00.
g) Errors in sampling or measuring 1-day data.

Criteria (a), (b), (c) and (d) comprise the "growth indication data", and were referred to after computation (to produce

"calculated data") and before subsequent data analysis, in order to assist in data selection.

7.59 All screened data, acceptable by criteria (a) to (g), were subjected to further analysis, and are computer-listed in Appendix A4.

Multiple regression analysis

7.60 It is frequently found that biological data can be related, either in raw or in logarithmic form, by equations which describe a straight line when plotted on a graph; thus in standard nomenclature:-

$$Y = a + bX \quad \text{or} \quad \log Y = \log a + b(\log X)$$

The latter can equally be expressed as:-

$$Y + aX^b$$

(this relationship in raw form may be a curve, but is reduced to a straight line in logarithmic form). A common example is the relationship between length and weight for salmonid fish where:-

$$\text{weight} = K \times \text{length}^3$$ (Brown 1957, Bowen & Studdard 1970)

(However, see Ricker (1973) for criticism of usually-quoted values for this exponent.) This relationship forms the basis of the condition factor (K) referred to earlier.

7.61 The above refers to a dependent variable (Y) related to only one independent variable (X). Where more than one independent variable is involved (X_1, X_2, X_3, etc.), the resulting graph must be "plotted" in n-dimensional space where n = number of independent variables. Where $n > 3$, this becomes difficult to comprehend, and impossible to display in a simple visual way.

7.62 The purpose of this study, as explained in Chapter 5, involved a number of X quantities, and their effect on a particular Y quantity identified as SER. For further analysis these X quantities were transformed to give an overall linear relationship. Expressing relationships in an n-dimensional straight-line form allows relatively easy comparison of different relationships, and selection of the one which gives the best-fitting

model, in order to predict SER in terms of the most appropriate X quantities; the technique is that of multiple regression analysis.

7.63 In the case of simple regression (one X quantity), prediction of Y from X values is usually governed by the regression of Y on X (this procedure has been criticised for fish work by Ricker (1973)); this is one of the two best straight lines drawn through the plotted data, calculated in well-known fashion by minimising the sum of squares of deviations from the line in the Y-direction (e.g. as in Bishop 1966), thus for n pairs of data:-

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

$$\Sigma d_y^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$$

$$\Sigma d_x d_y = \Sigma xy - \frac{(\Sigma x . \Sigma y)}{n};$$

then

$$\text{regression coefficient, } b = \frac{\Sigma d_x d_y}{\Sigma d_x^2}$$

and

$$r = \frac{\Sigma d_x d_y}{(\Sigma d_x^2 . \Sigma d_y^2)}$$

Now $\bar{x} = \frac{\Sigma x}{n}$ and $\bar{y} = \frac{\Sigma y}{n}$

and the regression equation is

$$y = \bar{y} + b\,(x - \bar{x})$$

Thus having calculated b, $\bar{x}$ and $\bar{y}$, a prediction of y can be made for any given value of x. Substitution of values for b, $\bar{x}$ and $\bar{y}$ allows calculation of the intercept, a, such that $y = bx + a$. When this line is plotted, the slope of the line is b. The quantity r derived above is the correlation coefficient, and expresses the degree of closeness of relationship between X and Y. This quantity can be tested for statistical probability of significance according to the magnitude of n.

7.64 In the case of multiple regression, each X quantity

nominated can be tested to derive similar information with respect to Y; the overall equation for prediction will be (for n X-quantities):-

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \ldots\ldots + b_nx_n$$

and the overall relationship will have a multiple correlation coefficient, R, corresponding in nature to r for simple regression, but of more complex derivation. Thus the procedure of multiple regression analysis involves:-

a) nomination of X quantities and Y,

b) calculation of R,

c) test of significance of R,

d) calculation of a and b_1, b_2, b_3..... b_n to define the equation,

e) test of significance of b_1, etc. by t-test ($t = \frac{b}{SE_b}$)

7.65 Now R can be thought of as a measure of the amount of Y variability explained, or accounted for, by the nominated X quantities. Since R can only take values between 0 and 1, these values can be very loosely translated into % of Y variability explained by the X quantities; thus if R = 0.75, the X quantities can be said, for intuitive comprehension, to explain approximately 75% of the Y variability. (Strictly, R^2 is the proportion of the squared deviations accounted for.) The remaining variability is expressed in a quantity known as the error sum of squares, which allows the setting of limits of accuracy to the multiple regression equation.

7.66 Examples of multiple regression used for fish physiology work can be found in Allen (1974) and Beamish (1974), and their examples illustrate a further complication in multiple regression. Whilst in simple regression the Y quantity can only be affected by X or some power of X, with increasing numbers of X quantities more possibilities occur. Y may be related to:

a) X_1, X_2, X_3, etc.,

b) powers of X (e.g. X_1^2, X_2^2 ; X_1^3, etc.),

c) interactions between X quantities or their powers; e.g.

X_1X_2, X_1X_3, X_2X_3 etc.,

$X_1^2X_2$, $X_2^2X_3$, $X_1^3X_2$, $X_1^2X_2^3$ etc.

Clearly, the more X quantities there are, the more of these possibilities there are, by geometrical progression. In order to deal with such a large range of possibilities, especially when working with a large number of data, it is necessary to use a digital computer.

7.67 Although a large range of possible effectors could thus be tested, it is important to note that many of the quantities generated by interactions as mentioned above are probably meaningless in any environmental sense, e.g. the cube of the temperature multiplied by the square of the number of fish is a valid interaction but somewhat difficult to appreciate or to relate to the situation in the tank. Thus, selection of meaningful quantities was equally as important as identifying significant ones.

7.68 As previously explained, Y for this analysis was SER. The nomination of X quantities was rather complex and several models of analysis were tested, in accordance with various hypotheses of effect. Two overall models (OM) were envisaged.

7.69 OM1 was characterised by deliberate avoidance of consideration of the number of fish in the tank, and hence the density (as earlier defined). Thus a basic question was posed: could the environmental effect on fish in the tank be independent of the actual number or density, and therefore was it capable of description by quantities related to purely chemical or physical effects? This model was as fundamentally mechanistic as possible, given that the organism was occupying a "black box" role (see Chapter 5). In this case the fish population in the tank was likened to a single ammonia-producing machine rather than a collection of them, dependent on variables apprehended by the population collectively rather than individually, and independ-

ent of differences between the fish in any one tank at a particular time. A property of this model would be that quantity of fish was adequately expressed by the weight, so that 50 fish of 2 g each would be equivalent to 2 fish of 50 g each.

7.70 OM2 took the number and density of fish into account, and thus considered a less mechanistic view of the situation.

7.71 Within the overall model, various combinations of possible X quantities could be tested, and analysed, by the use of a suitable package of computer routines. The following combinations were tested under OM1:-

a) temperature, loading, stocking, food fed, length; and squares of these quantities, and first-order interactions i.e. simple multiples (e.g. temperature x length);

b) logarithmic transformations of temperature, loading, stocking, food fed, and length.

Under OM2, the first combination tested was of the simplest quantities measured, i.e. temperature, number, flow, volume, length and food fed; then there was consideration of the calculated quantities such as loading, stocking and density; and subsequently consideration was given to more complex possible effector quantities such as mean free path (see Chapter 5).

7.72 Promising models for explanation of SER were computer-graphed to enable individual quantities to be examined in the context of the group. The first step was to identify in multiple regression analysis those quantities which were statistically significant at the level of $P = 0.05$, and then to calculate partially-corrected values of the dependent and independent variables (see Appendix C).

7.73 If, for example, there were three independent variables, three partially-corrected relationships would be derived; each one could be plotted for a different independent variable. The series of three graphs which such a process would generate is equivalent to a plot of the observations and the regression

equation in 3-D space (or, for n graphs of n quantities of X, in n-dimensional space). Each individual graph is equivalent to an observation of this space from a side corresponding to the particular independent variable, and looking in such a direction that the regression plane appears as a straight line (Aston 1972). Fig.7.7 shows the process involved in partial correction by using a simple model with two independent variables, which is appreciable on paper.

7.74 The graphs are useful for:
a) eye-examination of the closeness of fit to a straight line;
b) picking out of points which are well away from the line, allowing investigation of their particular circumstances;
c) checking whether points are relatively evenly distributed along the axes, and hence suggesting where further measurements may be taken.

COMPUTER PROCEDURES

Introduction

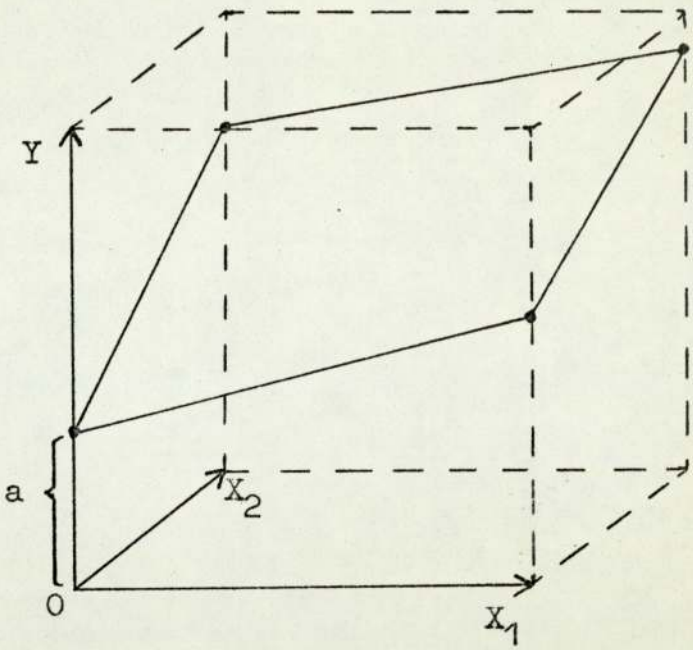
7.75 Handling large data arrays and subjecting them to complex analyses, with the limitations on time available, was a process demanding the use of digital computer time. All work was performed on the University of Aston ICL 1905E (later 1904S) machine using FØRTRAN programming language. A substantial amount of programming was required in early stages, but for multiple regression (analysis and presentation) standard software packages were employed.

7.76 At all times special code names were used for the various quantities dealt with. Those of major importance were required to have 6-character names (for the regression analysis package layout), and a list of these names is given below:-

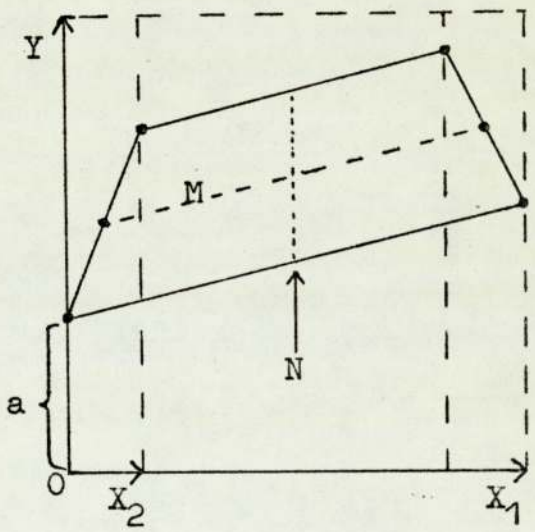
TØTAMM : TOA (concentration)
ENUMBR : number of fish
FLØWRT : rate of water flow through tank

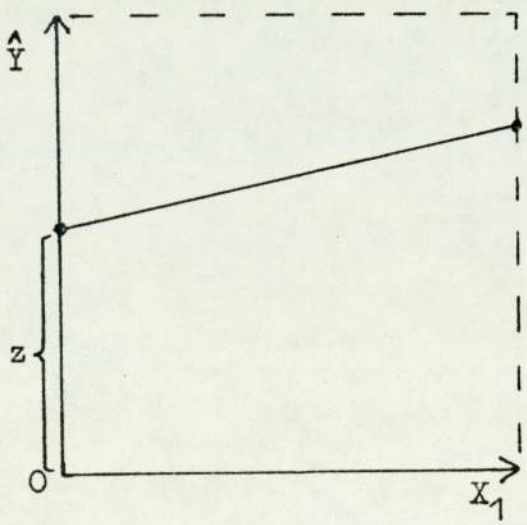
A) 3-D view of a regression plane given by:

$Y = a + b_1X_1 + b_2X_2$



B) Frontal view of (A) with depth perspective retained





C) Frontal view shifted to N in (B) and advanced to M;

$z = a + b_2t_2$

$\hat{Y}$ is partially corrected for X_1

(see Appendix C)

Figure 7.7 Partial correction procedure for Y

VØLUME : water volume in tank

WEIGHT : total mass of fish in tank

SIZELN : cumulative length of all fish in tank (see AVEENU)

AVERLN : mean length of fish in tank

SPEXRT : SER // EXRATE : water exchange rate:flow/volume

TEMPRT : temperature of tank water

DENSTY : number of fish per unit volume of water (density)

DNLØAD : mass of fish per unit flow of water (loading)

DNSTØK : mass of fish per unit volume of water (stocking)

FACTØR : loading per unit length of fish

GRUBFD : food fed in last 2 feeds of experimental DAY (G in para.7.55)

FDMEAL : total food fed in experimental DAY (F in para.7.55)

DNFACR : a loading term relating length to weight via the equation $W = KL^3$ (see para.7.60); quantity should be equivalent in effect to FACTØR above, and is given by: $\frac{\text{weight}^{2/3}}{\text{flow rate}}$ or $\frac{\sqrt[3]{\text{weight}^2}}{\text{flow rate}}$

FREPAT : mean freepath; a term relating the volume and the number of fish thus: $\frac{\text{volume}^{1/3}}{\text{number}}$ or $\frac{\sqrt[3]{\text{volume}}}{\text{number}}$

This is a measure of the theoretical average distance of separation between fish if they are randomly distributed through the tank.

STØLIN : stocking/loading interaction factor; $\frac{\text{weight}}{\text{flow x volume}}$

FDGØVN : feed-governing factor i.e. the quantity which determines the food fed: $\frac{\text{temperature x number}}{\text{mean length}}$

AVEENU : multiple of AVERLN and ENUMBR; same as, and used as alternative to, SIZELN; cumulative length

STKFAC : stocking per unit length of fish

DNSTAC : a stocking term related to STKFAC as DNFACR is to FACTØR (relating length to weight); it is given by: $\frac{\text{weight}^{2/3}}{\text{volume}}$ or $\frac{\sqrt[3]{\text{weight}^2}}{\text{volume}}$

7.77 The importance of these quantities is discussed in the RESULTS and DISCUSSION sections.

Calculation of UIA

7.78 The simplest calculation that was commonly required was the determination of UIA from measured data for TOA, pH and temperature. The relation is as follows:-

$$UIA = \frac{TOA}{1.0 + \text{antilog}\ (pK_a - pH)}$$

where

$$pK_a = \frac{(2835.76)}{T} - 0.6322 + (0.001225 \times T)$$

The latter equation for pK_a determination derives from, and uses, constants given by Robinson and Stokes (1969); T is the temperature on the Kelvin (Absolute) scale.

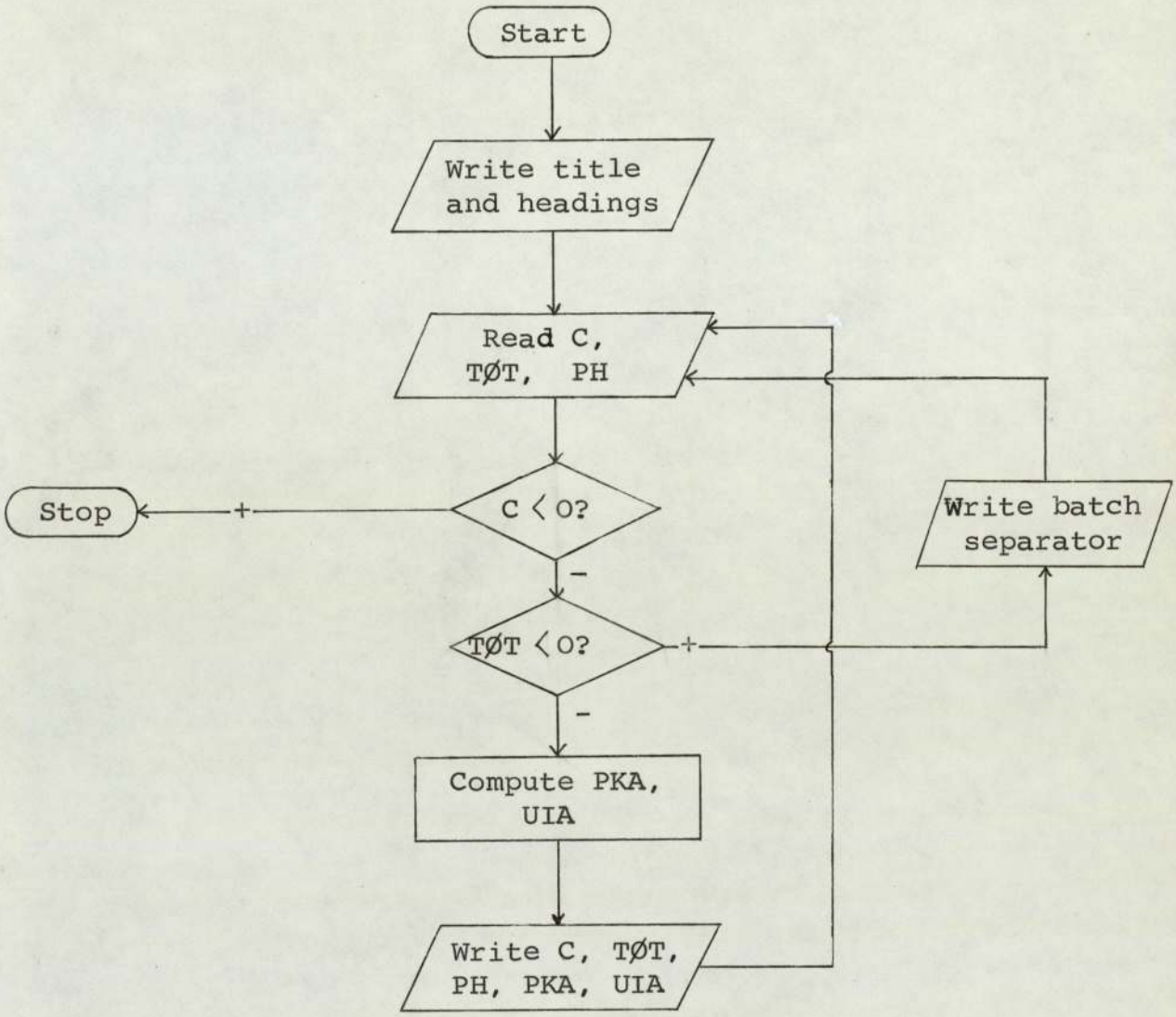
7.79 A simple program codenamed UIACALC was constructed to perform this calculation repeatedly. UIACALC is described by the flowchart of Figure 7.8 and was run under the SØFØR batch system for small FØRTRAN programs.

Calculation of intermediate weight and length data

7.80 As indicated in para.7.45, an estimate of intermediate weights and lengths was necessary to set alongside other 1-day data. To perform this estimation, program GRØWCALC was evolved over a series of trials, using 14-day data and 1-day food data to estimate conversion ratios and intermediate values as described earlier. Data was read in in separate complete matrices and was later accessed by a matrix indexing system as required.

7.81 The basic pattern of the simplest version of the program is given in Figure 7.9. However, in order to contend with mortalities, lack of data for particular tanks or days within the indexing system, or other problems, a complex program was required. The flowchart (Fig.7.10) is similarly complex, and has been reduced to a convenient pattern dispensing with the normal conventions used in Fig.7.8. However, apart from convenient changes in order of logic to allow easier handling of quantities, the flowchart covers the same procedures as are described in para.7.46 to 7.48 and Fig.7.2 to 7.5.

Figure 7.8 Flowchart for program UIACALC

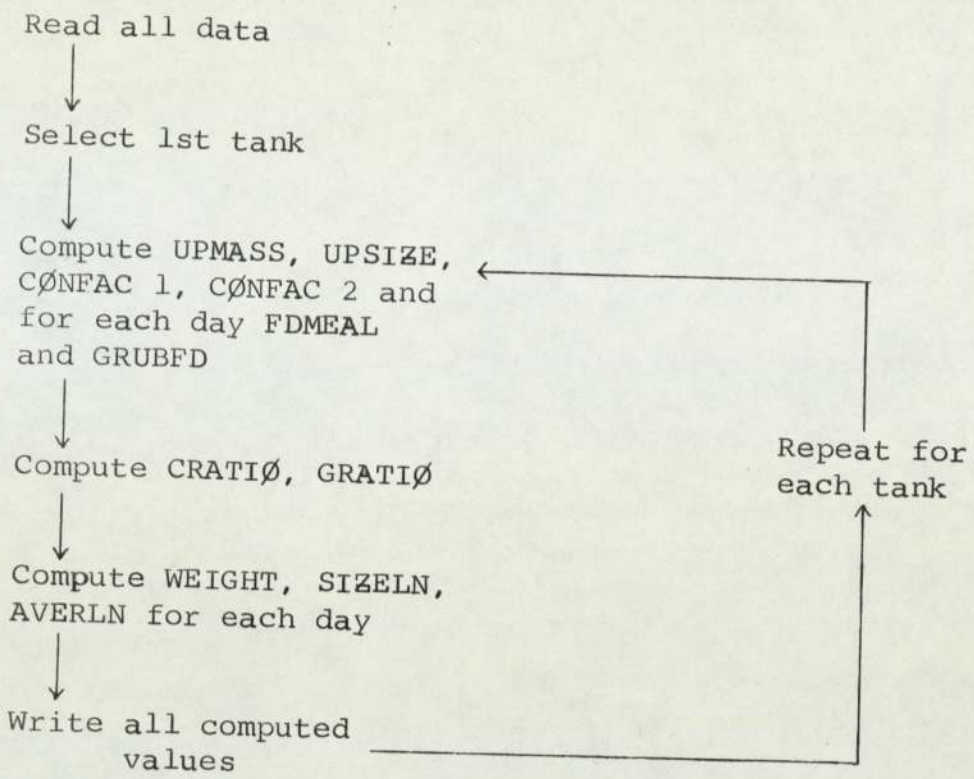


+	=	positive option	-	=	negative option
C	=	temperature	TØT	=	TOA
PKA	=	pK_a	PH	=	pH

Batch terminator dummy has -ve TØT

Data terminator dummy has -ve C

Figure 7.9 Basic pattern for GRØWCALC



UPMASS = $W_{14} - W_o$ (weight gain)

UPSIZE = cumulative-length gain

CØNFAC 1 = initial condition factor

CØNFAC 2 = final condition factor

CRATIØ = weight conversion ratio

GRATIØ = length conversion ratio

Figure 7.10 Flowchart for program GRØWCALC

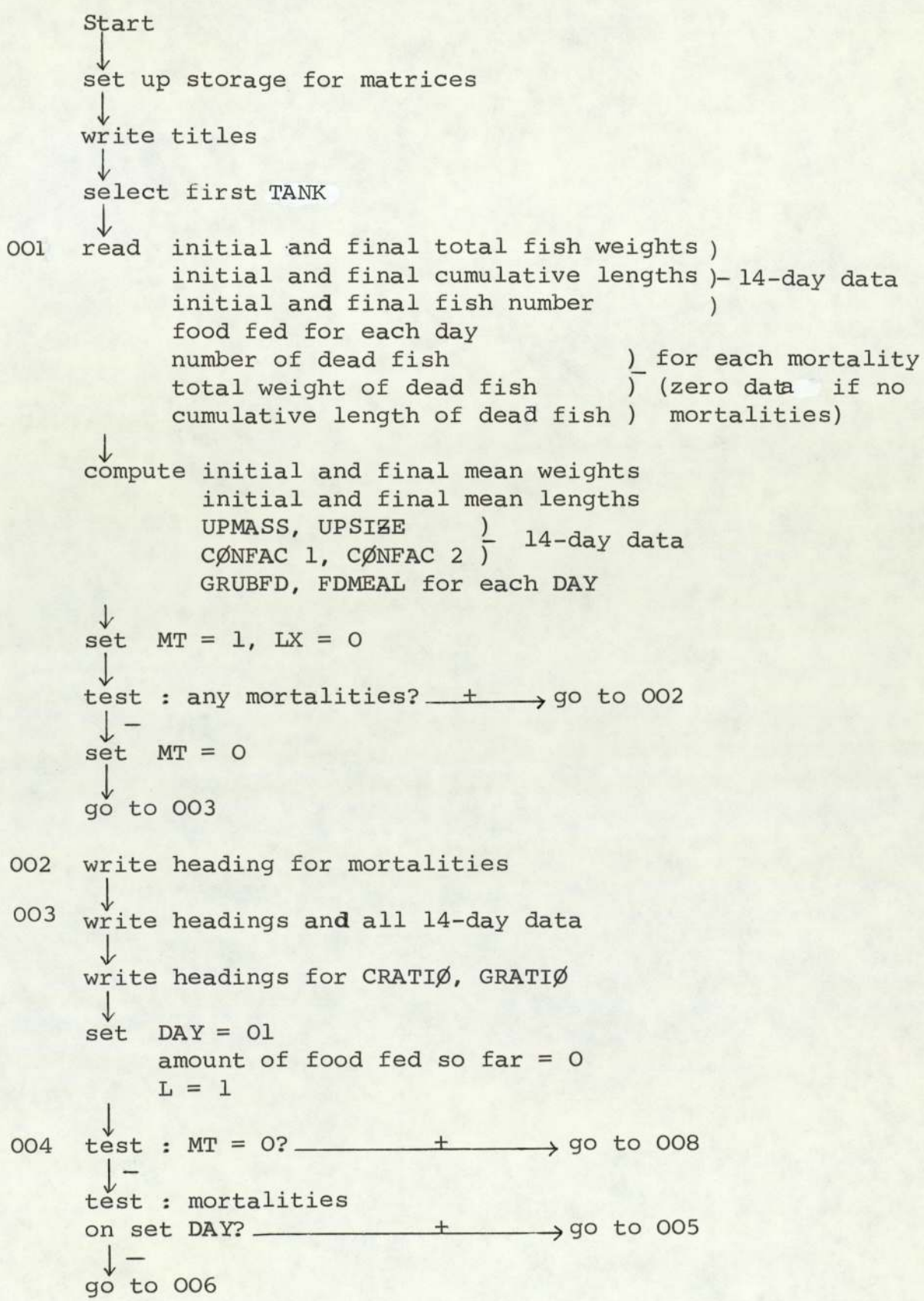


Fig. 7.10 continued

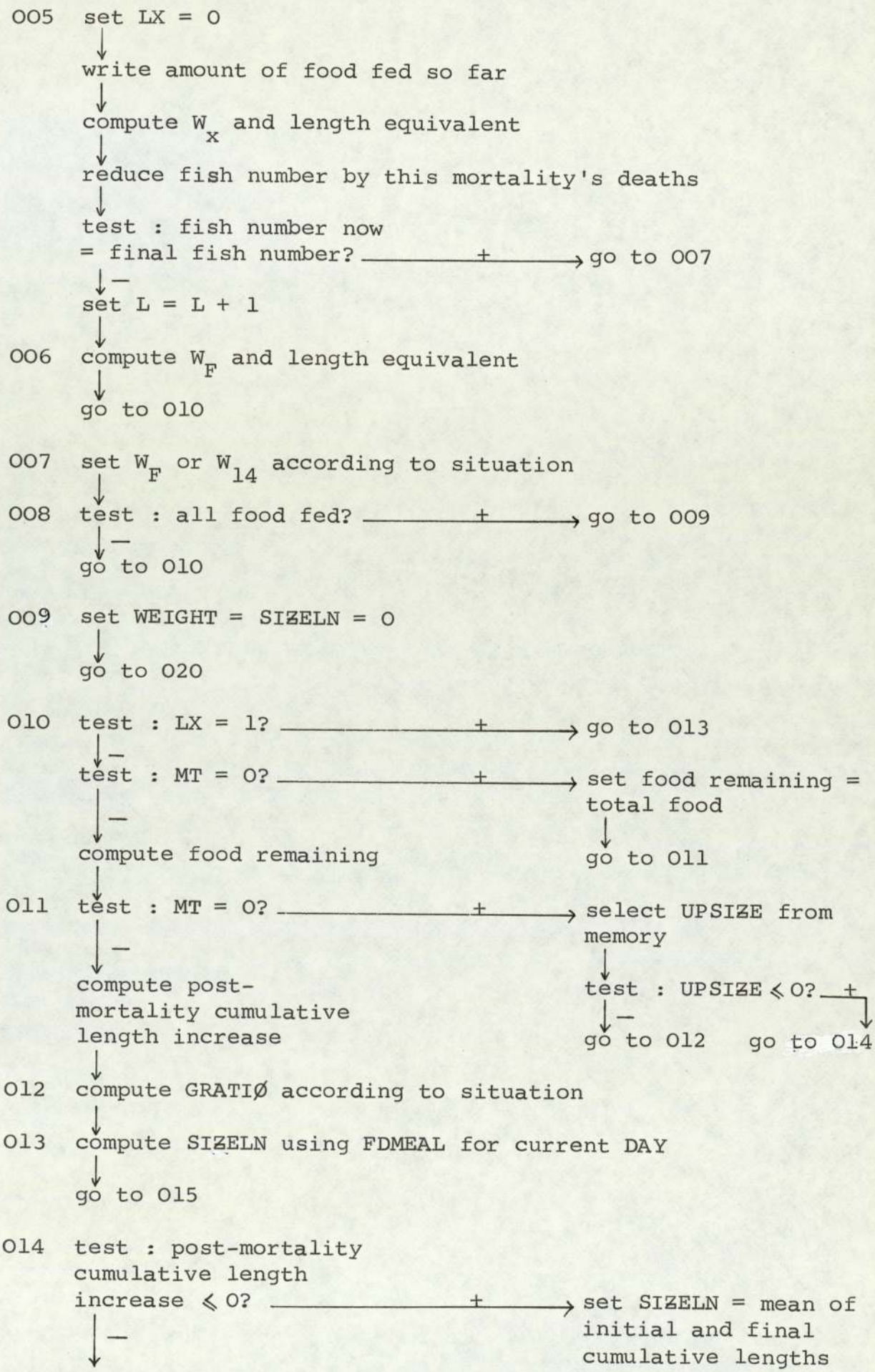


Fig. 7.10 continued

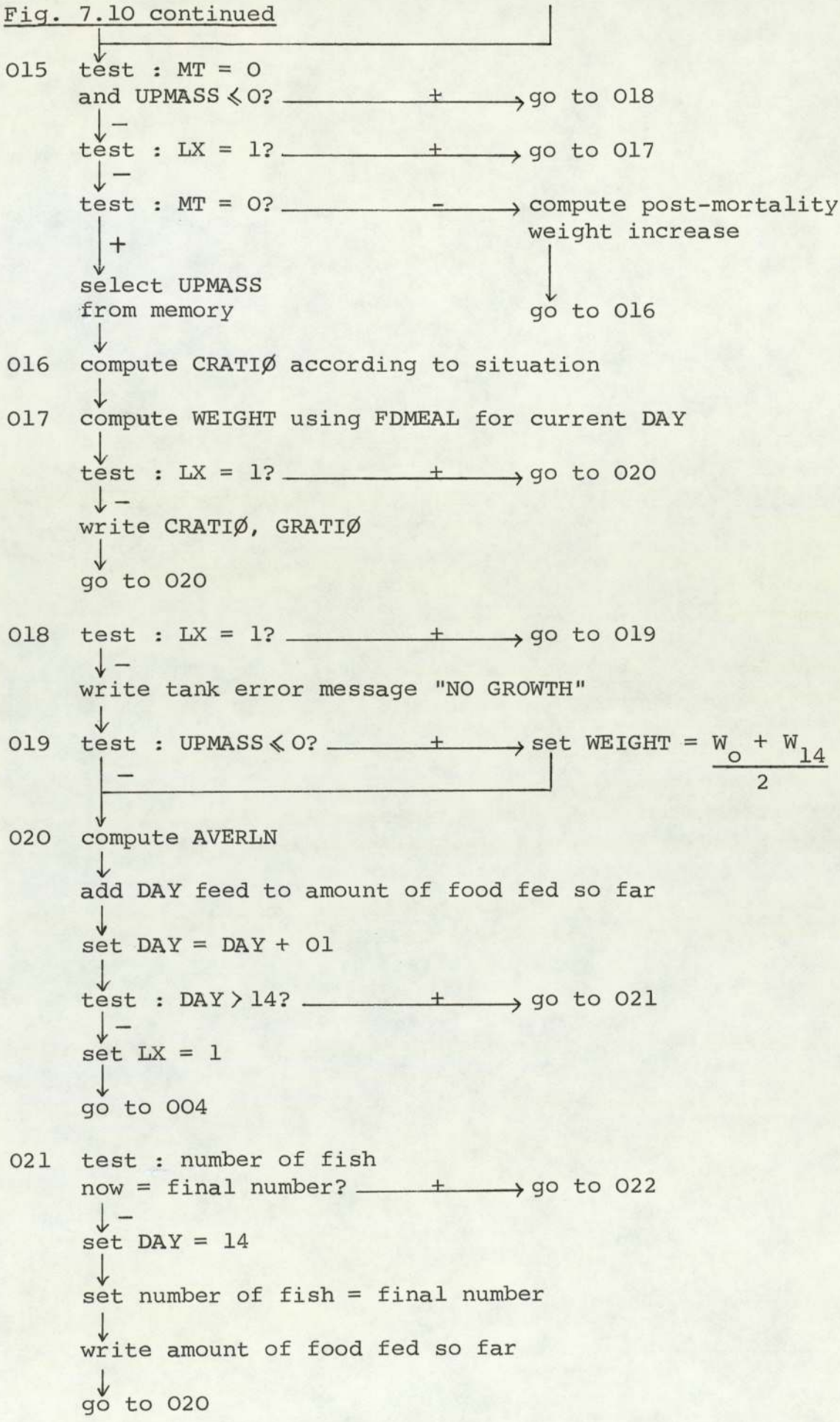


Fig. 7.10 continued

```
022  write headings for 1-day data
       |
       v
     write WEIGHT, SIZELN, AVERLN, GRUBFD, FDMEAL for each DAY
       |
       v
023  set TANK = TANK + 1
       |
       v
     test : TANK ⩽ 8? ----------+----------> go to 001
       |  -
       v
     stop
```

Notes:-

a) UPMASS, UPSIZE, CØNFAC 1 and 2, CRATIØ and GRATIØ are as defined in Fig. 7.9.

b) W_o, W_{14}, W_x and W_F are as shown in Fig.7.2 to 7.5 and described in para.7.46 to 7.48.

c) MT is mortality code. A distinction is drawn between a death (involving one fish) and a mortality (meaning an occurence of death involving one or more fish).

MT = 0 indicates no mortality:

MT = 1 indicates presence of one or more mortalities

d) LX is a repeater code for periods in between mortalities:

LX = 1 indicates that current CRATIØ and GRATIØ can be used for the next calculation of SIZELN and WEIGHT (skips irrelevant sections of program);

LX = 0 indicates that recalculation of CRATIØ and GRATIØ is required (at the beginning, or after a mortality) for the current tank.

e) L quantifies mortality; it is set to zero at the start, and is incremented by 1 for each mortality (not necessarily each death) for the current tank.

f) "Amount of food fed so far" refers to the total so far consumed at any particular point under consideration; at the end of consideration for one tank this quantity will have accumulated to the complete total fed (F_t).

g) As the program repeats itself for different DAYs and different tanks, quantities which have been calculated frequently need to be reset to zero or initial values, or updated to current values, to enable them to be re-used. For such operations the expression "set" is given in the flowchart, whereas straightforward calculations are expressed by "compute".

7.82 A sample of GRØWCALC output is shown in Fig.7.11. The growth indication data described in para.7.58 can be readily identified, and the required data for WEIGHT, SIZELN and AVERLN can be matched to any specified DAY.

Calculation of derived quantities

7.83 For the sake of pre-analysis information, a simple program entitled DERIVDATA was formulated to calculate values of SPEXRT, DNSTØK, DNLØAD and FACTØR from input values of 1-day data. The flowchart is given in Fig.7.12.

7.84 The programs GRØWCALC and DERIVDATA were later amalgamated into an overall program for pre-analysis treatment of E-series experiments, code-named ENVIRØDATA. Output from this program could be transferred to computer file storage when coded and stored in strings. The accumulated total storage of these coded data strings made up the content of the basic and calculated data matrices which form Appendix A3.

Multiple regression analysis

7.85 The data of Appendix A3 were stored, collected and edited on computer files to produce the single matrix of screened data which satisfied the criteria of para.7.58; this is given in Appendix A4 and was produced in correct format for the analysis requirements.

7.86 Multiple regression analysis, using the Appendix A4 data to supply quantities for independent variables with SPEXRT as the dependent variable, was carried out using the standard ICL Statistical Package (XDS3) software. Output includes the results of the analysis with multiple correlation coefficient, regression coefficients, statistical probability data, and limits and intercept of the regression equation. The same data could be reworked as many times as required, with specification of different independent variables.

7.87 Any individual multiple regression analysis carried out by the Statistical Package could be further scrutinised by means of a presentation program (see Appendix C).

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TANK	INITIAL WEIGHT (G)	FINAL WEIGHT (G)	UPMASS (G)	INITIAL SIZELN (CM)	FINAL SIZELN (CM)	UPSIZE (CM)	TOTAL FOOD FED (G)
5	530,0	628,0	98,0	346,5	354,5	8,0	95,0

XX
XX

INITIAL FISH NUMBER	FINAL FISH NUMBER	CONFAC1	CONFAC2	CRATIO	GRATIO
30,	30,	1,147	1,269	0,9694	11,8750

XX
XX

TANK	DAY	FDMEAL (G)	WEIGHT (G)	SIZELN (CM)	AVERLN (CM)	GRUBFD (G)
5	1	3,36	533,47	346,78	11,559	3,36
	2	7,72	541,43	347,43	11,581	2,68
	3	6,62	548,66	347,99	11,600	2,60
	4	7,34	555,83	348,61	11,620	3,44
	5	8,56	564,66	349,33	11,644	3,40
	6	8,02	572,93	350,00	11,667	2,92
	7	7,18	580,34	350,61	11,687	2,80
	8	5,66	586,18	351,09	11,703	1,46
	9	8,44	594,89	351,80	11,727	2,60
	10	7,06	602,17	352,39	11,746	3,16
	11	7,38	609,78	353,01	11,767	2,64
	12	6,56	616,55	353,57	11,786	2,60
	13	6,78	623,54	354,14	11,805	2,88
	14	4,32	628,00	354,50	11,817	0,00

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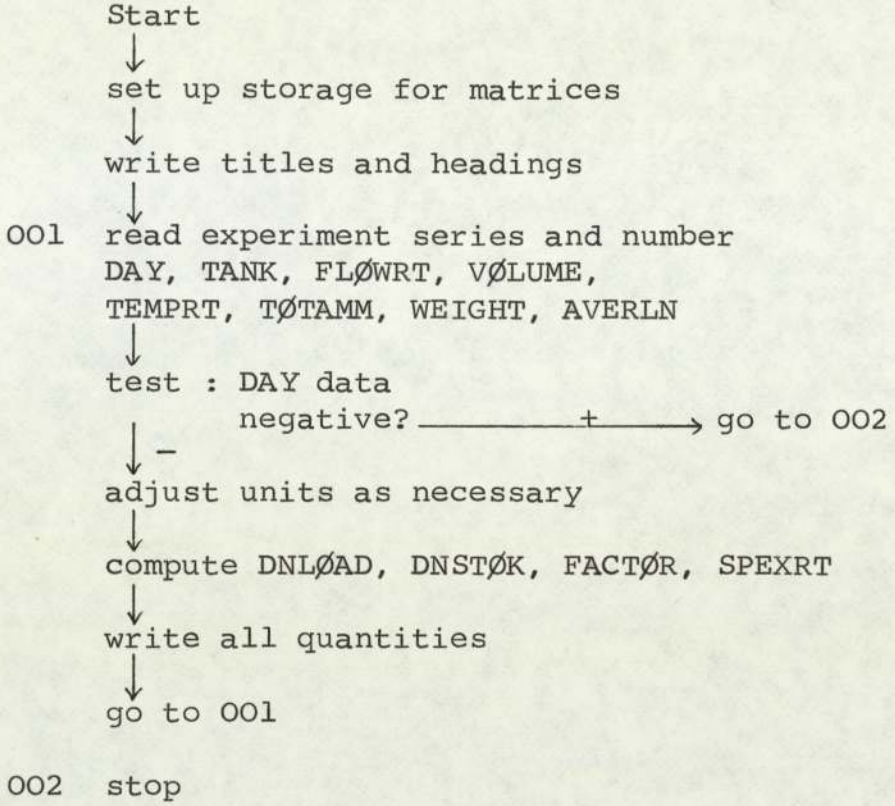
Figure 7.11 Sample of GRØWCALC output

Figure 7.12 Flowchart for program DERIVDATA



(Data terminator has negative value for DAY)

7.88 Basic and calculated data strings are given in total in Appendix A3. The screened data used for analysis are given in Appendix A4.

7.89 Table 7.3 lists the mean, minimum, maximum and standard deviation values of the quantities which formed the analysis observation matrix. Similar values are given in Table 7.4 for other quantities considered during analysis.

Individual correlations

7.90 During multiple regression analysis, the correlations between SPEXRT and the other individual quantities were produced as by-products. This enables one to gauge the efficiency of individual quantities as SPEXRT predictors under the conditions of these experiments (as well as enabling detection of high correlations between individual independent variables). A list of such correlation co-efficients is given in Table 7.5. It should be noted that with so many degrees of freedom, virtually any correlation coefficient is statistically significant.

7.91 Of the various quantities tested against SPEXRT, the best predictors are TEMPRT (temperature) in the positive sense, and DNFACR and DNLØAD (two different loading factors) in the negative sense. It is important to realise that this predictive ability lies only in that particular quantity taken alone; when groups of quantities are considered (as in multiple regression analysis) the situation is somewhat different. From the data of Table 7.5 it would appear that the best biophysical quantity on which to base simple "rule-of-thumb" prediction, for these experimental conditions is the loading factor which takes length into account by its relation with weight, DNFACR. It is interesting to note that the correlation between SPEXRT and FACTØR is relatively low, although FACTØR and DNFACR should be in theory different ways of calculating the same thing, provided the relationship between weight and length is constant. In the

Table 7.3 Summary data for observation matrix quantities

(n = 122)

QUANTITY	UNITS	MEAN	MINIMUM VALUE	MAXIMUM VALUE	STANDARD DEVIATION
SPEXRT	mg kg^{-1} h^{-1}	13.3751	3.829	34.845	7.05840
TEMPRT	^{o}C	9.97295	6.1	17.0	3.76612
ENUMBR	-	78.5164	19	350	87.9254
FLØWRT	ℓ min^{-1}	1.12267	0.228	3.000	0.671287
VØLUME	ℓ	34.5779	8.3	114.0	36.3247
AVERLN	cm	12.277	8.86	17.24	2.39057
DENSTY	ℓ^{-1}	2.71791	0.708	7.000	1.27894
DNLØAD	kg min ℓ^{-1}	1.52438	0.214	3.743	0.965343
DNSTØK	g ℓ^{-1}	57.4419	23.81	122.35	22.2050
GRUBFD	g	8.14139	1.46	30.12	7.21439
FDMEAL	g	21.3864	3.96	73.20	18.9725

Notes:-

a) Minimum and maximum values are given to the accuracy determined by readings.

b) Mean and standard deviation are given to the accuracy used by the computer during analysis (6 significant figures).

Table 7.4 Summary data for transformation quantities

(n = 122)

QUANTITY	UNITS	MEAN	MINIMUM VALUE	MAXIMUM VALUE	STANDARD DEVIATION
FACTØR	$kg\ min\ \ell^{-1}\ m^{-1}$	12.2161	1.98516	35.6781	7.41932
FREPAT	$ml^{1/3}$	0.613972	0.105258	1.21820	0.264166
EXRATE	min	27.2733	7.21717	74.4368	16.4905
AVEENU	m	9.67972	2.04820	33.1093	10.3046
DNFACR	$kg^{2/3}\ min\ \ell^{-1}$	1.28306	0.321354	2.25565	0.497329
STØLIN	$kg\ min\ \ell^{-2}$	0.0706914	0.0172581	0.170619	0.0448347
FDGØVN	$^{o}C\ cm^{-1}$	55.2109	17.0165	284.205	58.0796
STKFAC	$g\ \ell^{-1}\ cm^{-1}$	4.67125	2.20872	7.94283	1.47511
DNSTAC	$g^{2/3}\ \ell^{-1}$	5.50902	2.56906	10.2998	2.04448

Note:-
Values are given to the accuracy used by the computer during analysis (6 significant figures)

Table 7.5 Correlation of SPEXRT with other variables

VARIABLE	CORRELATION COEFFICIENT	
DNLØAD	- 0.600	(df = 120)
DNSTØK	- 0.473	
FACTØR	- 0.479	
DNFACR	- 0.635	
DENSTY	0.162	
FREPAT	0.377	
STKFAC	- 0.326	
DNSTAC	- 0.132	
STØLIN	- 0.278	
TEMPRT	0.778	
FDGØVN	0.102	
GRUBFD	- 0.274	
AVERLN	- 0.494	
EXRATE	- 0.425	
AVEENU	- 0.317	

conditions of these experiments the correlation between FACTØR and DNFACR was 0.818, showing variability in the length/weight relationship. A similar "equivalence" between STKFAC and DNSTAC showed a correlation of only 0.690.

7.92 The next-best biophysical predictor was DNLØAD, the simple loading factor (weight/flow), whereas the stocking factor DNSTOK (weight/volume) was relatively poor, and was exceeded in predictive capability by the simple length of the fish (AVERLN). All of the quantities so far mentioned had a negative correlation with SPEXRT, showing that as the loading of fish increased, ammonia excretion was depressed. However, temperature (which had the highest correlation of all) was positively correlated, indicating that ammonia excretion increased with increasing temperature.

Multiple regression analysis (OM 1)

7.93 Under the analytical procedure of OM 1 (see DATA ANALYSIS section), several different additive models were tried, using various combinations of the supposedly basic influences: temperature, some loading factor, stocking, the food and the squares and first-order interactions of these quantities. GRUBFD was chosen in preference to FDMEAL for consideration because it seemed likely that the excretory rate as sampled at 1330 would be most affected by the food fed at 1000 and 1200 on the same day, and much less by feeds on the previous day which fell within the same experimental DAY. (Substitution tests between these quantities showed no difference in the final analysis). Stocking and loading were both built into the models, but three different ways of expressing the loading were tested: (i) as DNLØAD + AVERLN, (ii) as DNFACR, (iii) as FACTØR. The basic pattern of the additive models was :-

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \quad \ldots\ldots \text{(simple quantities)}$$
$$+ b_{1.2}x_1x_2 + b_{1.3}x_1x_3 \quad \ldots\ldots \text{(simple interactions)}$$
$$+ b_{1.1}x_1^2 + b_{2.2}x_2^2 + b_{3.3}x_3^2 \ldots\ldots \text{(squares)}$$

7.94 In addition, multiplicative models were tested, using log transformations of SPEXRT, TEMPRT, DNLØAD, DNFACR, FACTØR, AVERLN and GRUBFD. (In this case no interactions or squares were included owing to their difficulty of comprehension.) The multiplicative models had a basic pattern thus:-

$$\log y = \log a + b_1 \log x_1 + b_2 \log x_2 + b_3 \log x_3 \ldots.$$

or:-

$$y = a.\, x_1^{b_1}.\, x_2^{b_2}.\, x_3^{b_3} \ldots\ldots$$

Again the three possibilities of DNFACR, FACTØR and (DNLØAD, AVERLN) were tested as loading factors.

7.95 Results from the best additive and the best multiplicative models are shown in Table 7.6 and the regression coefficients of significant independent variables are given. It was found that in the full analysis the quantities DNFACR and FACTØR are interchangeable, with no effect on the resulting predictive equation, and that in both the additive and multiplicative types, the inclusion of both DNLØAD and AVERLN was preferable to the alternative expressions of loading, giving a higher overall multiple correlation coefficient. The equation suggested by the results for the best additive model (1) is:-

$$y = 48.622 - 2.620x_1 - 1.504x_2 - 0.324x_3 + 0.004x_3^2 - 0.018x_4^2 + 0.259x_1x_2 - 0.490x_1x_5 + 0.133x_1x_4 - 0.027x_2x_3 \ (\pm 6.376)$$

- where

x_1 = TEMPRT, x_2 = AVERLN, x_3 = DNSTØK,
x_4 = GRUBFD, x_5 = DNLØAD

(Cl_{95} given for one observation in parentheses.)

7.96 This model accounts for a high proportion of the variability in the data, having R = 0.899, and surpasses the corresponding multiplicative model (2) which has R = 0.808:-

$$\log y = 1.086 + 0.921 \log x_1 - 0.476 \log x_2 - 0.230 \log x_3 \ (\pm 0.268)$$

or:-

$$y = \frac{12.19x_1^{0.9}}{x_2^{0.5}\ x_3^{0.2}} \qquad (* 1.854)$$

Table 7.6 Multiple regression analysis OM 1 results

		X*	b
MODEL	(1) Additive	TEMPRT	- 2.6195272
		AVERLN	- 1.5037104
Y	SPEXRT	DNSTØK	- 0.3235561
R	0.899	$DNSTØK^2$	0.0042644
a	48.6218979	$GRUBFD^2$	- 0.0167329
ESS	1161.53	TEMPRT x AVERLN	0.2585517
SE_y	3.22037	TEMPRT x DNLØAD	- 0.4904210
Cl_{95}	6.376	TEMPRT x GRUBFD	0.1328849
		DNSTØK x AVERLN	- 0.0274240
MODEL	(2) Multiplicative	log TEMPRT	0.9213403
		log AVERLN	- 0.4757199
Y	log SPEXRT	log DNSTØK	- 0.2302780
R	0.808		
a	1.0859537		
ESS	2.16965		
SE_y	0.135598		
Cl_{95}	0.268		

Notes:-

a) X* = variables significant at level P = 0.05

b) b = regression coefficient

c) R = multiple correlation coefficient

d) a = intercept (log value for multiplicative model)

e) ESS = error sum of squares

f) SE_y = residual error = $\sqrt{\frac{ESS}{df}}$

g) CL_{95} = 95% confidence limits for observations = SE_y x 1.98 (since 1.98 is the t-statistic for P = 0.05 with df $\doteqdot$ 120)

h) Values are given as produced by computer, except for CL_{95} (which was calculated separately).

This approximates to:-

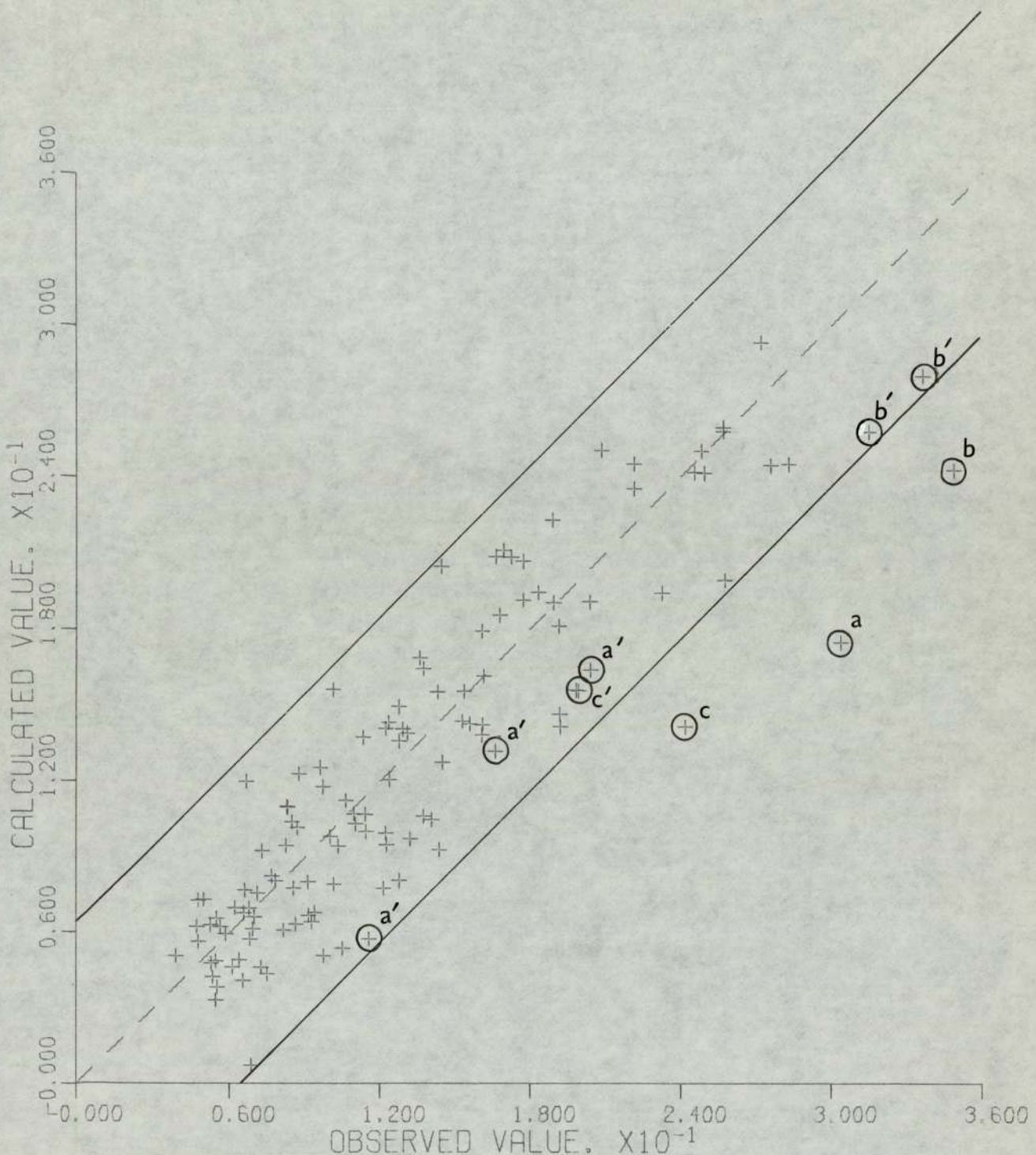
$$y = \frac{12x_1}{\sqrt{x_2} \cdot \sqrt[5]{x_3}}$$

7.97 Both of these models were subsequently submitted to the multiple regression presentation procedure, to produce graphical illustration of the models (Appendix C). Graph 7.2 (for model 1) shows values of SPEXRT calculated from the model ($\hat{y}$) plotted against observed values (y). The points are fairly well distributed about the mean line (dashes), with three particularly 'wild' points a, b and c (in descending order of deviation from the line), which fall outside the 95% confidence limits for y (solid lines - see Table 7.6).

7.98 Investigation of points a, b and c revealed special circumstances about their measurement. For points a and c an excessive amount of food had been fed to the fish in those particular tanks over the 24 h before TOA measurement (due to miscalibration of feeders by overshoot, see Chapter 7, para.7.27); this probably resulted in excessively high observed values. For point b the calibration values of TOA seemed to be unusually high compared with the values on other days; this could also account for an extra-high observed value. It was decided to remove a, b and c readings from the data matrix because of the doubts due to these circumstances. Following Wilson (1952), who maintained that:

"if a given circumstance is once used to justify discarding a discordant result, the occurrence of the same circumstance must cause rejection whenever it happens and whatever the result", - a thorough search was made for similar occurrences of these conditions. The case of b (high calibration) was true for the other readings of that day (b' in Graph 7.2), but was not otherwise found. The circumstances of a and c (sudden change in amount fed) were noted on these related occasions :- (see Graph 7.2)

a' : other tanks on the same day as for a also suffered 'overfeeding' to the extent of 56%, 52% and 50% increases over



Graph 7.2

OBSERVED V CALCULATED.

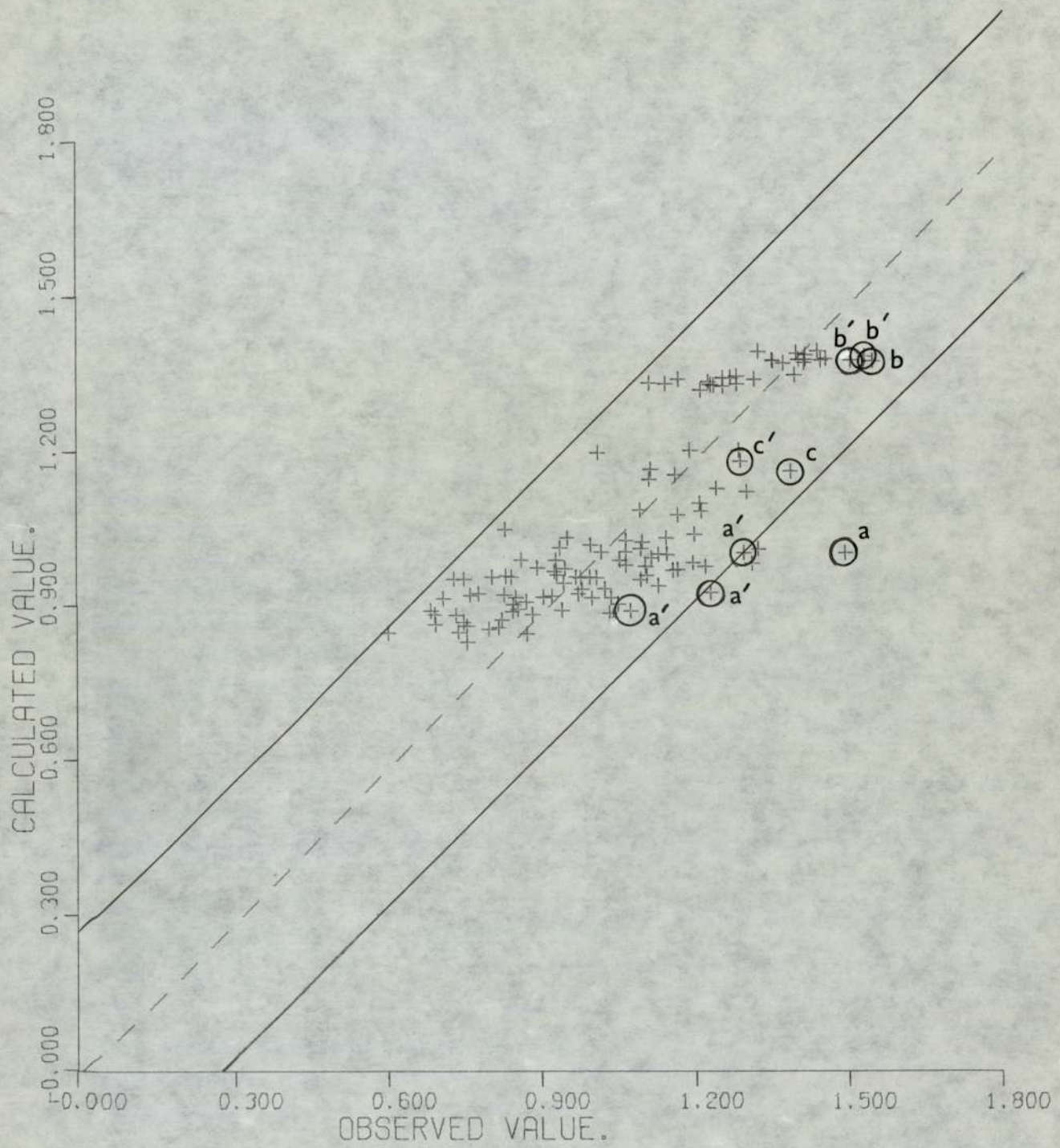
the previous DAY (a had a 50% increase);

c' : another tank suffered similarly on the same day as c, recording a 44% increase (c had 40% increase).

No other cases of exceptional 'overfeeding' occurred, and checks for 'underfeeding' by overshoot calibration of feeders revealed no instances where decrease was of a similar magnitude to the increases in a and c.

7.99 The corresponding graph for the multiplicative model (2) is shown in Graph 7.3, with points a, a', b, b', c and c' indicated. Some more 'wild' points are noted on this plot, but since this model is inferior to model 1, it was not further pursued: the data had already been checked for unusual circumstances. Comparison between the two graphs shows that (if points a, b and c are ignored), the points behave differently with respect to convergence: in Graph 7.2, there is neither a notable convergence nor a notable divergence of points as the values increase; however, in Graph 7.3, there is evidence of some convergence of points towards the higher values. It can be concluded that the standard deviation of Y is not dependent on the magnitude of the quantities; had that been so, there would have been appreciable divergence of scatter with increasing values in Graph 7.2, and the log transformation, which closes up higher values but opens out lower values, would have been more suitable as a model. In practice, the additive model (1) is superior on these grounds as well as its expression of a higher R value (Table 7.6)

7.100 For further analysis, points a, a', b, b', c and c' were eliminated from the observation matrix, and the regression analysis and presentation re-worked for the additive model. Corrected analysis results are given in the first part of Table 7.7, and it can be seen that an improved model (3) is generated, with R = 0.915 as opposed to R = 0.899 in model 1 (i.e. less of the variability in the data remains unaccounted for). Graph 7.4 shows the 'observed' against 'calculated' plot for model (3), and two points of interest arise: there are a few points just



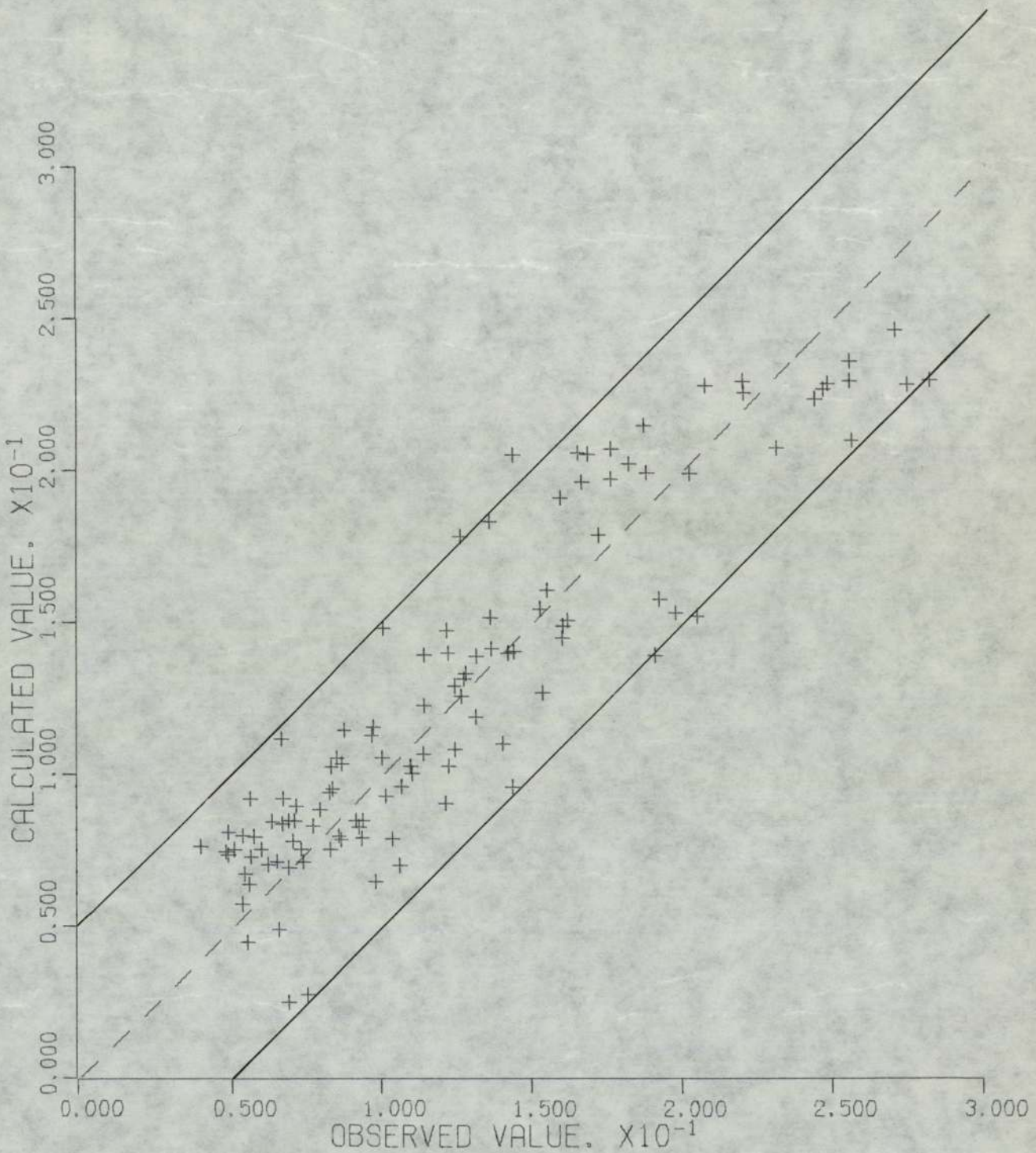
Graph 7.3

OBSERVED V CALCULATED.

Table 7.7 Multiple regression analysis OM 1 (corrected) and OM 2 results

		X*	b
RESULTS	OM 1 (corrected)	DNSTØK	- 0.3125522
MODEL	(3) Additive	$GRUBFD_2$	1.1121440
Y	SPEXRT	$TEMPRT^2$	0.0232537
R	0.915	$DNSTØK^2$	0.0018541
a	21.5339584	$GRUBFD^2$	- 0.0262277
ESS	691.956	DNLØAD x AVERLN	- 0.3275169
SE_Y	2.55497		
CL_{95}	5.059		
RESULTS	OM 2	$ENUMBR_2$	0.0427383
MODEL	(4) Additive	$TEMPRT^2$	0.0414127
Y	SPEXRT	$ENUMBR^2$	0.0001978
R	0.917	TEMPRT x FREPAT	0.4164954
a	5.7522365	ENUMBR x DNSTØK	- 0.0013503
ESS	676.424		
SE_Y	2.51430		
CL_{95}	4.9783		

(Notes apply as for Table 7.6)



Graph 7.4

OBSERVED V CALCULATED.

outside the Cl_{95} lines on this plot also, and it seems possible that at high observed values, the calculated values are tending to level off; the latter phenomenon may indicate the influence of some variable which has not been taken into account, especially since all observed values above about 23 mg kg^{-1} h^{-1} have low calculated values, causing points which fall well below the best-fit line.

Multiple regression analysis (OM 2)

7.101 Bearing in mind the additional factors envisaged under OM 2 (see DATA ANALYSIS section), and the possible influence of un-measured variables detected in OM 1 model (3), attention was focussed on the OM 2 analytical procedure, where the fundamental innovation was a separate consideration of the number of fish in the tank, especially as expressed in the factors ENUMBR, DENSTY and FREPAT. Once again, a battery of different models (all additive) were tried out in multiple regression analysis.

7.102 The introduction of ENUMBR and FREPAT for consideration seemed to remove the direct effects of food factors (GRUBFD or FDMEAL) from the list of significant quantities, and a simple model in terms of TEMPRT, ENUMBR, FREPAT, AVERLN and DNSTØK gave a promising result (R = 0.854) for unrevised data (i.e. including a, a', b, b', c, c'). When squares and first-order interactions were taken into account, R rose to 0.882.

7.103 Final modification was performed by using the revised observation matrix (without a, a', b, b', c and c') for the model described above. This final model (4) is detailed in the second part of Table 7.7, and results in a small improvement on model (3), having R = 0.917. This final model (4) has the advantage of an intuitively realistic intercept value ('a' in the Table) as compared with models (1) and (3); about 6 mg kg^{-1} h^{-1} seems a reasonable possibility for a basal excretion rate with most of the influencing factors at a minimum. Accordingly, a full presentation treatment was performed on model (4) (see Appendix C).

7.104 Graph 7.5, the 'observed' against 'calculated' plot, shows a fairly balanced distribution about the line although the extent of deviation of some points from it emphasises the limits of accuracy of the model.

7.105 By examination of Graph 7.5 and the other graphs also generated (see Appendix C), a number of other points became clear, and the overall results are detailed below.

Summary of results of multiple regression analysis

7.106 The best equation found for SPEXRT estimation was as follows (Cl_{95} for one observation given in parentheses):-

$$y = 5.7522 \times 0.0427x_1 + 0.0002x_1^2 + 0.0414x_2^2$$

$$+ 0.4165x_2x_3 - 0.0014x_1x_4 \quad (\pm 4.9783)$$

- where y = SPEXRT, x_1 = ENUMBR, x_2 = TEMPRT, x_3 = FREPAT, x_4 = DNSTØK

7.107 This model was selected by the best multiple regression analysis of the data available ($R = 0.917$) one limitation on its use is evident in the relatively wide and poorly-explained variability in SER in situations where <100 fish are present.

7.108 Since:

$$\text{FREPAT} = \frac{\sqrt[3]{\text{volume}}}{\text{number}} \quad \text{and} \quad \text{DNSTØK} = \frac{\text{weight}}{\text{volume}},$$

then the model allows a fairly reliable estimate of SPEXRT in terms which involve mathematical manipulation of only four measured quantities; temperature, number of fish, volume of water and weight of fish.* Manipulated correctly, these variables can also account for the food fed, and the majority of variability found in measured SPEXRT (according to TOA concentrations), under the conditions of experiment.

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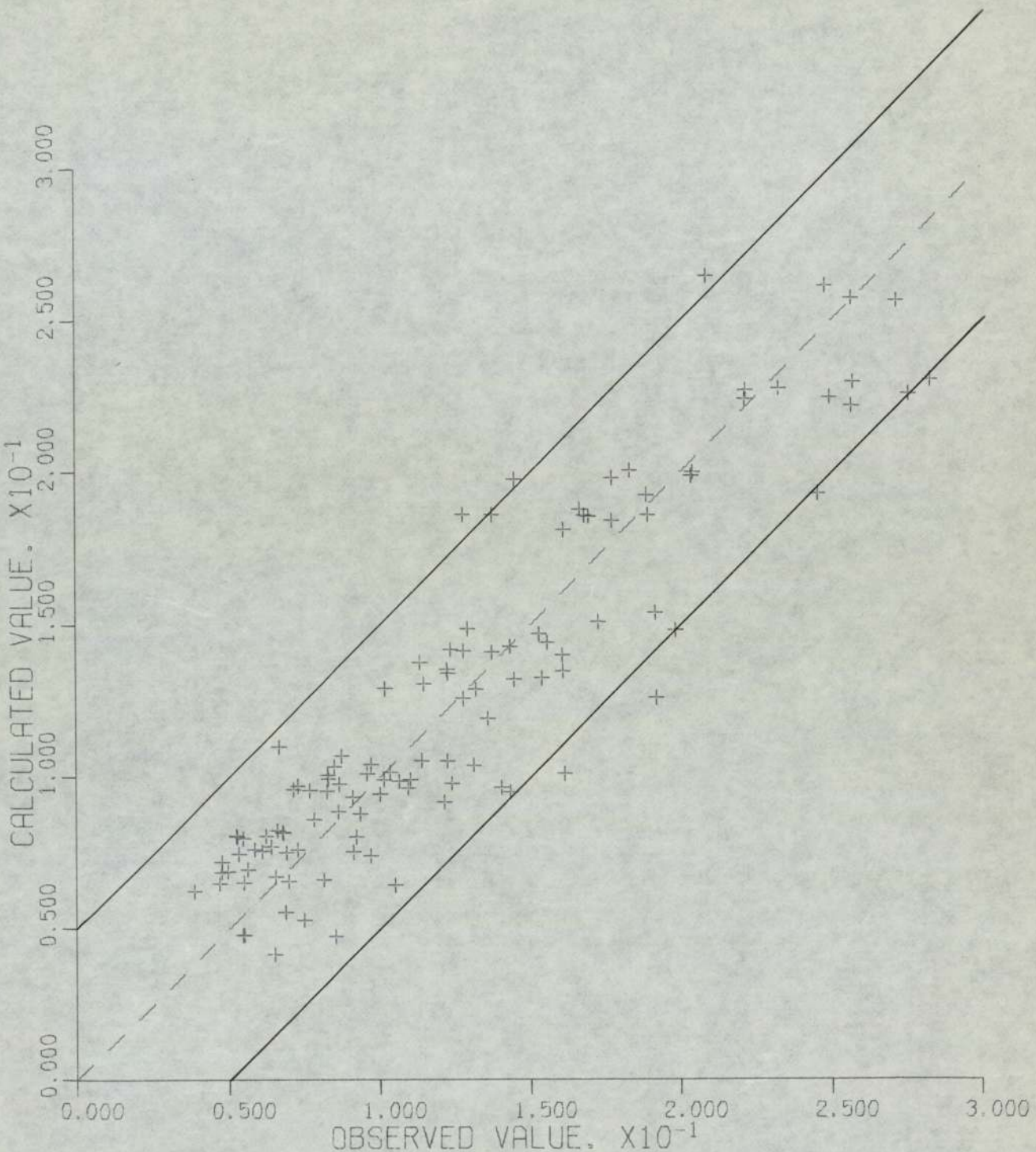
* See Appendix C for notes on independence of these quantities.



Graph 7.5

OBSERVED V CALCULATED.

7.109 The full E-series experiments were superior to the pilot experiments in several important respects:- (Ref. Chapter 6 DISCUSSION).

a) food consumption was maximised and, except for a few cases of accidental "overfeeding" (where food may not have been fully consumed) (see RESULTS section), the assumption of full consumption was justified by observation;

b) inauguration stresses (due to weighing out fish at the start of experiment) were minimised by improvement of technique and time was allowed for fish to settle down before readings were taken;

c) estimation of weight was improved to provide values for each experimental DAY;

d) a representative TOA reading was taken at the same point of the diurnal photoperiod cycle, in all cases (1330); if there is a diurnal excretory cycle this will have standardised its effect;

e) there was no sudden change in temperature or photoperiod during the changeover from holding to experimental conditions (due to HC2);

f) measurements were taken over a longer period;

g) tank size and configuration were improved, eliminating the extreme degree of 'crowding' mentioned in para.6.46.

7.110 With these improvements, a fairly reliable system was created for meaningful measurements of SER. The good hydraulic properties of the circular tank provided adequate mixing to ensure a fairly reliable sample from the effluent outflow (as contrasted with the raceway outflows used by Burrows: raceways have poor mixing qualities (Burrows & Chenoweth 1955)).

7.111 The final model for SER estimation, evolved from the results of E-series experiments, represents a surprisingly good explanation of the situation, bearing in mind that it is based only upon four measurable quantities. These quantities will affect the fish internally as suggested in Fig.5.6

(Chapter 5), and the external effect on TOA concentration will be by way of the changes in metabolism. However, it is almost certain that they will be unable to account thus for the complete total of variability in ammonia production.

7.112 This area of doubt is more closely examined in Appendix C. It seems probable that the best explanation of the lack of accuracy of the model is due to unmeasured quantities. Where unexpected deviation from the calculated (theoretical) SER values takes place, a hitherto intangible variable is suggested, which may be conveniently referred to as stress. In this particular instance, one possible type of stress might be quantitatively (and empirically) defined by the unexplained variability in excretion rate when the effects of normal biophysical parameters, and their measurement errors, have been taken into account. In the case of the final model for SER, this would be expressed in, and estimated by, the error limits of the equation (which also includes the measurement errors).

7.113 Summary of conclusions

1. The variability in SER is adequately explained ($R = 0.915$) by an additive model (3) which ignores the number of fish in the tank (OM 1), and hence allows SER description by quantities related to purely chemical or physical effects, i.e. treating the tank as a single ammonia-producing machine (see para.7.69).

2. The variability in SER is marginally better explained ($R = 0.917$) by an additive model (4) which takes the number of fish into account, together with three other basic quantities: temperature, fish mass and water volume.

3. The multivariate relationships which these models describe are sufficiently good ($R > 0.9$) to use as predictors of SER, within the limits of the experimental conditions, for future similar experiments.

4. Doubt exists as to the effect of number of fish below a value of about 100, but this does not seriously disturb the final model (4).

5. The error in observed SER values as compared to calculated (predicted) values may be interpreted as including a quantitative component attributable to stress, as well as measurement errors.

6. Future experiments are required to investigate more values of temperature, fish number and stocking, in order to verify the overall findings.

PART 4

TOLERANCE OF FISH

TO FISH EFFLUENT

8. INTRODUCTION TO AMMONIA TOLERANCE

LITERATURE REVIEW AND TOLERANCE THEORY

Toxicity and tolerance

8.1 Compounds which adversely affect the physiology of living organisms have long been termed toxic, principally when death results if they are allowed to act unchecked. Much literature has been devoted to identifying concentrations which produce measurable effects. This work is most advanced in medicine, but during this century attention has also focussed on other species. Fish have received much attention, especially in the particular context of pollution.

8.2 During this work, a variable terminology has grown up to describe toxicity; standard medical terminology has been involved, but recently some new definitions have been coined.

8.3 The effects associated with death are usually referred to as acute toxicity; and the quantity of compound causing death under stated conditions is the lethal concentration. Frequently the most important conditions involved are:

a) % of subjects dying (e.g. LC-50 indicates a lethal concentration at which 50% of subjects are killed);

b) time-span over which the effect occurs (thus 24 or 48 h LC-50);

c) temperature at which the effect occurs.

8.4 In many studies, however, one is not considering death; if suitable curative measures are to be taken, the identification of less severe criteria is clearly important. This kind of effect is described as sub-acute, sublethal or chronic toxicity, the latter emphasising that long periods of time may be involved. The looseness of the "medical" terminology (with its implication of time involved rather than effect caused) is unsatisfactory, especially since the criteria of effect may vary widely. For this reason, in common with several authors (e.g. Sprague 1971, Lloyd 1972, Webb & Brett 1972, 1973, Schulze-Wiehenbrauck 1974)

the term sublethal toxicity will be preferred in this account, and will be used to describe the whole range of adverse effects, short of death.

8.5 In contrast to the "negative" definition of chemical effects (emphasising adverse effects), it is possible to view the situation from the opposite end, and consider to what extent physiology is maintained as normal, despite the presence of a poison; or in what ways the physiology may be adjusted to cope with the substance. This is the phenomenon of tolerance, and it becomes increasingly important as the concentration involved decreases from lethal levels. For instance, when trying to establish "safe" levels of known pollutional chemicals to allow to be present in a waterway, either of two questions may be answered:-

a) At what level can no adverse effect whatsoever be detected?

b) What is the level up to which some physiological criterion of normality (e.g. breeding capability) is maintained?

The first question assumes that a level exists at which experimental and control tests will not differ, whatever the physiological criterion used; given the complexity of living organisms and their environmental interactions, this may often be an artificial assessment, since environmental variations are part of most organisms' "normal" conditions of life. At very "low" concentrations of the chemical, its effect may be lost in a complex pattern of interactions with other variables. The second question above assumes that a sufficiently sensitive criterion can be identified so that its maintenance will also guarantee preservation of more general qualities e.g. growth. This may lead to difficulties in assessing whether the criterion is of the correct sensitivity for the particular objectives envisaged.

8.6 In fish farming, there are probably two major types of objective borne in mind when assessing the fish environment. In one case the farmer may be most interested in the breeding

capability of the fish; but in the more generally important case, the farmer's objective is couched in growth terms. This may be expressed in different ways, according to circumstances; sheer speed of growth may be the objective in some cases, whereas in others the efficiency with which food is converted into fish flesh is more important. In the latter situation, the gross conversion ratio may well express the ratio between major outlay (food materials bought) and income (fish sold) assuming sales match production. Thus a suitable measure of tolerance may in many cases be the maintenance of growth rates or conversion ratios. Similarly, when considering recycling of water, there may be levels of dissolved excretory products which can be allowed to persist because they do not prevent the maintenance of economic growth rates or conversion ratios. If so, then a filtration unit need only remove excretory products down to such levels: complete removal may be unnecessary (if indeed practicable). The implications of this on filter unit cost may be important, particularly where filter efficiency is related to unit size as may be the case with biological filtration.

Ammonia as a toxicant

8.7 Given that ammonia is an acceptable index substance for dissolved excretory products, it is important to assess ammonia as a chemical toxic to fish, before considering it from the point of view of tolerance.

8.8 It has been recognised for about thirty years that the described toxic effects of ammonia on fish are due to the free radical NH_3^o (UIA) as opposed to the ion NH_4^+ (Wuhrmann & Woker 1948). Since that time much work has been done, especially on the lethal toxicity of UIA to fish, and the work is substantially reviewed in the FAO Report on ammonia and inland fisheries (EIFAC 1970), from which the expression below is taken:-

$$\% \text{ UIA in TOA} = \frac{100}{1 + \text{antilog } (pK_a - pH)}$$

where

pK_a = negative log of ionisation constant (dependent on temperature: determined by formula given in Robinson & Stokes 1969 [following Lloyd & Herbert 1960]).

8.9 The study of ammonia as a toxic substance can be split into two major areas: (a) studies in which fish are exposed to made-up solutions of ammonium compounds, and (b) studies in which adverse effects are described in conditions which imply that ammonia is the culprit. The literature of (a) is widespread and often scientifically rigorous, and has been extensively reviewed by EIFAC (1970); the major points of which are worth recapitulating. They include the early recognition of the importance of pH (Wuhrmann Zehender & Woker 1947, Wuhrmann & Woker 1948), contrasting with the approach of Grindley (1945), who calculated NH_3 as a molecular fraction of NH_4Cl or $(NH_4)_2SO_4$. The effects of oxygen (Downing & Merkens 1955, Merkens & Downing 1957, Lloyd 1961a), free CO_2 (Lloyd & Herbert 1960), temperature and other quantities (Lloyd 1961b, EIFAC 1970) on the lethal toxicity have been well described, so that given a chemical description of a water supply, the threshold lethal levels (in which prolonged exposure kills 50% of the fish) can be estimated (Lloyd 1961b). More recently Ball (1967) has compared the susceptibility of several species of fish to UIA and found little difference at around threshold values, with species differences more clear at higher levels (possibly indicating different modes of action in causing death).

8.10 Work has gradually extended into the sublethal region, following the spread of estimates for safe levels of ammonia (e.g. as reported in Ball 1967), couched in terms of LC-50. Recognition of the possibility of different physiological effects at different sublethal levels has prompted more recent workers to define sublethal criteria of toxicity independently of LC-50 (Reichenback-Klinke 1967, Fromm & Gillette 1968, Lloyd & Orr 1969, Schulze-Wiehenbrauck 1974).

8.11 Studies of adverse effects on fish in culture systems

have generally assumed that, because of the proven toxic effects of ammonia solutions in laboratory studies, ammonia is the culprit whenever high excretory levels would be expected and adverse effects occurred, with a strong tendency to indict ammonia especially for growth losses. Thus Brockway (1950) advises that 0.3 mg ℓ^{-1} "ammonia" affects blood oxygen content without specifying conditions or UIA content, and links this to probable effects caused by metabolic products in general. His quoted criterion of 0.1 mg ℓ^{-1} ammonia (TOA) as a "maximum that should be tolerated in waters used for fish culture" has often been quoted (e.g. Spotte [1970]), but has only recently been properly examined.

8.12 Early studies on metabolic product effects frequently involved simultaneously the problems of stocking (weight per unit volume), and subjective estimates of "stress" (Philips et al 1950, 1951); and growth-rate studies linked to measures of fish density or crowding cited ammonia as the agent of adverse effects (Kawamoto Inouye & Nakanishi 1957, Kawamoto 1958, Yashouv 1958), based on the reasonable assumption that denser collections of fish would give higher local ammonia levels, assuming that excretion rate was maintained. The point at issue, however, was whether ammonia, specifically, was the cause of growth losses under the particular conditions applying. From foregoing discussion, it is clear that

(a) pH and temperature, by their determining effect on UIA, could permit or prevent toxicity; and

(b) the knowledge that UIA can be toxic in controlled laboratory studies does not rule out other factors present in the fish culture situation as being involved in growth inhibition, either instead of, or as well as, UIA effect.

Such other factors could include:

a) direct action by other chemicals excreted,

b) feedback effect of stocking, loading or some measure of "crowding" (see Chapter 7), as a bio-physical effect,

c) direct or interactive effects of other variables allowed to operate on the system (e.g. oxygen content, temperature,

lighting regime, "disturbance stress", etc.). It is at this point that the more recent combination of laboratory experiments supplementing field observations has come into being.

8.13 Kawamoto (1961) followed the growth of carp (Cyprinus carpio) in an ammonia solution (held at roughly constant pH), compared to controls, and recorded a definite growth disturbance, although the data were variable and there was some good growth. He found that oxygen consumption of carp dosed with the growth disturbing ammonia solution displayed an altered pattern, being decreased at low temperatures but increased at high temperatures. His work, assuming that the considerations of (b) and (c) in para.8.12 can be ruled out, shows that, for carp, ammonia can have an adverse effect on growth. The question however arises of whether the effect is due to TOA or UIA. Kawamoto measured ammonia as TOA (this is assumed, since no mention is made of a distinction between TOA and UIA), and pH remained relatively low (6.25 - 6.72) so that dissociation would be low. Carp were exposed first to 0.3 mg ℓ^{-1} TOA (0.15 - 0.40 μg ℓ^{-1} UIA) and later to 1.2 mg ℓ^{-1} TOA (1.30 - 2.90 μg ℓ^{-1} UIA). There was most growth disturbance in the latter phase which coincided with the 4-fold rise in TOA (7- or 8-fold rise in UIA). Thus it seems that, at least for carp, either UIA or TOA could be causing growth inhibition at these levels.

8.14 For salmonids, more detailed consideration of the role of excretory products largely springs from the work of Burrows (1964), who looked at the effects of stated UIA levels on the gill histology and swimming stamina of chinook salmon (Oncorhynchus tshawytscha). Citing instances of growth rates being 10% lowered in "ammonia-dominated" raceways (see Chapter 5), Burrows goes on to nominate ammonia as a weakening agent which also predisposes fish to disease, notably bacterial gill disease (although Larmoyeux & Piper (1973) stress that oxygen depletion may also be involved). All of his work was conducted at pH 7.8, and three dosing solutions (0.3, 0.5 and 0.7 mg ℓ^{-1}

TOA) were used in tests of gill damage effect. Burrows estimated these at 6, 10 and 14 $\mu g\ \ell^{-1}$ UIA at 6°C; and 8, 12 and 18 $\mu g\ \ell^{-1}$ at 14°C. All produced gill hyperplasia, the severity of effect being correlated with concentration. Trussell (1972) has since pointed out that Burrows' UIA values are overestimates, and they should be approximately halved. The effect at "6 $\mu g\ \ell^{-1}$" has given rise to a common quoting of 5 $\mu g\ \ell^{-1}$ as the maximum acceptable UIA value for salmonid rearing (Liao & Mayo 1972, 1974). The order of magnitude of UIA involved is that which might have caused growth disturbance in Kawamoto's (1961) carp, and reinforces his findings as to the dangers of ammonia to sensitive species, among which chinook salmon can clearly be included.

8.15 More recently, the rainbow trout itself has been the subject of experiments, and at Bozeman Center studies on serial use of fish tanks generated interest in the combined effects of ammonia accumulation and oxygen depletion, (Larmoyeux & Piper 1973). Report on the effect of ammonia alone has been provided by Smith (1972) and Smith & Piper (1975). A preliminary six-week study of dosed NH_4OH at high oxygen levels indicated no loss of growth, at a concentration of 0.8 - 1.0 mg ℓ^{-1} TOA, (about 8 to 10 $\mu g\ \ell^{-1}$). These values of UIA are of the order of, or higher than, those causing growth disturbance and gill damage in chinook salmon (Burrows 1964); this indicates that rainbow trout may well be less susceptible to sublethal UIA toxicity.

8.16 Smith then performed a 12-month study at three TOA levels (0.6, 1.2 and 1.6 mg ℓ^{-1}) at 10°C and pH 7.75, using excreted ammonia, kept steady by adjusting the fish loading. Growth and stamina were measured, and internal organs histologically examined (see below), and in overall terms Smith could find no effects on growth except in his highest concentration (1.6 mg ℓ^{-1} TOA) and then only after 6 months' continuous exposure (Smith & Piper 1975). UIA values were about 6, 12 and 17 $\mu g\ \ell^{-1}$, in the various tanks. The "danger zone" for growth

disturbance is thus about 17 $\mu g\ l^{-1}$, corresponding to the 1.6 mg l^{-1} TOA tanks. The data support Smith's contention that Spotte's (1970) adherence to the 0.1 mg l^{-1} TOA limit is not applicable, and his further comment that it may be economically unfeasible to keep ammonia this low is particularly interesting, especially when the possible costs of biological filtration or other methods of achieving such a standard are taken into account.

Criteria of adverse effect of UIA

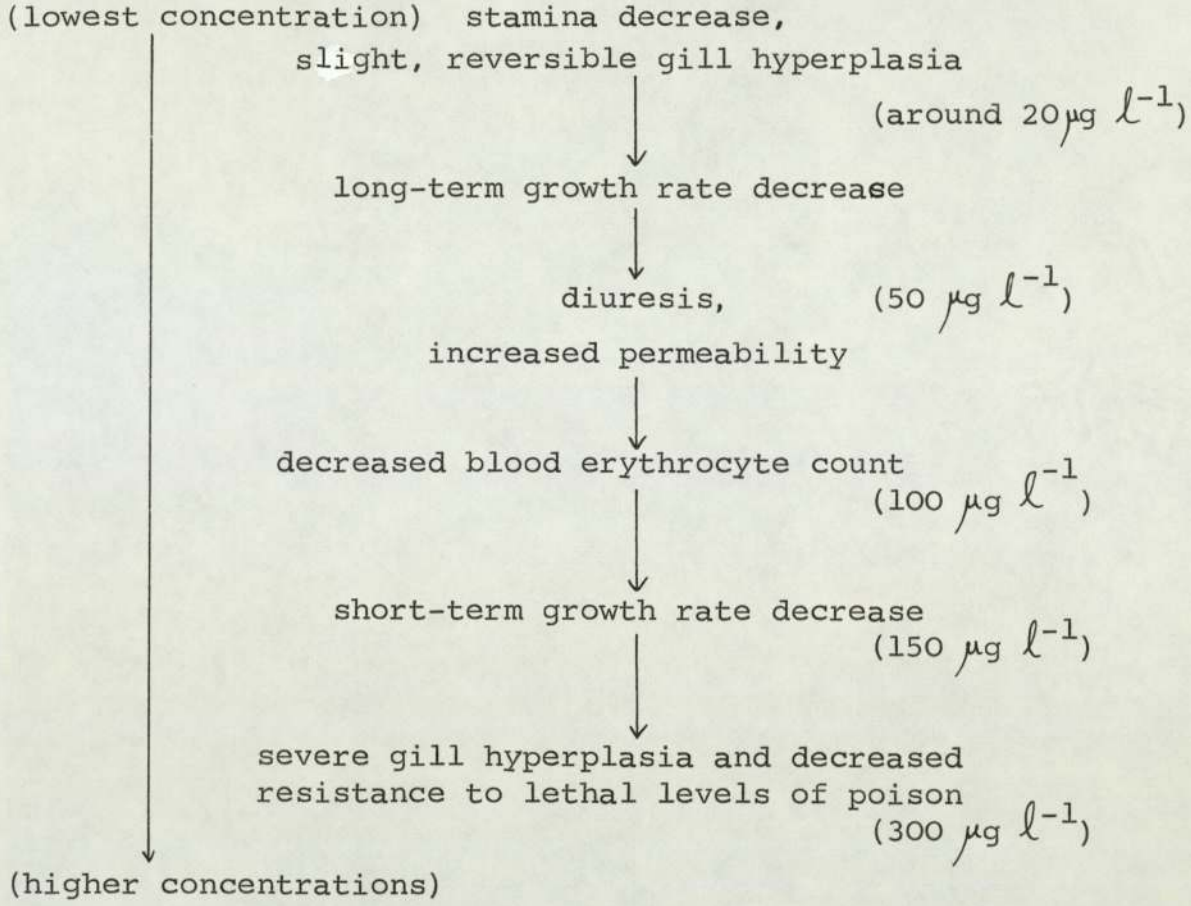
8.17 In recent studies of sublethal toxicity in dosage experiments, a variety of criteria of sublethal toxic effect have been used, and some of these are summarised in Table 8.1, for work directly using rainbow trout. It can be seen that

a) different effects can be associated with different levels, and

b) there can be controversy even over application of the same criterion.

Perhaps the difference between Smith (1972) and Schulze-Wiehenbrauck (1974) in their interpretation of growth rate changes lies either in the fact that Schulze-Wiehenbrauck's tests were limited to 7 weeks (Smith's ranged over 12 months) or that different sources of UIA were employed: Smith used excreted ammonia, whilst Schulze-Wiehenbrauck used a mixture of NH_4Cl and NaOH. It is probably that the 12-month study would reveal differences not found in 7 weeks, but with the extra passage of time, other variables may have had more opportunity to affect results, e.g. feedback effects of ammonia. Schulze-Wiehenbrauck maintains that the damage done to growth rates and food conversion which is observed at high fish densities is not due to ammonia, and that 100 $\mu g\ l^{-1}$ UIA can be regarded as harmless for young rainbow trout. This contrasts sharply with Smith's findings.

8.18 A possible interpretation of the results of Table 8.1 is that the various criteria represent different stages of toxicity in an ascending order such as:



At the top end of this range, lethal concentrations would be being approached (WPRL 1968, Lloyd & Orr 1969), and variations in conditions (including exposure time) might alter the exact UIA values involved at any stage, or indeed cause changes of position of effects in the scale.

8.19 In particular, Schulze-Wiehenbrauck's comment throws up doubts over the role of such factors as density of fish, stocking, etc. in either (a) modifying effective values, or (b) exerting effects of their own. Smith & Piper (1975) mention constant loading values used in their trials, but size and density certainly will have changed greatly over a growth period of 12 months. It is interesting that the one test which gave Schulze-Wiehenbrauck a definite difference in growth rate between controls and those treated with 170 $\mu g\ l^{-1}$ UIA, was that where highest stocking values were used (although still low, between about 10 and 20 g l^{-1}) in company with highest loading (1.1 to 2.0 kg min l^{-1}). This might lead to suspicion of a type (a) effect (above).

Table 8.1 Criteria of sublethal ammonia toxicity for rainbow trout

CRITERION OF TOXICITY	LOWEST EFFECTIVE CONC UIA ($\mu g\ l^{-1}$)	FOUND	NOTES
gill hyperplasia	> 300	Reichenbach-Klinke 1967	
gill hyperplasis and liver lesions	17	Smith & Piper 1975	
decreased blood erythrocyte count	100	Reichenbach-Klinke 1967	
diuresis (increased permeability)	90	Lloyd & Orr 1969	a)
decrease in stamina	17	Smith 1972, Smith & Piper 1975	b)
decrease in growth rate (weight)	170	Schulze-Wiehenbrauck 1974	c)
lowered food consumption			
decreased resistance	500	Schulze-Wiehenbrauck 1974	c)

Notes:-

a) Lloyd & Orr (1969) estimated no diuretic effect to occur at about 46 $\mu g\ l^{-1}$

b) Smith (1972) stressed that growth rate decrease was only found after 6 months continuous exposure

c) Schulze-Wiehenbrauck (1974) had doubts about his measured effects at 170 $\mu g\ l^{-1}$; he found an <u>increase</u> in resistance (to subsequent lethal UIA levels) at about 130 $\mu g\ l^{-1}$.

8.20 Sprague (1971) and Lloyd (1972) have reviewed sublethal effects of pollutants, and the problems of establishing "safe" water quality criteria. Sprague mentions that growth should always be monitored, but that it is not always a sensitive indicator. He also mentions disadvantages in relying on swimming speed/stamina trials or behavioural effects, encourages the possibility of measurement of scope for activity (see below), and cites reproduction and controlled ecological production experiments as particularly good assays. Sprague distinguishes between estimates of "safe" levels due to projections back from lethal concentrations (e.g. as reported in Ball [1967]), and those due to direct measurement of other sublethal criteria. Lloyd (1972) follows Sprague in advocating consideration of as many aspects as possible when setting water quality criteria, and warns of the danger of treating fluctuating concentrations of a toxicant (especially at sublethal levels) as the same as a steady dosage. He cites the combination of laboratory studies (Lloyd & Orr 1969) and field observations in arriving at a recommended standard for UIA in river water, as reported in EIFAC (1970). This standard is 25 $\mu g\, \ell^{-1}$ UIA, at which no diuretic effect should occur (Lloyd & Orr 1969), and which allows a safety margin for fluctuations.

8.21 It might be pointed out here that the work of Lloyd & Orr (1969) and Fromm & Gillette (1968), which described effects of ambient ammonia on excretion, was performed with fish which would almost certainly be losing weight due to lack of feeding before and during experiment. Also the fish were large and treated singly or in pairs. Although the relevance of these studies to setting of water quality standards for rivers may be great, it is important to contrast this type of work with that of Smith (1972) and Schulze-Wiehenbrauck (1974), who used populations of young fish which were feeding and actively growing. Clearly the latter type of experiment comes closer to the situation of the commercial fish farmer.

8.22 Nevertheless, the EIFAC (1970) standard of 25 $\mu g\ l^{-1}$, for no diuretic effect, seems a good basis on which to assume lack of short-term UIA effects in experiments, as in Chapters 6, 7 and 9. Although it is slightly above Smith's (1972) effective concentrations for stamina, histopathology and growth-rate decrease, these effects were found over a long period of time; and for the limited purposes referred to for this study, 25 $\mu g\ l^{-1}$ was accepted as the UIA concentration causing minimum effect on general physiology, including growth.

8.23 From this brief survey of the toxic effects attributed to ammonia, several points arise for consideration when the active growth situation of fish culture is envisaged:-

a) There is still a requirement to disentangle the effects of ammonia in general, and UIA in particular, from other factors, e.g. as described in para. 8.12.

b) There is a requirement for identification of the appropriate criteria of sublethal toxicity that should apply in a given situation.

c) There is, in particular, the necessity of assessing physiological responses of fish exposed to these culture environment effects.

Measurement of sublethal toxicity

8.24 Appendix D deals in detail with the rationale behind the use of the EC-50. This figure is equivalent to LC-50 in concept; EC-50 represents median effective concentration, the concentration at which 50% of subjects show a particular defined response under stated conditions. Whereas LC-50 implies the "lethal response" (death), an EC-50 can be defined for any desired quantity, e.g. loss of growth rate or loss of conversion rate. Since a level can be defined where 50% of subjects are affected, there can theoretically be an EC-0 where none are affected; in practice a true EC-0 is unobtainable. For this reason "no effect" values, or "safe" levels, such as the EIFAC UIA standard, are referred to in this study as IC_{max} (maximum ineffective concentration).

8.25 Use of the EC-50 does not imply necessarily the knowledge of the mode of action of the chemical involved; the concept is equally applicable to any desired criterion of sublethal toxicity, or tolerance.

Excretory materials

8.26 Since this appreciation of tolerance is independent of mode of toxic action, it can thus be independent of exact chemical identity of the toxicant. Provided that the toxicant supply is of relatively stable composition, any feature of it which is conveniently measurable as a concentration will suffice for describing an EC-50. Such an example is the excretory output of fish. This has been tacitly assumed to be ammonia; but as shown in Chapter 5, ammonia is not the only product; it is merely an indicator of nitrogenous excretory strength. Thus the question may be asked, is it possible and valid to determine the EC-50 for nitrogenous excretory materials taken together (using ammonia as an indicator), rather than to determine it for artificial ammonia solutions such as are used in most dosing experiments?

8.27 Herein lies a major difference between the fish-farming situation and the dosage experiments on ammonia toxicity: what is really important is not the effects of UIA, but the effects of dissolved nitrogenous excretory products in total. If Schulze-Wiehenbrauck (1974) is correct, and UIA is not responsible for growth losses when below 100 $\mu g\ l^{-1}$, then it is quite possible that some other excretory component is, quite aside from the effects of loading or stocking, etc. Putting this another way: what difference is there in growth effect between the dissolved nitrogenous excretory output of a fish tank and a made-up ammonia solution in dosage experiments?

8.28 It would seem that a useful approach to the consideration of the effect of recycled fish-farm water on the fish involved would be to investigate the tolerance of fish to their own dissolved nitrogenous excretory products (using ammonia as an index).

8.29 Finally, if as Schulze-Wiehenbrauck suggests, UIA is unimportant below about 100 $\mu g\ l^{-1}$, then within a reasonable pH range, TOA should be perfectly reliable as the ammonia index value, and TOA concentrations should suffice as indicators of dissolved nitrogenous excretory strength; TOA itself may be effective against growth rate, although the evidence to date (e.g. Burrows 1964) seems to suggest not.

INTRODUCTION TO T-SERIES EXPERIMENTS

Simulated recycled effluent

8.30 A scheme was prepared for investigating the growth-inhibition effects of the dissolved nitrogenous excretory products of rainbow trout in circular tanks in culture conditions.

8.31 Basic to this scheme was the provision of a source of such products. There were two possible sources:-

a) recirculation of the fish tank effluent back to the same tanks;

b) intallation of one series of tanks as an "effluent factory", with the effects of the effluent measured in a second series.

Because of the associated control problems of (a) (i.e. accumulation effects, deoxygenation of water problems, requirement for regulation of bleed-in water to a closed system), it was decided to use method (b) and set up an effluent "factory" of tanks serving a test series. The products of the "factory" required two major treatments before delivery to the experimental tanks: removal (screening) of solid wastes, and aeration to prevent interference by low oxygen effects.

8.32 Water produced by the "factory" tanks, and subsequently screened and aerated, is referred to as SREF (or simulated recycled effluent), and the design of T-series experiments is fundamentally a preliminary stage in the exploration of the growth-tolerance of rainbow trout to SREF.

Aims of study

8.33 Originally, the intention was to explore tolerance to SREF in terms of EC-50, as described in the preceding section. In

practice, shortage of time allowed for just two preliminary growth studies which can be viewed as pointing the way to further experiments. Fish from holding tank environments (and thus acclimated to low ambient inflowing TOA) were exposed to SREF in experimental tanks, whilst similar populations of fish under otherwise similar conditions were treated with control (inflow) water. SREF was quantified in terms of TOA measured, and information was sought on a series of effects:-

a) growth rate)
b) appetite) — over a 6-week growth trial
c) conversion ratio)

As only two experiments were possible, only two levels of SREF could be investigated.

8.34 Originally the intention was to chemically alter the SREF to a pH at which ammonia-dissociation would be higher. However, preliminary investigation with "natural" methods of raising pH, such as crushed calcareous shell (recommended in Burrows & Combs (1968) and Spotte (1970) for maintenance of pH in closed-system biological filter beds), proved ineffective. Clearly their effects in a closed system depend on accumulation as the water is recycled. Direct chemical dosing with alkali was then considered, using NaOH or a combination of NaOH and Na_2HPO_4. However, the quantities of chemical needed to meet the theoretical requirements of the fairly swift water flow proved excessively high. At very high alkali concentrations, the rate of drip-feed addition to the factory effluent would have been so slow as to require special equipment to maintain a correct feed (e.g. see Schulze-Wiehenbrauck 1974).

8.35 In practice it was decided to leave the pH of the SREF unaltered, thereby avoiding problems of chemical alteration. Whilst this limits the validy of the present study to soft water of relatively low pH (see Chapter 1), this is not necessarily too important. Modern fish-farming ventures, such as those of Shearwater Fish Farming, may well make use of soft water of low pH. The major difference is that almost all the

ammonia present is ionic, with negligible UIA: if Schulze-Wiehenbrauck is correct in his assessment of UIA growth-effects, this will not matter since the other factors will be causing any growth effects found. Also the subsidiary question arises of growth effects due to NH_4^+ itself: assuming negligible UIA, how concentrated can NH_4^+ become before growth is affected? This is, however, a separate question from the effects of SREF.

8.36 In addition to the growth parameters listed in para.8.44, several other criteria of effect were considered for investigation:-

a) haematocrit (as a measure of general health of the blood);
b) oxygen consumption capacity (as a measure of overall metabolic rates, or (in extreme) of damage to gills (frequently reported for UIA toxicity);
c) histological examination of selected organs, especially gills, liver and kidney (based on the known effects of UIA, as in Flis (1968), Smith & Piper [1975]).

These measures are all clearly measures of toxicity rather than of tolerance; they were considered from the point of view of additional evidence to support or contradict tolerance measures; in the case of contradiction, the way would be open for more rigorous toxicological assessment.

8.37 With regard to these additional parameters, Sprague (1971) remarks that haematocrit is useful only in so far as one can define "normal" or healthy values: in the present case no attempt is made to compare haematocrits on an absolute scale, only between control and experimental tanks. On oxygen consumption, Sprague laments the lack of experiments evaluating toxicants in terms of scope for activity (see Appendix D). It is important to emphasise that in the current study no full attempt to describe a stress in terms of oxygen consumption was made, although this is highly desirable once EC-50 levels have been roughly assessed. Instead, a few sample experimental and control fish were taken at the end of one of the T-series experiments, and allowed to settle in identical conditions to some-

where near a base level of activity (i.e. approximately the physiological 'standard metabolism', although the precise level is unimportant provided it was the same for all fish). Then their oxygen consumption rates were measured over a short period. This process demanded only a crude form of respirometer, but since the results were intended to be comparative (between experimental and control fish) and not definitive, this was acceptable for the purposes in hand. The only results envisaged as being important would be if there was a great difference between experimental fish and control fish (e.g. an order of magnitude), which might indicate a need for further assessment either by respirometry or toxicological methods.

8.38 In view of these considerations, it was decided not to proceed with histological assessment unless other measurements indicated a requirement to do so. (However, tissue samples were taken, fixed and wax-embedded in case of need.)

8.39 These two growth trials were envisaged as preliminary explorations only, and the conclusions drawn from them are consequently only pointers towards further examination at some future period.

9. TOLERANCE EXPERIMENTS

PREPARATION

Introduction

9.1 Basic and structural facilities were as described in Chapter 3, with some differences to meet the requirements of T-series experiments.

9.2 Facilities were required for:-

a) production of a fish effluent preferably containing high TOA,

b) screening out of effluent solid waste,

c) distribution of the screened effluent to experimental tanks,

d) a duplicate system for control tanks.

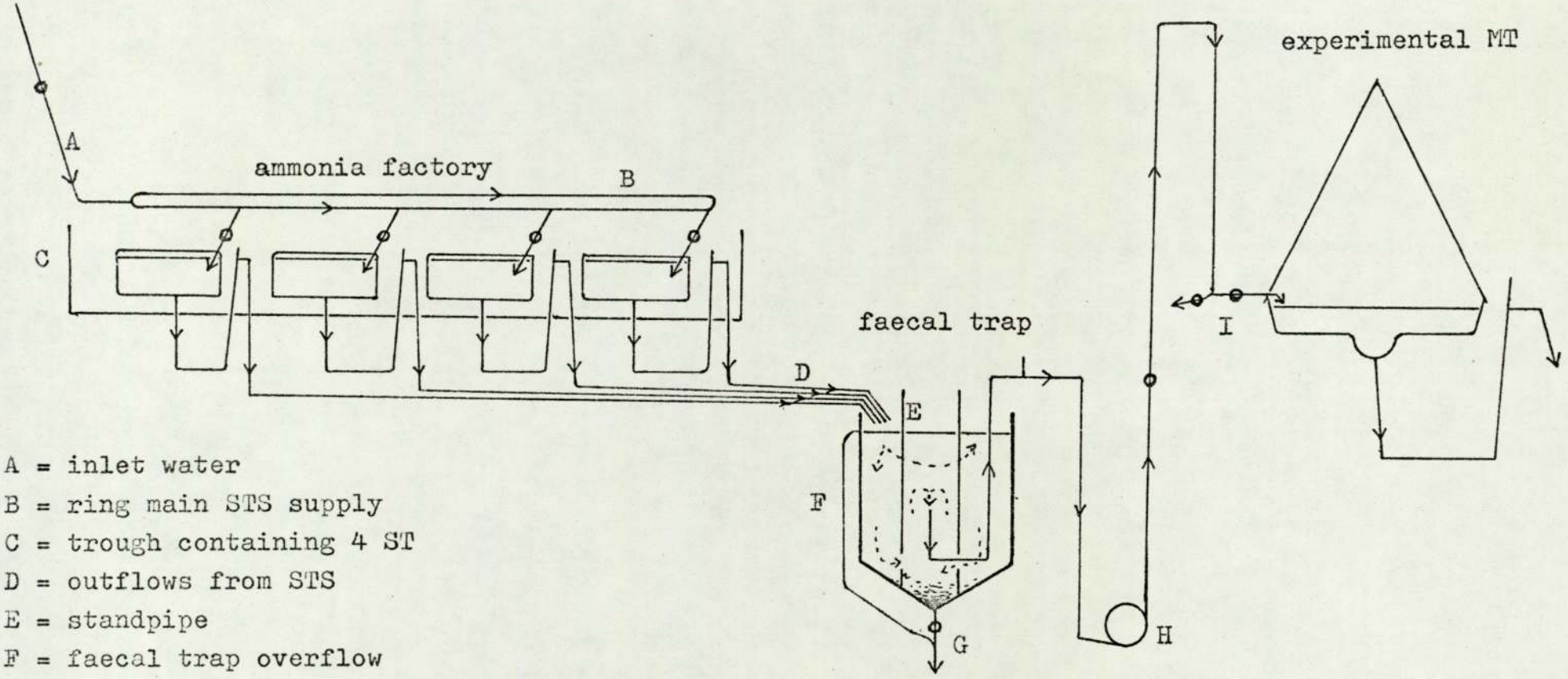
9.3 Figure 9.1 shows in diagrammatic form the system devised. Effluent water from four heavily-loaded tanks ("ammonia factory") was conducted to a faecal trap for solid-settling, and then pumped to experimental tanks. The system was duplicated from the faecal trap stage onwards for control water taken direct from inlet supply.

STS arrangement

9.4 Four ST type 2 were employed, clamped to the floor of the trough, with external overflow levelling, overhead lighting, blackout curtains and U-tubes.

9.5 It was necessary to have ready access to the fish in the tanks, and so the shroud used in other systems was replaced by the original lid across the tank top. Two holes pierced each lid, one to provide entrance for an air-line and diffuser stone, and a second for food. The latter had a tight-fitting plastic funnel-shaped cup inserted into the hole.

9.6 Fig.9.2 shows the major modifications in the STS when used for T-series experiments. The trough was maintained empty of water, acting as a safety overflow reservoir and helping to screen tanks. Above each tank twin feeders dispensed food; they were controlled by the automatic system and mounted on support arms attached to the STS framework. Food was dispensed through the tank lid receiving cup.



A = inlet water
B = ring main STS supply
C = trough containing 4 ST
D = outflows from STS
E = standpipe
F = faecal trap overflow
G = debris drainage
H = pump
I = inlet to experimental tanks (only one shown)
ø = control clip

Figure 9.1 T - series system : basic plan

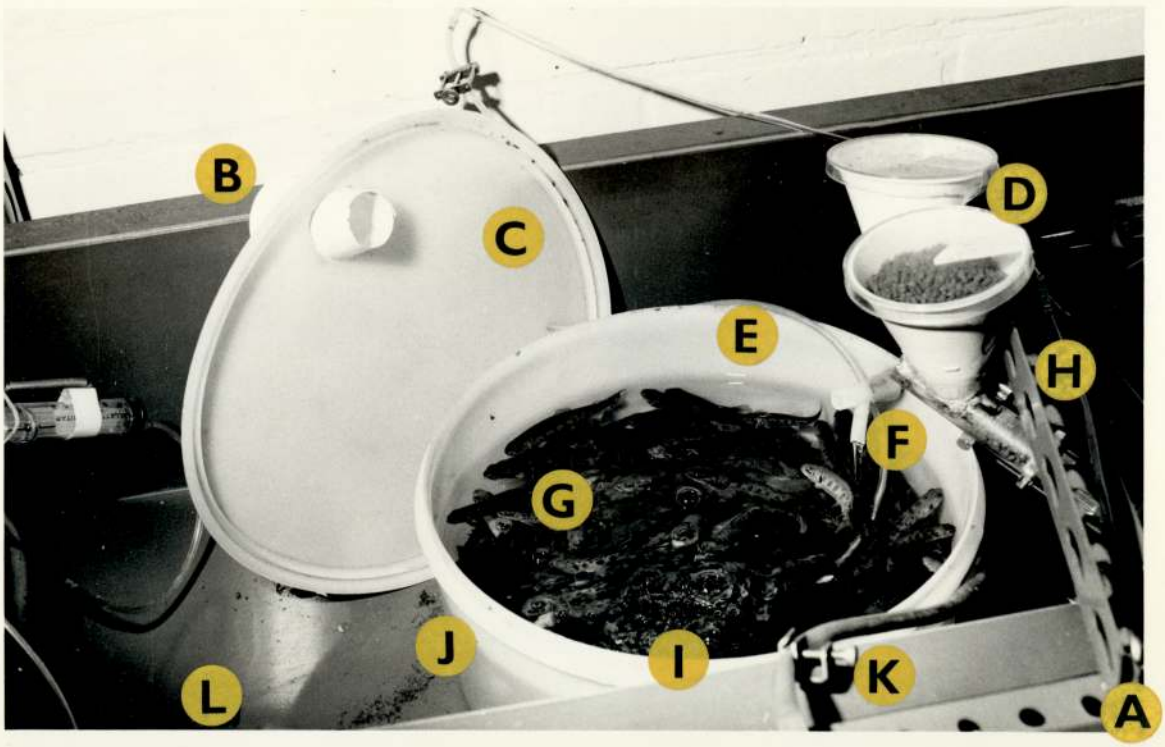


Figure 9.2 STS in T-series configuration

A STS framework
B Food receiving cup
C Tank lid (removed)
D Twin feeders for the tank
E Air line supplying tank through lid
F Densely-packed small fish
G Tank water inlet
H Feeders support arm
I Bubbles from diffuser stone
J ST with high water level
K Water inlet control at front
L Trough (empty of water)

9.7 U-tube arrangement was as for the MTS, the plastic tap of pilot experiments being unnecessary. At the overflow level controls, the outflow from each tree was continued directly into an individual effluent line (13 mm plastic tubing), which conducted effluent direct into the faecal trap. (The STS gutter was only used during cleaning operations and for trough overflow). Each effluent line was interrupted at convenient points by short rubber tubing sections at which the line could be opened, and a cleaning brush could be inserted, in between experiments or as required (the clear plastic allowed debris build-up to be observed).

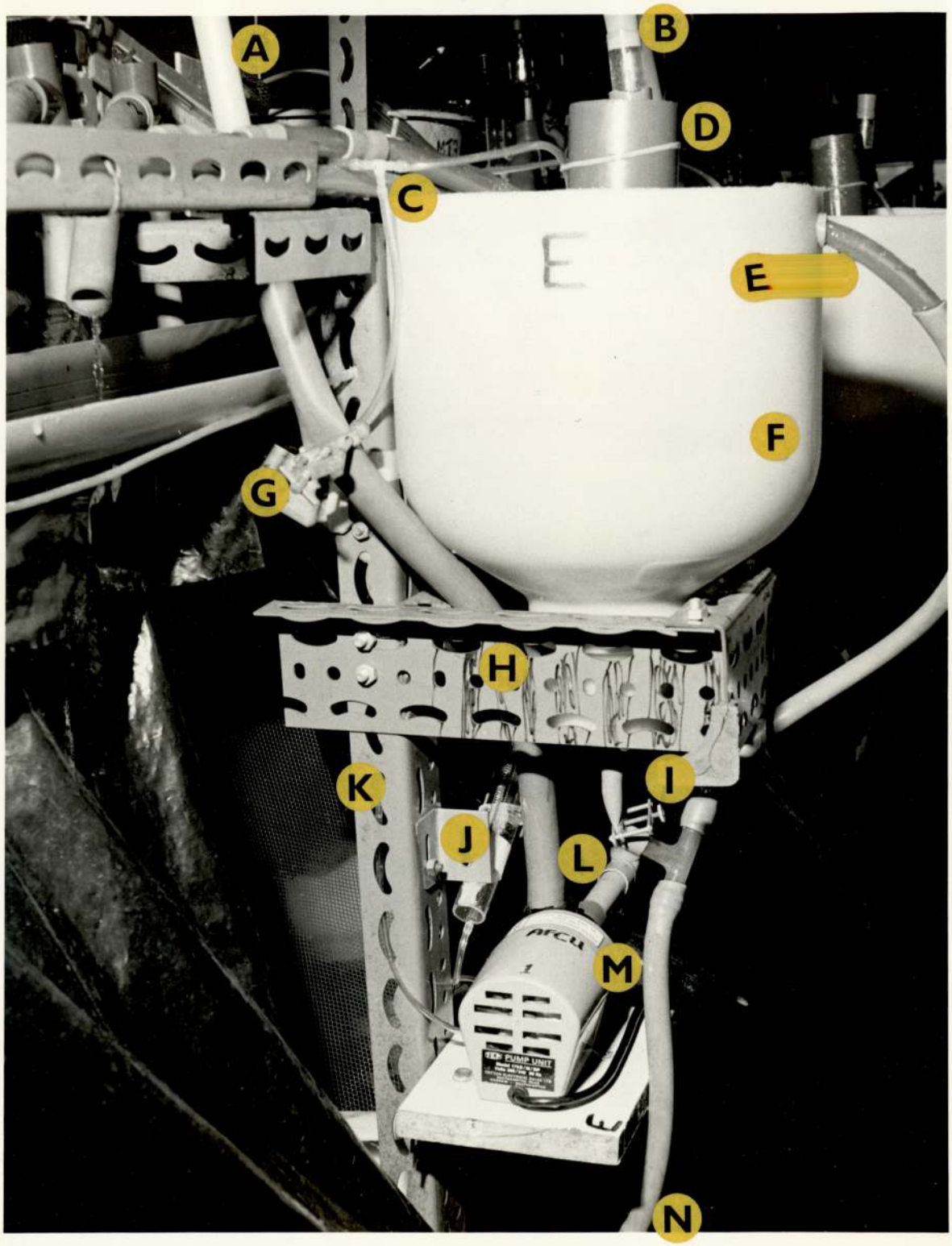
9.8 Individual ST effluent lines were used in preference to a larger common line in case of temporary loss of flow due to debris, which would thus only affect one tank at a time, instead of cutting off the complete flow. This factor was important for the continuity of pumping (see below).

Faecal trap and MTS arrangement

9.9 Four MT from the system were turned over from E-series experiments: the remaining four were maintained in use for E-series experiments, in company with some of the LTS.

9.10 Figures 9.3 and 9.4 show the equipment for screening and distributing STS effluent. Effluent entered the faecal trap from the individual lines at an angle, and so caused circular flow round the trap, during which faeces and any uneaten food wastes sedimented to the bottom. Here they could be removed by using a drain clip (Fig.9.3). The trap water level was controlled by overflow from a point 3 cm from the top, since inflow to the trap always exceeded the pumped outflow.

9.11 The trap was of high-density polyethylene, cylindrical over most of its height (25 cm) but tapering to an outflow point. The top diameter was 27 cm, and the volume approximately 11.4 ℓ. Overflow and drain lines were of 13 mm tubing. The trap was supported by a square section of framework around the bottom, bolted onto the major arch-leg of the main framework.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9.12 Inside the trap a vertical PVC standpipe (6 cm diameter, 36.5 cm height) was placed, with a ring of holes around the lower end allowing water entry (see Fig.9.1). Inside the standpipe a 2 cm diameter PVC exit pipe was positioned, with mouth opening vertically upwards, 9 cm from the bottom of the standpipe. This pipe passed through a slot in the side of the standpipe and conducted SREF upwards and out over the rim of the trap (see Fig.9.1) and then down to the pump below the trap (Fig.9.3) At the highest point (next to the trap rim), a "visitube" branch led vertically upwards for 2 cm and was securely bunged. This access branch could be used for filling the system and pump-priming; being of transparent plastic it also afforded a monitoring point for checking that the system was full of water (without air locks) during operation. Small bubbles could ascend to this point without blocking the exit tube; since the trap was directly aerated by two diffuser stones this was important.

9.13 SREF was pumped by a Totton Model 175B/M/DP electric pump into an ascending water pipe of 2 cm rubber tubing. The pump was screwed to a piece of wooden board securely bolted onto the main framework 20 cm below the trap. With the pump at the bottom of the system, there was a danger of spillage affecting it, but the danger of loss of pump water supply was minimised. In practice, relatively little splash-water found its way to the pump, and a tissue matting between pump and board absorbed it.

9.14 Control of the pumped SREF was exercised by a large Hoffman clip on the ascending water pipe. With a combination of control at this point and an extended head through which to push water the pump was effectively "throttled down" to give the required delivery.

9.15 The ascending SREF pipe was continued upwards by a PVC pipe (Fig.9.4) to a head of 120 cm above the pump. At this point it was conducted over the MTS superstructure girder and continued as a descending PVC pipe ending in a glass T-piece 5 cm above the rims of MT1 and 2. The cross-arms of the

Figure 9.4 MTS in T-series configuration

A Descending PVC SREF pipe

B Ascending PVC SREF pipe (cross-pipe above)

C MT1 (experimental tank)

D MTS superstructure framework: beyond, MT3 and 4 (control tanks)

E Normal MT2 inlet (closed off) behind shroud

F T-series inlet to MT2 passing through shroud; control clip beside T-piece

G T-series inlet to MT1 passing through shroud (control clip not shown)

H Tubing supplying volume indicator (see Chapter 3)

I MTS drainage gutter

J Faecal trap aeration line

K Four individual effluent lines from STS discharging into faecal trap

L Volume indicator tube and scale (see Chapter 3)

M MTS trestle framework

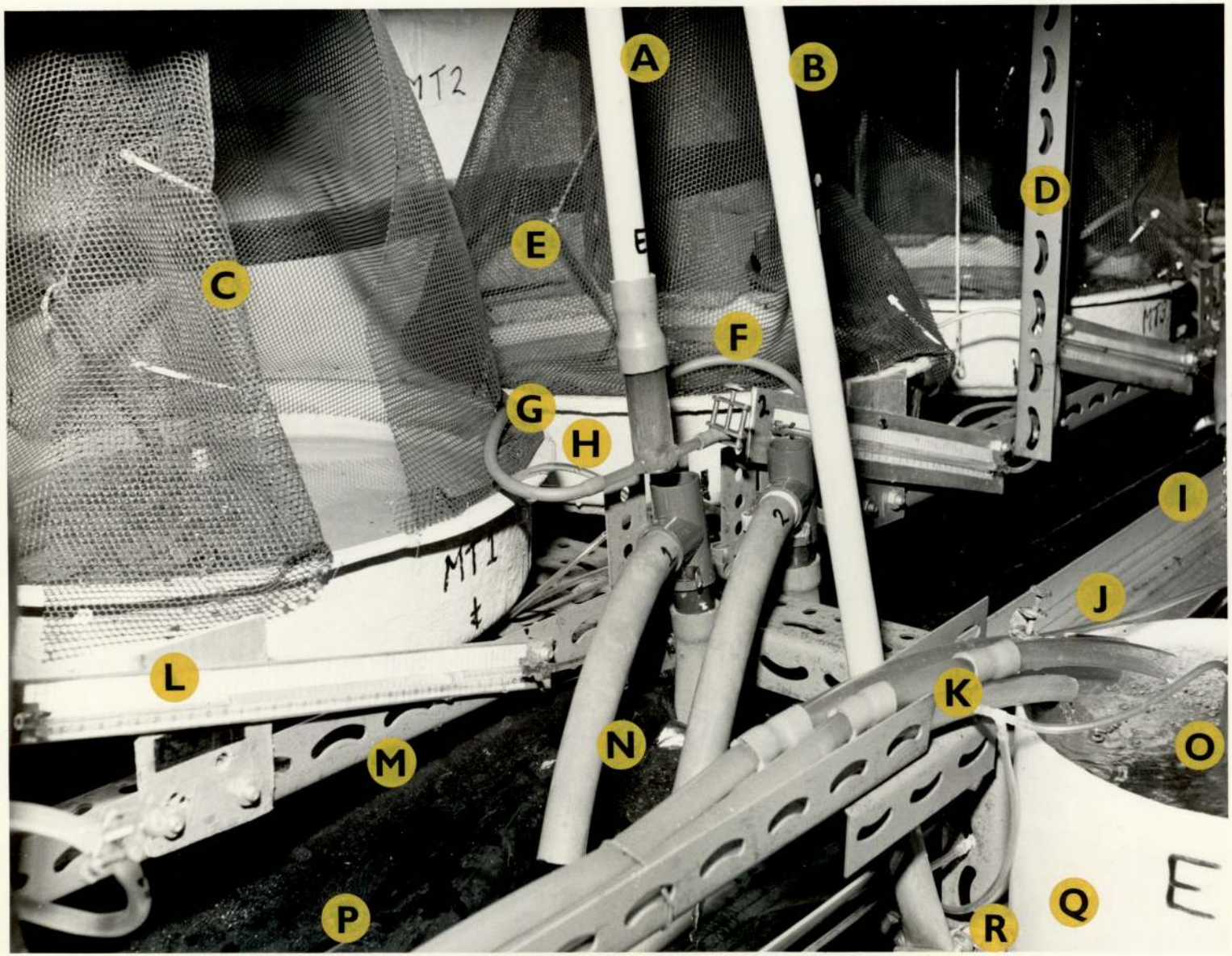
N MT1 overflow discharge pipe to gutter

O Faecal trap standpipe

P Blackout separator between MTS and LTS

Q Faecal trap

R Main control clip on ascending rubber pipe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MT2
MT1
E

T-piece were of 6 mm diameter, and from the arms short sections of rubber tubing (with control clips) conducted SREF to the experimental tanks, passing through the shrouds just above the tank rims, and supplying water at an angle for peripheral flow. (The normal MT inlets were closed off).

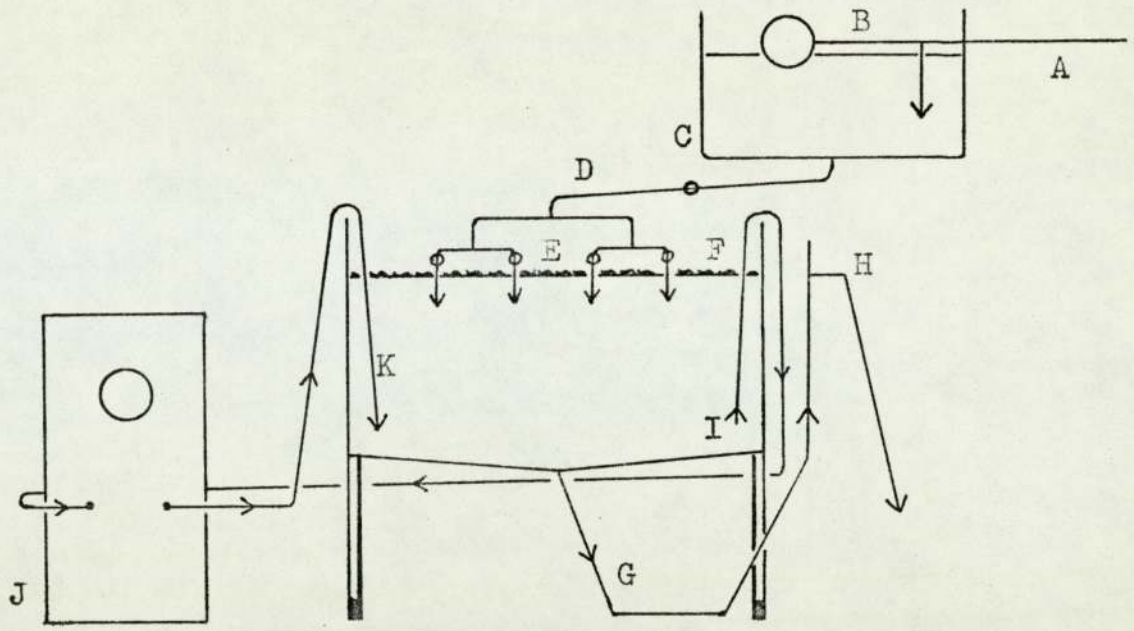
9.16 The system as described served two experimental tanks, MT1 and 2. Two further tanks, MT 3 and 4, were the controls. Their normal water supplies were also shut off, and water from a specially-inserted branch line off the MTS supply was used. This supply was controlled by a Hoffman clip, and conducted by 13 mm plastic tubing along the front of the MTS to discharge into a duplicate faecal trap, pump, and delivery system (mounted in a similar way to the first). The single water line to the control trap could handle enough water to match the experimental system's flow in four separate lines. In both systems the trap supply was greater than the pump output, the excess flowing to waste via the overflow tube.

9.17 The feeders and feeding attachments of MT 1, 2, 3 and 4 were removed, since feeding was by hand.

LT 1 adaptation

9.18 At the end of each T-series experiment, a large-capacity water-bath was required, for oxygen consumption tests, equipped with a supplementary controlled water supply. This was achieved by conversion of LT 1 as in Fig.9.5. The feeder and shroud were removed and normal inlet supply closed off. A 2 ℓ-capacity cistern was installed on a supporting framework approx. 30 cm above the tank. From this cistern one supply line (6 mm tubing) led downwards and branched by means of T-pieces into four equal supplies, each controlled by a clip. These supplies could discharge into the LT or into any apparatus placed in it, when a water supply was connected to the cistern ballcock valve. The flow to the tank escaped via the normal tank outlet/overflow system.

9.19 As a separate circuit, two 13 mm plastic tubes connected



A = inlet water supply
B = ballcock valve
C = cistern header tank
D = main supplementary supply
E = 4 branch supplementary supplies
F = expanded polystyrene pieces
G = LT outlet
H = overflow level control
I = outflow to chiller
J = chiller/thermocirculator
K = inflow from chiller
ø = control clip

Figure 9.5 Plan of arrangement of LT1 adapted as water-bath

the LT water-bath with the chiller/thermocirculator used in pilot experiments. This circuit controlled the water-bath temperature, aided by a layer of expanded polystyrene pieces spread across the LT water-surface. These pieces provided thermal insulation between the water and the air above, and the water-bath could easily maintain a desired low temperature. Black-out curtains were retained for screening-off LT 1.

Equipment sources

9.20 Manufacturers of important items are listed below:

Water pumps	- Totton Electrical Sales Ltd. Southampton.
Faecal traps (purchased as container bottles; bottoms removed)	- Jubb Containers
Glassware	- Glassblowers, Department of Physics, University of Aston
Oxygen measurement aquaria (see METHODS section) (to specification)	- Workshop, Dept. Biological Sciences, University of Aston

METHODS AND MATERIALS

Fish

9.21 In each experiment, two fish batches were used, all from Lots 03 and 04 pooled. The smallest fish (FCAT 1, 2 and 3) were used in STS, since smaller fish metabolise (and hence excrete ammonia) at a higher rate, weight for weight, than larger fish.

9.22 Fish in the MTS were all of FCAT 3. All fish selected had a healthy appearance and good maintenance record; all had been held at Aston for at least three months before use.

Feeding

9.23 During experiment, all MTS fish were fed Trout No.4 (floating) pellets. STS fish were fed a mixture of Salmon No.2 and No.3 (sinking) diets.

9.24 The STS feeding regime was as determined by the automatic procedure used for E-series experiments (see Chapter 7), with

five automatic feeds per day, dispensed from the twin feeders mounted over each ST. Amounts were calculated according to temperature from Table 1.3 (Chapter 1) and the twin feeders calibrated accordingly.

9.25 For the MTS fish, a measure of appetite was required (see Chapter 8) and so fish were fed by hand "ad-lib", through the tank shroud, until satisfied, twice during each day. Food in measured amounts was weighed out daily into small plastic containers, one per tank, and at each feed several pellets were sprinkled into the water flow in the tank, for each tank in turn. All these pellets were usually eaten at once, and then a few more were dropped in, from the appropriate container, again for each tank in turn. This process was repeated as necessary for each tank until at least four pellets were left uneaten between one visit and the next. This was taken as a signal of satiation. Experience with this method of feeding has confirmed that even excess pellets are usually consumed within the next 15 min or so; and the error due to loss of pellets rarely exceeds about 0.5 g if the feeding is carefully carried out (P.Smith, personal communication). After feeding, the containers were re-weighed and the food consumed found by difference; records were kept for each tank. In all cases consumption was excellent.

Tank fish densities

9.26 For the STS, the objective was to produce an effluent containing as much TOA as possible. This was done by crowding the tanks as full as seemed reasonable with small fish; although loading was not extremely excessive due to the low fish weight, stocking and fish density were high. In experiment TO1, the STS population density was 150 fish per tank, or about 15 fish ℓ^{-1}, and in TO2 this was increased to 200 fish per tank (20 fish ℓ^{-1}). Since the STS water flow averaged 0.5 ℓ min^{-1} per tank, the loading and stocking values would be in the region of 1.0 kg min ℓ^{-1} and 50 g ℓ^{-1} respectively, although they would fluctuate as fish grew. As this was a highly stressful environment,

and the fish employed were growing slowly, there was a steady mortality of one or two fish per tank per day. This was made up from the Lot 03/04 reserve stocks. It should be stressed that the sole function of the STS was as a fish effluent "factory" and the physiology of the fish was not studied in any other respect.

9.27 In the MTS, a minimal stress was required. Numbers were restricted to 20 in each tank, and so with water level maintained almost full and a flow of about 0.75 ℓ min $^{-1}$ through each tank, the following figures would roughly apply at the start of each experiment:

density : 2.0 fish ℓ^{-1}

stocking: 20 g ℓ^{-1}

loading : 0.3 kg min ℓ^{-1}

(The latter two values increased as fish grew.)

Sorting/weighing

9.28 Weighing took place fortnightly over the 6-week period of each experiment. Procedures were broadly as described in Chapter 7, but with the major difference that all fish were individually weighed to the nearest 0.1 g. Individual lengths were also measured in TO2. For individual weighing, anaesthetised fish were carefully "blotted" on damp absorbent paper to remove excess water, then placed on a dampened pad (tared) on the balance. The procedure could be speedily combined with length measurement, and the time that each fish was exposed to sorting stress (exclusive of anaesthetising)would not exceed 1 minute. In all cases fish recovered swiftly from anaesthetic and exhibited no side-effects, eating quite normally on the following day.

Procedure

9.29 After initial sorting (during an afternoon) each experiment lasted for 42 days. During this time, the fish were weighed again on day 14, day 28 and finally on day 42. Fish were not re-sorted on these occasions, but maintained as the same tank population throughout. During TO1, fish were weighed with empty guts (not fed on the morning previous to weighing) in the

interests of optimium comparability between weights. This had the disadvantage of interrupting the feeding schedule and thus the growth pattern. For TO2, the feeding schedule was maintained throughout, so that fish were weighed with guts in various stages of digestion. It was felt that the error thus introduced would average out over a tank population, and still allow comparability between experimental and control tanks. Initial sorting was on 19/11/74 for TO1 (final weighing 31/12/74), and on 13/1/75 for TO2 (final weighing 24/2/75).

9.30 Water samples for TOA measurement were taken as for E-series experiments. Volume and flow through tanks were also monitored daily and adjusted when necessary to preserve environmental conditions; fluctuation in flow occurred due to build-up of debris in the effluent lines, faecal trap and pumping circuit, hence daily monitoring and debris-removal was necessary. Pump operation, aeration, temperature, lighting, STS condition and MTS condition were also monitored daily. pH was monitored on every occasion of TOA measurement.

Haematocrit measurement

9.31 At the end of each experiment, two further tests were carried out on selected experimental and control fish; haematocrit readings and crude measurement of oxygen consumption capability. For haematocrit, a majority of fish from each tank (12) were removed to an aerated holding bucket one or two days after final sorting; in the meantime they had been maintained under experimental conditions. Each fish was anaethetised lightly in a bucket of MS.222 solution at 80 mg l^{-1}, killed by severing the backbone just behind the cranium, and the tail was cut off at a point just behind the vent. The cut surface was swiftly "blotted" with absorbent tissue (since dilution with water causes haemolysis), and blood collected from the caudal artery in a heparinised micro-haematocrit tube; this was allowed to fill to about two-thirds of its length when held horizontally. In this way sufficient blood could be obtained for measurement. (For TO2, duplicate tubes were taken for each fish). Micro-

haematocrit tubes were sealed at the clean end by careful rotation in a micro-burner flame (without affecting the blood), allowed to cool, placed in a haematocrit centrifuge, and spun at a frequency of 20 Hz for 7 min. They were then placed on a micro-haematocrit reader apparatus and the % volume of red cells read off on the scale and recorded. (This method follows the recommendations of Snieszko [1960].)

Oxygen uptake measurement

9.32 The apparatus used consisted of four modified small rectangular aquaria, each of volume about 7 ℓ and equipped with a sealed lid with inlet and outlet ports (Fig.9.6). For use, the aquaria were placed in the LT water-bath previously described (para. 9.18) with four supplementary water supplies connected to the inlets and under flow. The surrounding tank water level was below the central port in the lid. Two fish were selected from each MT (experimental and control) on the day of final weighing, care being taken to make sure that they were among neither the largest nor the smallest, but otherwise selected randomly. Each pair was placed in an aquarium through the central port, and the bung was inserted, thus sealing the aquarium and freeing it from air-bubbles. The LT water level was then raised (by adjusting the overflow) to cover the aquaria, which were weighted on top to ensure that they remained submerged.

9.33 The aquaria were screened from one another with sheets of opaque plastic, and the LT water-bath screened from surroundings by blackout curtain. The fish were left in darkness without food at a constant low temperature (about 6^{o}C) for 24 h, served by a flow of clean water from the cistern. After 24 h, a 50 ml syringe with flexible tubing attached was inserted into each aquarium outlet port, and a water sample removed for dissolved oxygen analysis. This was done in darkness, with as little fish disturbance as possible. The water supplies were then closed off and the outlet ports bunged, and the system left for 2 h exactly. Then a second water sample was taken from each aquarium and the fish were then removed from the aquaria and weighed individually.

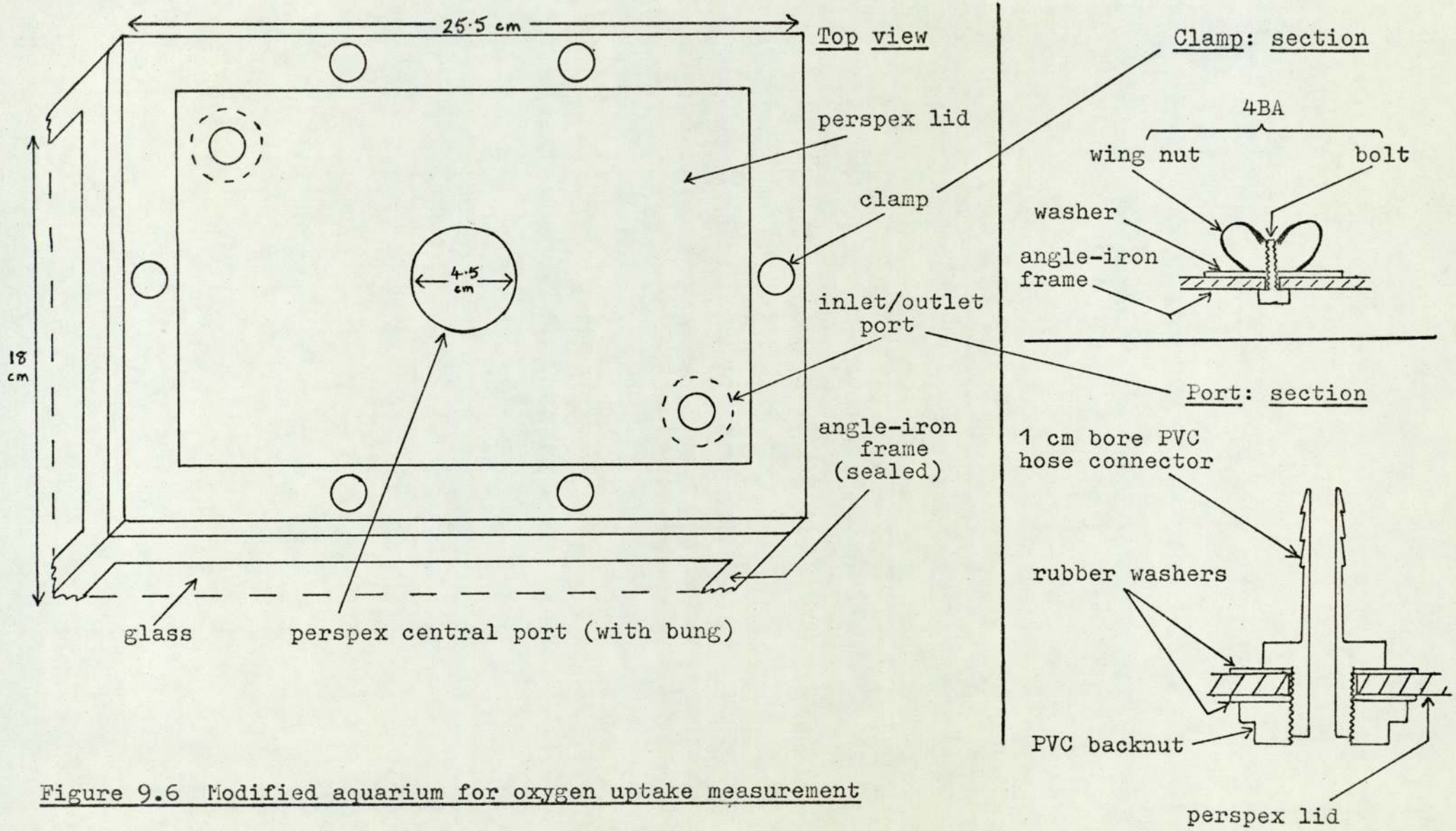


Figure 9.6 Modified aquarium for oxygen uptake measurement

Subsequently the full volume of each aquarium was determined accurately. From these measurements the fish oxygen uptake during the 2 h was estimated.

RESULTS

TOl conditions

9.34 Several quantities in the experimental conditions were variable, and required assessment in case of differential effects on experimental and control tanks. The major factors were temperature, flow, volume, stocking, loading (which were similar in all tanks), and inflow excretory product (which was intentionally different between experimentals and controls).

9.35 Temperature, volume and flow were measured at intervals, as was TOA in the water inflow to the tanks. Intermediate weight estimations were made, to correspond with these values, using the process described in Chapter 7. Thus appropriate values of stocking and loading could be calculated. All conditions are summarised, and their statistical analyses described, in Table 9.1. A multiple range test (Duncan 1955) was used to partition significant results and identify significant differences between tanks. Volume and stocking were found to differ significantly between tanks, but the test showed that the differences were due to individual tank fluctuations and did not represent a difference between experimental tanks on the one hand and control tanks on the other. Thus for volume:

$$\text{MT } \underline{1 < 4 < 3} \ll 2$$

- where

underlining indicates no significant differences, and a double magnitude sign represents a significant difference ($P = 0.05$). Similarly, for stocking:

$$\text{MT } \underline{3 < 2 < 4} \ll 1$$

(Although not analysed, it is clear that for TOA:

$$\text{MT } \underline{3 \doteq 4} \ll \underline{1 \doteq 2})$$

9.36 This type of analysis assumes that results from different days are completely equivalent, and that time (stage of experi-

Table 9.1 Environmental conditions in TO1

Quantity	Units	MT	MEAN	SEM	MINIMUM	MAXIMUM	ANALYSIS
Temperature	(^{o}C)	1	7.606	0.092	7.1	8.5	F = 2.2490
		2	7.611	0.091	7.1	8.5	P > 0.05
		3	7.361	0.087	6.9	8.0	NS
		4	7.378	0.084	6.9	8.0	
Volume	(ℓ)	1	9.46	0.21	8.4	11.1	F = 4.8917
		2	10.22	0.17	9.5	12.2	0.001 < P<0.01
		3	9.71	0.05	9.3	10.2	**
		4	9.67	0.10	9.3	10.9	
Flow	(ℓ min^{-1})	1	0.565	0.040	0.221	0.916	F = 1.4003
		2	0.571	0.033	0.229	0.851	P>0.05
		3	0.651	0.028	0.444	0.945	NS
		4	0.594	0.031	0.425	1.034	
Stocking	(g ℓ^{-1})	1	25.69	8.64	20.8	32.9	F = 7.5183
		2	21.96	6.30	19.0	27.5	P<0.001
		3	21.86	6.77	17.5	26.7	***
		4	22.00	5.21	18.5	25.7	
Loading	(kg min ℓ^{-1})	1	0.4924	0.0608	0.211	1.328	F = 2.4451
		2	0.4365	0.0505	0.216	1.190	P>0.05
		3	0.3402	0.0221	0.189	0.518	NS
		4	0.3773	0.0240	0.174	0.562	
TOA	(μg ℓ^{-1})	1, 2	359.7	13.3	268	445	(not analysed)
		3, 4	(<50)	-	(<50)	(<50)	

Table 9.1 continued

Notes:-

a) n = 18

b) MT1 and 2 are experimental, MT 3 and 4 control.

c) TOA was measured for each inlet supply (experimental and control), and not for all four tanks; all control values were always measured as below 50 $\mu g\ l^{-1}$ and so their accuracy is highly dubious and the values probably negligible; since the difference between these values and those of the experimental tanks is so large (by design), no analysis was performed.

d) The analysis of variance tested for significant differences between the four tanks for each environmental condition; probabilities are expressed against a null hypothesis; for the F values: df = 3,68.

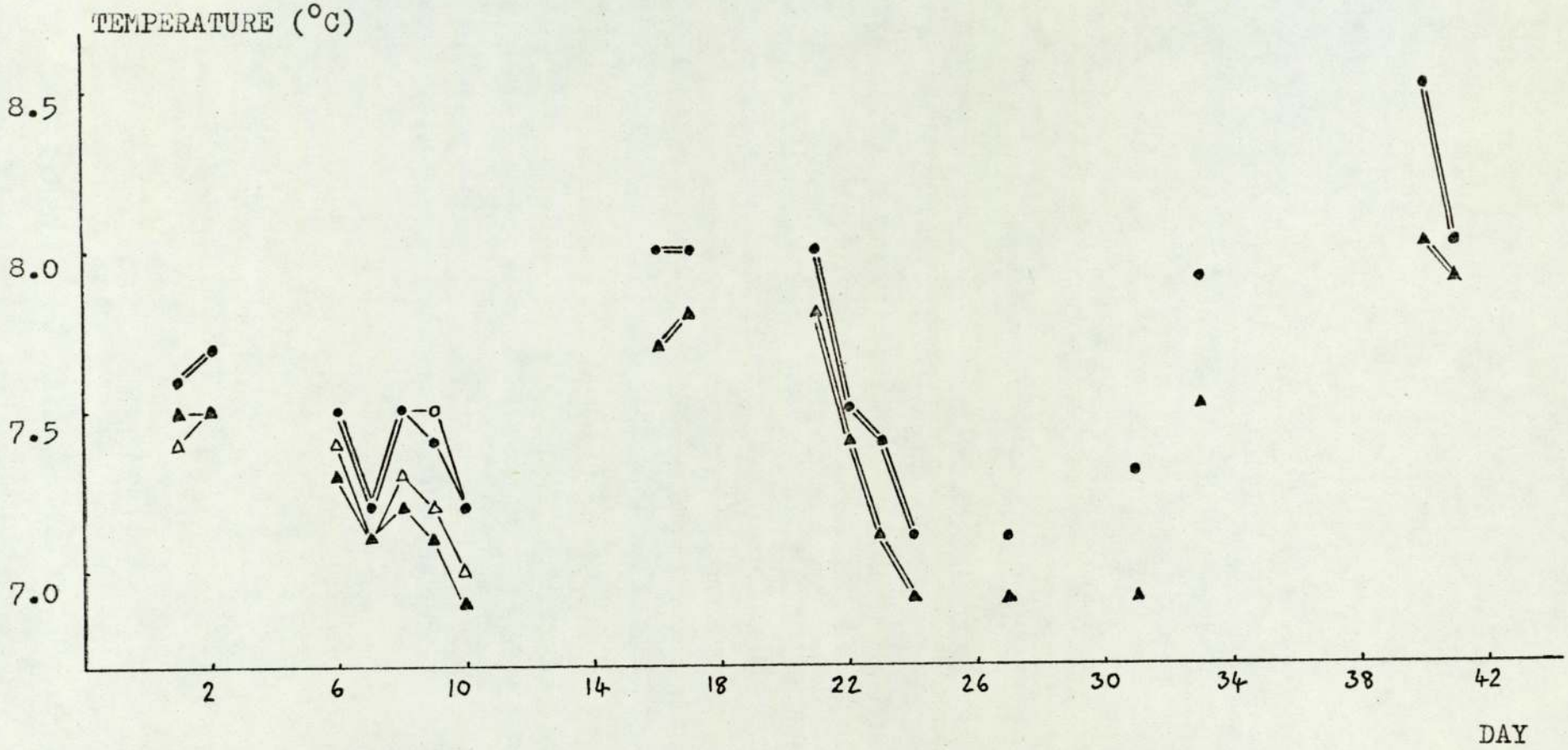
ment) had no effect; so that mean values for environmental conditions are compared in the context of their variances according to tank only. However, it must be recognised that experimental and control tanks might have differed if time were taken in account. Accordingly, this was checked and only temperature (Graph 9.1) shows a clear and consistent difference between experimental and control tanks. This, although small in relation to the total variability of the quantity in each tank, must be considered in any further analysis. (see DISCUSSION section).

9.37 pH was monitored and was consistently lower in the experimental tanks (mean pH 6.60, minimum 6.25, maximum 6.80), as would be expected from the pilot excretion experiment results. Control values were: mean pH 7.02, minimum 6.80, maximum 7.30. The effect of this pH difference in altering UIA would be insignificant; TOA in control tanks was too low for there to be appreciable UIA, whilst higher TOA in the experimental tanks, at lower pH, would still cause negligibly small UIA; i.e. orders of magnitude below the diuresis IC_{max}.

TO1 measured effects

9.38 For experiment TO1, weight was the only growth criterion measured directly, and fish weight results are summarised in Table 9.2. No mortalities occurred. Within each tank, the passage of time and growth opened out the range of weights about the mean for each weighing, as shown in Graph 9.2 for MT 1. Mean and SEM values are shown for all tanks and phases in Graph 9.3; it is clear that there is some divergence of the lines, and this was subsequently statistically tested.

9.39 Feeding was measured in terms of the food weight consumed, and daily records were kept for each tank; Table 9.3 gives a summary of food consumption data. As there were a few unavoidable occasions when feeding was missed, the trends are better appreciated by "ironing out" the effects of these occasions and the subsequent "compensation" feeding on the days following.



Graph 9.1 TO1 temperature results

Table 9.2 Weight results for TO1

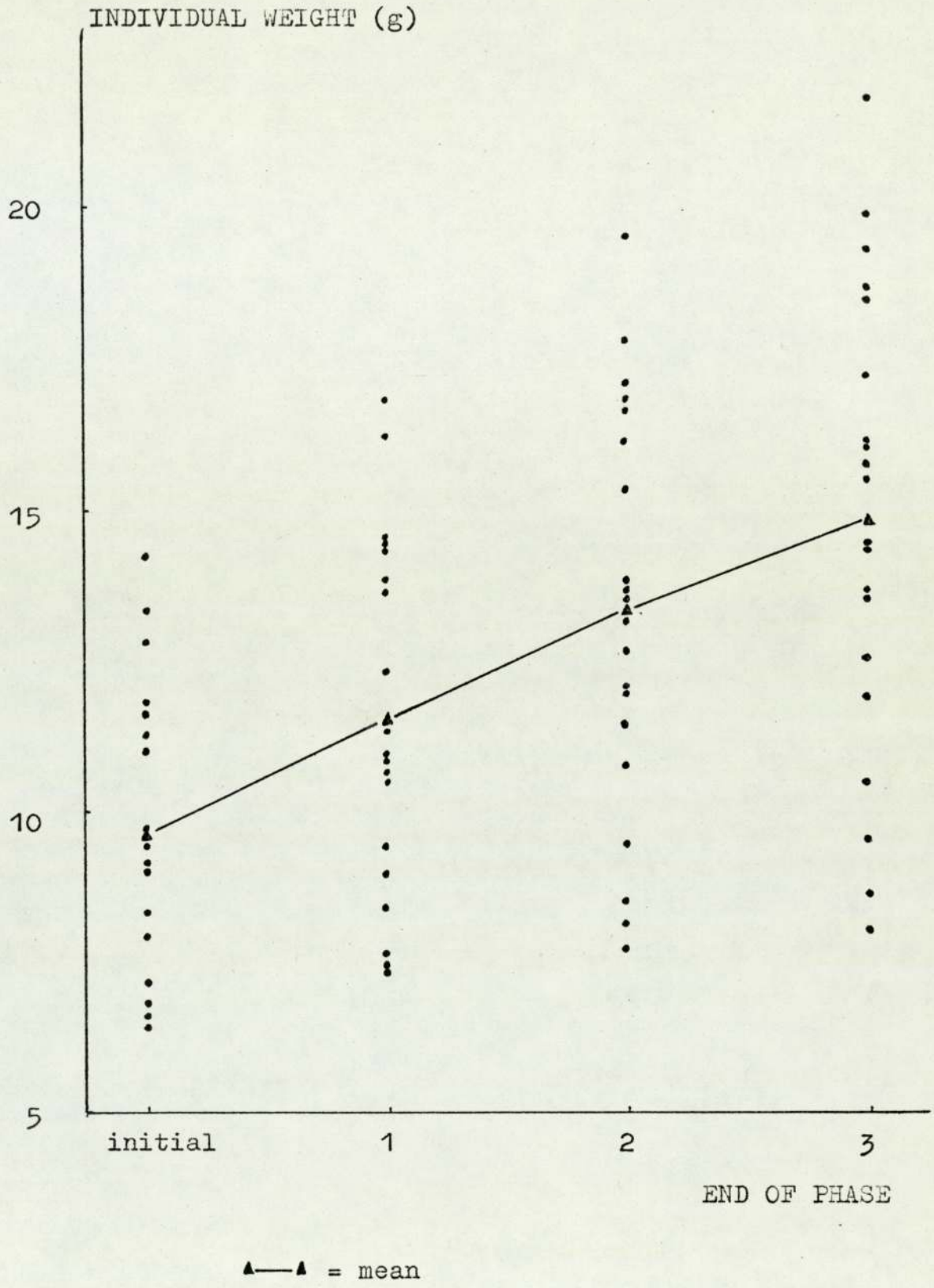
MT	INITIAL	END OF PHASE 1	END OF PHASE 2	END OF PHASE 3/FINAL
1	9.600	11.515	13.285	14.775
(EXPTL)	0.525	0.647	0.760	0.863
	6.4	7.3	7.7	8.0
	14.2	16.8	19.5	21.8
2	8.960	10.715	12.175	13.740
(EXPTL)	0.495	0.629	0.780	0.924
	6.0	7.5	8.1	8.3
	13.4	17.1	19.7	22.4
3	8.670	10.130	11.410	12.835
(CONTL)	0.452	0.535	0.740	0.890
	6.0	6.9	6.8	7.2
	12.8	15.9	18.5	20.6
4	8.905	10.070	11.510	12.830
(CONTL)	0.422	0.590	0.650	0.805
	6.5	6.6	7.6	7.7
	13.4	15.4	18.5	21.4

Notes:-

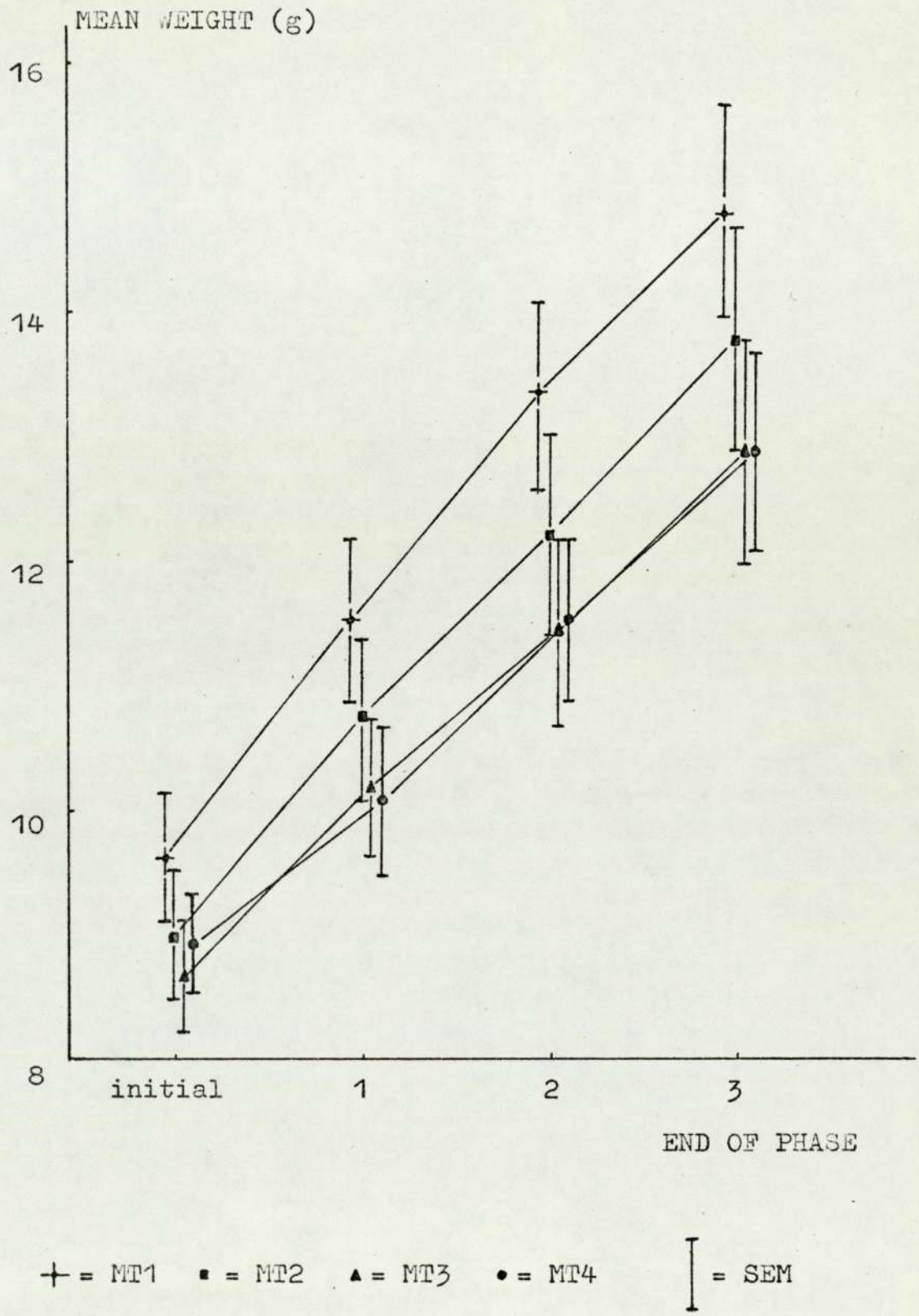
a) All values are in g; EXPTL = experimental; CONTL = control

b) Each cell contains values for mean, SEM, minimum and maximum, respectively. (n = 20)

c) Analysis of variance on the initial data for the four tanks indicates that there was no significant difference in the spread of mean weights between tanks at the start of the experiment. ($F = 1.4518$; $df = 3,76$; $P > 0.05$)



Graph 9.2 T01 individual weights of fish



Graph 9.3 T01 mean weights of fish

Table 9.3 Food consumption in T01

PHASE	MT 1	2	3	4
1	44.6	40.5	38.1	37.8
2	46.2	40.6	39.8	39.0
3	45.4	43.2	40.4	42.3
OVERALL TOTAL	136.2	124.3	118.3	119.1
OVERALL DAILY MEAN	3.243	2.960	2.817	2.836

All values in g

Table 9.4a Gross conversion ratios in T01

PHASE	MT 1	2	3	4
1	1.164	1.154	1.305	1.622
2	1.305	1.390	1.555	1.326
3	1.523	1.380	1.418	1.602
OVERALL	1.316	1.300	1.420	1.517

Analysis of variance : phases - $F = 1.3301$; $df = 2,6$; $P > 0.05$ NS
tanks - $F = 1.2718$; $df = 3,6$; $P > 0.05$ NS

Table 9.4b % weight increase in T01

PHASE	MT 1	2	3	4
1	19.95	19.59	16.84	13.08
2	15.37	13.63	12.64	14.30
3	11.22	12.85	12.49	11.47
OVERALL	53.91	53.35	48.04	44.08

Analysis of variance: phases - $F = 7.7155$; $df = 2,6$; $0.01 < P < 0.05$*
tanks - $F = 1.1601$; $df = 3,6$; $P > 0.05$ NS

This was done by taking a moving average set of values (span 3 days) and such a set is plotted both for MT1 (experimental) and MT3 (control) in Graph 9.4. (The duplicate experimental and control tanks' values were extremely similar to those illustrated.) Feeding data were further analysed as indicators of appetite as described in the DATA ANALYSIS section.

9.40 Gross conversion ratios were calculated for each tank for each period, and are shown in Table 9.4a. A two-way analysis of variance indicated that there was no significant difference due to tanks, nor due to phase of the experiment. Percent weight increase was calculated as

$$\left(\frac{\bar{x}_1 - \bar{x}_o}{\bar{x}_o} \right) \times 100$$

- where $\bar{x}_o$ = initial) mean weight for one phase
$\bar{x}_1$ = final)

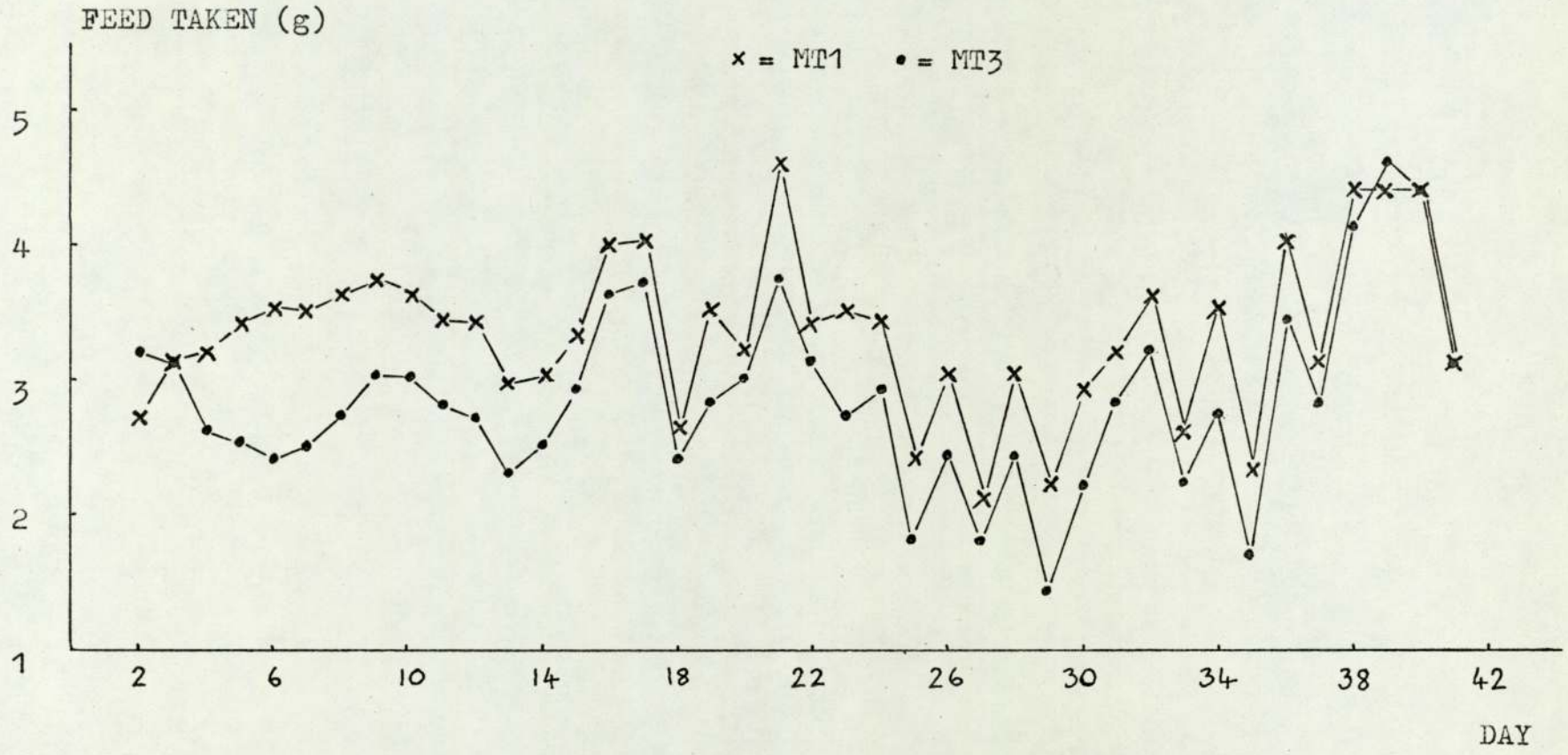
This showed no significant difference between tanks, but there was a difference according to phase of the experiment.

9.41 During haematocrit measurement, some tubes were lost due to breakage in the centrifuge, but the results from the remainder are summarised in Table 9.5a, followed by a comparison of the means for the tanks, using t-tests (Table 9.5b). MT1 was found to differ significantly from the other three tanks, but no other significant results were found.

TO2 conditions

9.42 As with TO1, experimental conditions were statistically tested for differences between experimental and control tanks. As length of fish was measured (as well as weight), the intermediate weight and length estimations could be used to determine the length-related loading factor (FACTØR in Chapter 7), and so this also was tested. Table 9.6 gives a summary of conditions and their analyses. Values for MT3 (control) are missing, since a structural failure on the 19th day caused the tank to be closed down.

9.43 The Duncan test was again applied to the significant



Graph 9.4 T01 food consumption: 3-day means

(plotted on middle day)

Table 9.5a Haematocrit from fish in TO1

	MT			
QUANTITY	1	2	3	4
MEAN	49.80	43.46	42.57	42.00
SEM	1.50	2.31	2.18	1.36
MINIMUM	40	36	33	38
MAXIMUM	57	64	50	53
n	10	11	7	11

Values are expressed as % red blood cells in the volume of blood.

Table 9.5b Comparison of TO1 haematocrit values

MT	1	2	3
4	$0.001 < P < 0.01$ **	$P > 0.05$ NS	$P > 0.05$ NS
3	$0.01 < P < 0.05$ *	$P > 0.05$ NS	
2	$0.01 < P < 0.05$ *		

$(df = n_1 + n_2 - 2)$

Values are given as probability results from t-tests between values from the tanks indicated.

Table 9.6 Environmental conditions in TO2

QUANTITY	UNITS	MT	MEAN	SEM	MINIMUM	MAXIMUM	ANALYSIS
Temperature	(^{o}C)	1	6.815	0.171	6.1	8.4	F = 1.3057
		2	6.792	0.170	6.1	8.4	P>0.05
		4	6.485	0.142	5.9	7.7	NS
Volume	(ℓ)	1	9.90	0.13	9.1	11.1	F = 5.9386
		2	10.37	0.10	9.7	11.0	0.001<P<0.01
		4	10.11	0.04	10.0	10.4	**
Flow	(ℓ min^{-1})	1	0.510	0.039	0.199	0.795	F = 1.7706
		2	0.534	0.041	0.209	0.755	P>0.05
		4	0.598	0.017	0.464	0.667	NS
Stocking	(g ℓ^{-1})	1	27.79	0.67	24.7	31.4	F = 0.1938
		2	28.09	1.04	21.4	33.2	P>0.05
		4	27.33	0.85	21.8	31.4	NS
Loading	(kg min ℓ^{-1})	1	0.5849	0.0531	0.317	1.128	F = 2.4978
		2	0.5972	0.0579	0.333	1.084	P>0.05
		4	0.4650	0.0154	0.349	0.521	NS
Loading factor	(kg min ℓ^{-1} m^{-1})	1	5.883	0.568	3.27	11.92	F = 2.0503
		2	5.893	0.590	3.48	11.41	P>0.05
		4	4.700	0.134	3.71	5.27	NS
TOA	(μg ℓ^{-1})	1, 2	698.2	60.5	370	1180	(not analysed)
		4	(<50)	-	(<50)	(<50)	

Notes:- (a) n = 13;(b) Notes (b) and (c) for Table 9.1 also apply here;(c) For analysis F values, df = 2,36

quantity, volume, and indicated:

$$MT1 < \underline{4 < 2} \qquad (1 \ll 2)$$

Thus the significant difference lay between experimental tanks 1 and 2, and not between experimental and control.

9.44 Temperature, volume and flow were checked against time, and once again only temperature shows a consistent difference between environmental and control tanks.

9.45 pH monitoring gave the following values for TO2:-

MT1/2 : mean 6.59, minimum 6.25, maximum 6.85

MT4 : mean 7.35, minimum 7.00, maximum 7.75

UIA values were negligible.

TO2 measured effects

9.46 Weight and length results are shown in Table 9.7. No mortalities occurred. Mean weights are plotted against time in Graph 9.5 and against mean lengths in Graph 9.6, where the lines for all three tanks appear to be in good agreement, the slight divergence of MT4 in phase 1 being removed by the end of the experiment. This suggests that all fish in TO2 can be considered as a homogeneous population.

9.47 Food consumption results were treated similarly to those of TO1; they are summarised in Table 9.8. Gross conversion ratios are shown in Table 9.9a and % weight increase values in Table 9.9b. Two-way analysis of variance indicated that neither quantity was significantly different, according to tanks or phases.

9.48 Haematocrit results are summarised in Table 9.10a followed by a comparison of means by t-test in Table 9.10b. No significant differences were found.

9.49 Results from the oxygen consumption test are shown in Table 9.11. Although only single values were obtained, and hence the tests have no statistical validity, the similarity in the values does not suggest any difference between experimental and control tanks. (For comparison, Davis (1956) quotes a range of 84 to 727 mg kg^{-1} h^{-1} oxygen consumption in salmonids under hatchery conditions.)

Table 9.7 Weight and length results for TO2

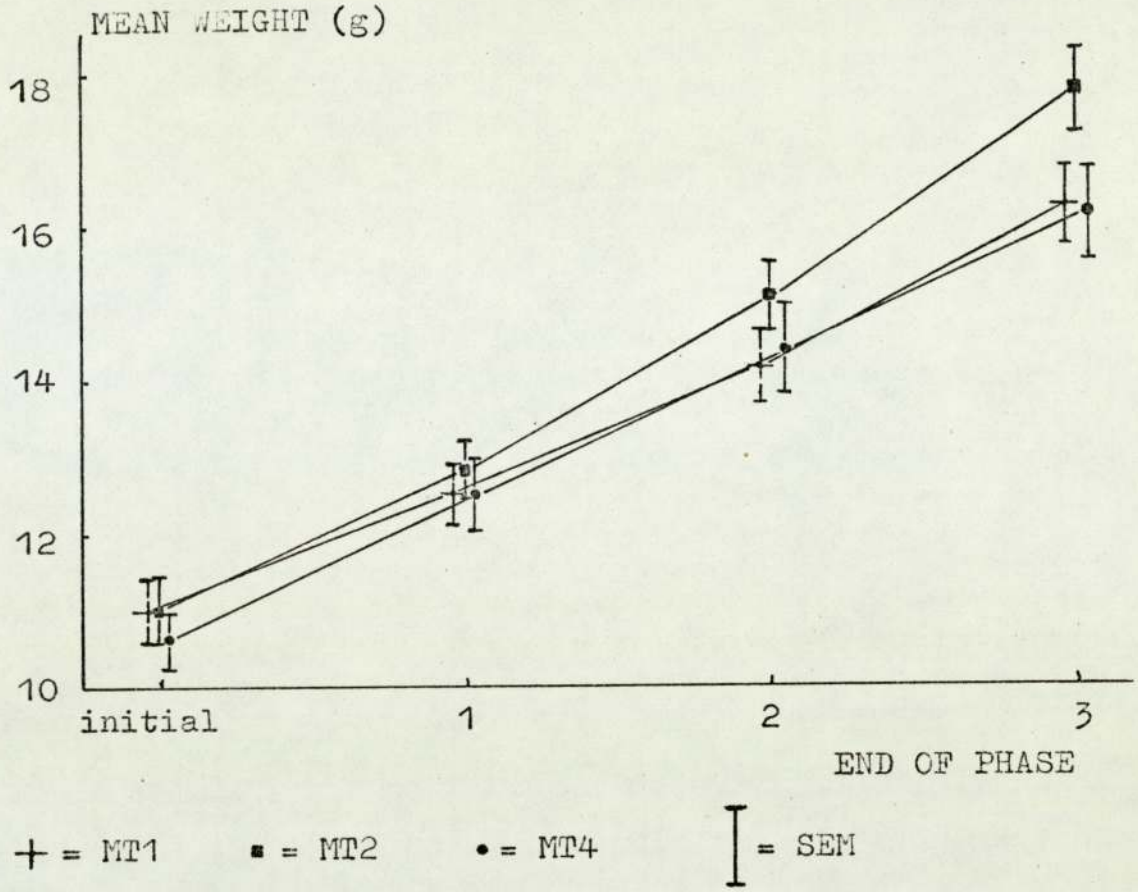
MT	WEIGHT (g)				LENGTH (cm)			
	INITIAL	END PHASE 1	END PHASE 2	END PHASE 3/FINAL	INITIAL	END PHASE 1	END PHASE 2	END PHASE 3/FINAL
1	10.970	12.460	14.165	16.330	9.425	9.675	10.050	10.525
	0.386	0.421	0.457	0.539	-	-	-	-
	7.1	8.5	9.8	10.8	8.5	8.5	9.0	9.0
	14.3	15.6	17.1	20.3	10.0	10.5	11.0	11.5
2	11.020	12.760	15.120	17.820	9.450	9.750	10.250	10.800
	0.365	0.405	0.449	0.566	-	-	-	-
	7.8	8.5	10.2	11.9	8.5	8.5	9.0	9.5
	14.5	16.0	18.7	22.9	10.0	10.5	11.0	11.5
4	10.565	12.470	14.430	16.245	9.300	9.525	10.000	10.450
	0.360	0.451	0.541	0.630	-	-	-	-
	7.2	8.7	9.3	10.4	8.5	8.5	9.0	9.0
	13.6	15.8	19.2	22.0	10.0	10.5	10.5	11.5

Notes:-

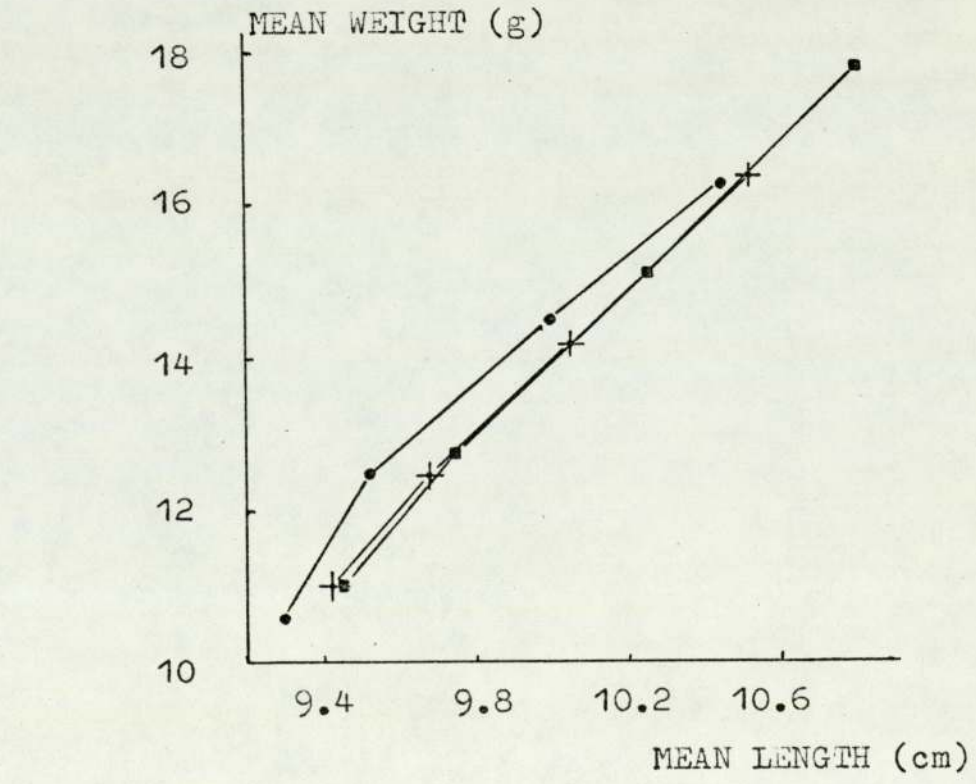
a) Each cell contains values for mean, SEM, minimum and maximum, respectively (n = 20).

b) SEM values for length are omitted, as length was measured in 0.5 cm classes.

c) Analysis of variance on initial weight data indicates no significant difference between tanks ($F = 2.2036$; $df = 2,57$; $P > 0.05$).



Graph 9.5 TO2 mean weights of fish



Graph 9.6 Length and weight of fish in TO2

Table 9.8 Food consumption in TO2

PHASE	MT 1	MT 2	MT 4
1	37.8	44.4	44.4
2	45.2	54.2	53.0
3	56.4	63.1	58.7
OVERALL TOTAL	139.4	161.7	156.1
OVERALL DAILY MEAN	3.319	3.850	3.717

All values in g

Table 9.9a Gross conversion ratios in TO2

PHASE	MT 1	MT 2	MT 4
1	1.268	1.276	1.165
2	1.326	1.148	1.352
3	1.303	1.169	1.617
OVERALL	1.300	1.189	1.374

Analysis of variance: phases - F = 0.5672; df = 2,4; P>0.05 NS
tanks - F = 1.1016; df = 2,4; P>0.05 NS

Table 9.9b % weight increase in TO2

PHASE	MT 1	MT 2	MT 4
1	13.58	15.79	18.03
2	13.68	18.50	15.72
3	15.28	17.86	12.58
OVERALL	48.86	61.71	53.76

Analysis of variance: phases - F = 0.0872; df = 2,4; P>0.05 NS
tanks - F = 1.5676; df = 2,4; P>0.05 NS

Table 9.10a Haematocrit from fish in TO2

QUANTITY	MT 1	2	4
MEAN	38.33	39.92	36.64
SEM	1.10	1.70	1.54
MINIMUM	34	32	27
MAXIMUM	45	53	45
n	12	12	11

Values are expressed as % red blood cells in the blood volume.

Table 9.10b Comparison of TO2 haematocrit values

MT	1	2
4	$P>0.05$ NS	$P>0.05$ NS
2	$P>0.05$ NS	$(df = n_1+n_2-2)$

Values are given as probability results from t-tests between values from the tanks indicated.

Table 9.11 TO2 oxygen-consumption test results

MT	MEAN TITRE (ml)			DO FALL (mg l^{-1})	BOX VOLUME (l)	FISH WEIGHT (g)	OXYGEN CONSUMPTION (mg kg^{-1} h^{-1})
	INITIAL	FINAL	CHANGE				
1	2.86	2.47	-0.39	1.505	7.05	32.6	171.4
2	2.85	2.48	-0.37	1.504	7.00	27.5	191.3
4	2.84	2.36	-0.48	1.951	7.08	36.7	188.1

Notes:-

a) Temperature = 6.0°C.

b) Titre is in ml of N/80 thiosulphate solution required during Winkler estimation; value given is mean of two estimations; INITIAL indicates before 2 h of test run (see METHODS section), and FINAL indicates immediately afterwards.

c) DO (dissolved oxygen) FALL is given by:- $\frac{\text{titre difference} \times 101.6}{\text{volume of sample titrated}}$

d) FISH WEIGHT is the sum of the two individual weights.

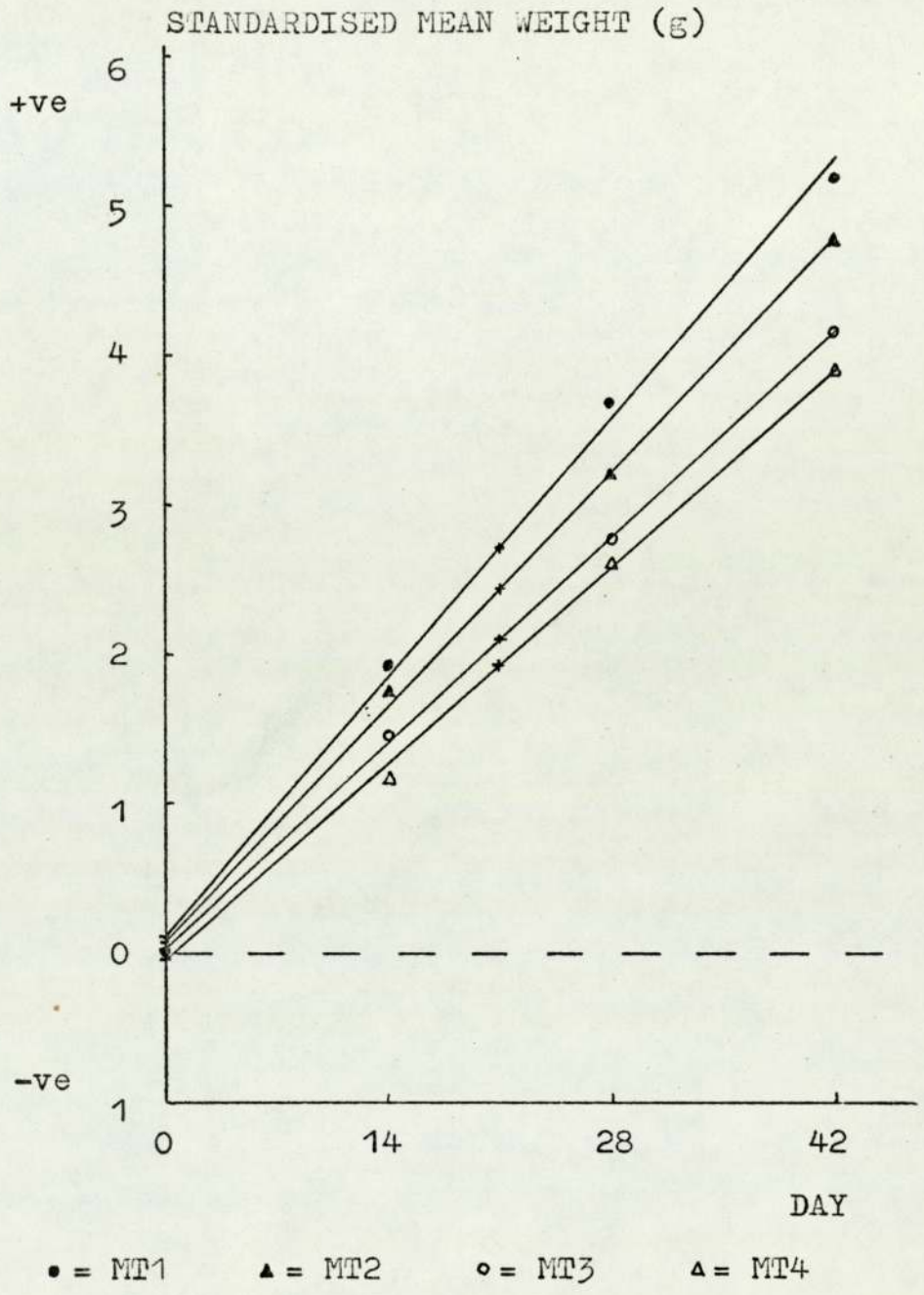
DATA ANALYSIS

9.50 All calculations were performed on an Olivetti Programma P101 desk computer unless otherwise stated.

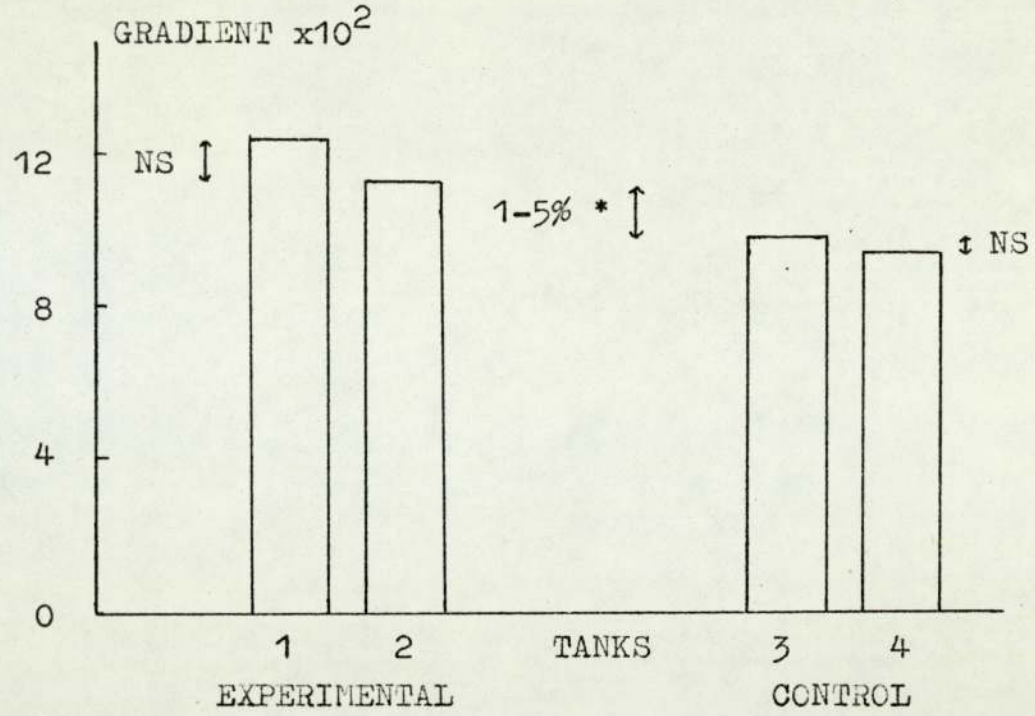
Analysis of growth in T01

9.51 Graph 9.3 showed some growth line divergence in T01, and in order to assess the importance of this, the slope differences between the lines were statistically tested. This demanded two steps; (a) regression of the points to define an equation which would reveal the overall slope, and (b) statistical test of the slope differences. For (a), the simplest method was to perform a regression analysis on the untransformed mean weight data. However, when Haskell (1948) compared growth curves for salmonids, including using simple weight data, log transformation, and cube root transformation, his recommendation was to transform mean weight values to their cube roots to give the best straight-line. In the present case, all three methods were used, and the best straight line selected by comparison of correlation coefficients. Slopes for the four tanks were then compared statistically according to a standard process (Cole 1975).

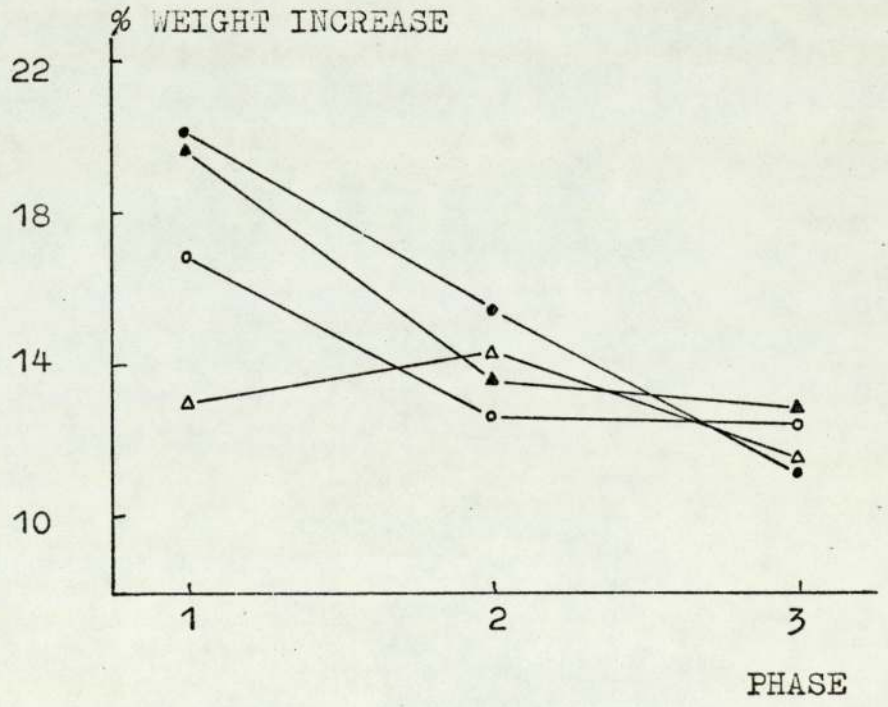
9.52 Table 9.12a shows the correlation coefficients, and the one selected is that with the highest average value over the four tanks; in this case simple mean weight. Values for this quantity are plotted against time, with regression lines, in Graph 9.7, having first been standardised to make the graph clearer. (The standardisation is equivalent to assuming that fish in all tanks started off at the same mean weight, artifically set to zero.) Limits are not given for the regression lines or predicted Y values, since the lines are only for comparison and not for prediction. Table 9.12b gives details of the regression lines and the slope analysis; and the significant difference between the experimental and control groups is illustrated in Graph 9.8. Similar analyses performed on log and cube root transformations show no significant differences.



Graph 9.7 Regression of standardised mean weight with time for T01



Graph 9.8 Gradient differences for T01 regression lines



Graph 9.9 T01 % weight increase by phase

9.53 This analysis was carried out on the ICL 1905 computer using program GRADIENTS (flowchart in Fig.9.7). The following calculations were performed, from sets of data input for time, mean weight, log mean weight, and cube root of mean weight; for each tank:-

(x = time and y = weight data)

a) $\Sigma x,\ \bar{x},\ \Sigma x^2,\ \frac{(\Sigma x)^2}{n}$

b) SS_x (sum of squares) $= \Sigma x^2 - \frac{(\Sigma x)^2}{n}$

s_x^2 (variance) $= \frac{SS_x}{n-1}$

c) $\Sigma y,\ \bar{y},\ \Sigma y^2,\ \frac{(\Sigma y)^2}{n}$

d) $SS_y = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$

$s_y^2 = \frac{SS_y}{n-1}$

e) $\Sigma xy,\ \frac{(\Sigma xy)^2}{\Sigma x},\ \frac{\Sigma x.\Sigma y}{n}$

f) $SS_{xy} = \Sigma xy - \frac{\Sigma x.\Sigma y}{n}$

g) s_{xy}^2 (covariance) $= \frac{SS_{xy}}{n-1}$

h) $R = \frac{s_{xy}^2}{\sqrt{s_x^2 . s_y^2}}$

$b = \frac{s_{xy}^2}{s_x^2}$

$a = \bar{y} - b\bar{x}$

Then for each pair of tanks (denoted as 1 & 2):-

i) $s^2_{y_1.x} = \frac{n_1 - 1}{n_1 - 2}(s_{y_1}^2 - b_1^2 s_x^2)$

$s^2_{y_2.x} = \frac{n_2 - 1}{n_2 - 2}(s_{y_2}^2 - b_2^2 s_x^2)$

} population variances

j) $F_{12} = \dfrac{s^2_{y_1.x}}{s^2_{y_2.x}}$ (tested for significance, df = n_1-1, n_2-1)

Assuming F is not significant, and thus population variances are $\pm$ equal, then:

k) $$s^2_{y.x.p} = \frac{(n_1 - 2)s^2_{y_1.x} + (n_2-2)s^2_{y_2.x}}{n_1 + n_2 - 4}$$

(pooled variance)

l) $$s^2_{(b_1-b_2)} = s^2_{y.x.p.}\left(\frac{1}{[n_1 - 1]s_x^2} + \frac{1}{[n_2-1]s_x^2}\right)$$

(variance of b_1-b_2)

and, finally:

m) $$t = \frac{b_1 - b_2}{\sqrt{s^2_{(b_1 - b_2)}}}$$

(df = $n_1 + n_2 - 4$)

9.54 In all cases, for both TO1 and TO2, the F-test at (j) proved non-significant, and the t statistic was thus valid, and its probability could be checked against the usual t distribution.

9.55 Percent weight increase is illustrated in Graph 9.9. Overall % weight increase is lower in control tanks, and % weight increases for different phases. The trend is clearly towards a lower value in all tanks as expected; fish once having passed their earliest growth phases tend to decelerate in growth rate. There is no clear difference btweeen experimentals and controls. Further analysis of % weight increase can be achieved by plotting it against the mid-phase mean weight (i.e. $\frac{\bar{x}_o + \bar{x}_1}{2}$

where $\bar{x}_o$ = initial) mean weight for one phase).
$\bar{x}_1$ = final)

Such a plot (Graph 9.10) for TO1 shows a suggestion of a difference between the estimated average for experimental tanks and the average for control tanks. This suggests a possibility of

Figure 9.7 Flowchart for program GRADIENTS

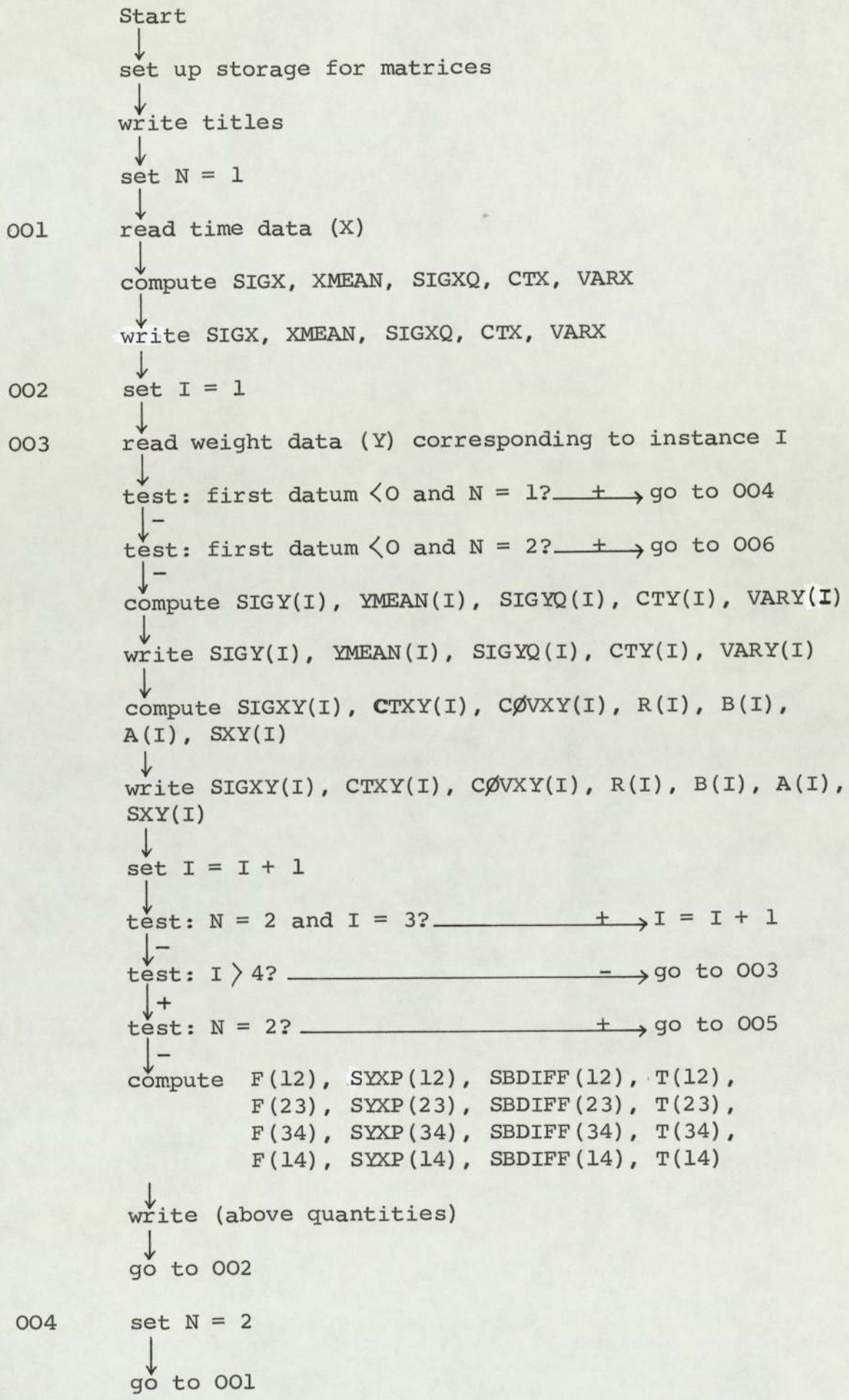


Fig. 9.7 continued

```
005     compute  F(24), SYXP(24), SBDIFF(24), T(24),
                 F(41), SYXP(41), SBDIFF(41), T(41),
                 F(21), SYXP(21), SBDIFF(21), T(21)

        go to 002

006     stop
```

Notes:-

a) Codes refer to the quantities described in para. 9.53 as follows:-

X : x	Y : y
SIGX : $\lesssim x$	SIGY : $\lesssim y$
XMEAN : $\bar{x}$	YMEAN : $\bar{y}$
SIGXQ : $\lesssim x^2$	SIGYQ : $\lesssim y^2$
CTX : $(\lesssim x)^2/n$	CTY : $(\lesssim y)^2/n$
VARX : s_x^2	VARY : s_y^2

SIGXY : $\lesssim xy$

CTXY : $(\lesssim x . \lesssim y)/n$

CØVXY : s_{xy}

R : R

B : b

A : a

SXY : $s^2_{y.x}$

F(12) : F_{12}

SYXP(12) : $s^2_{y.x.p}$

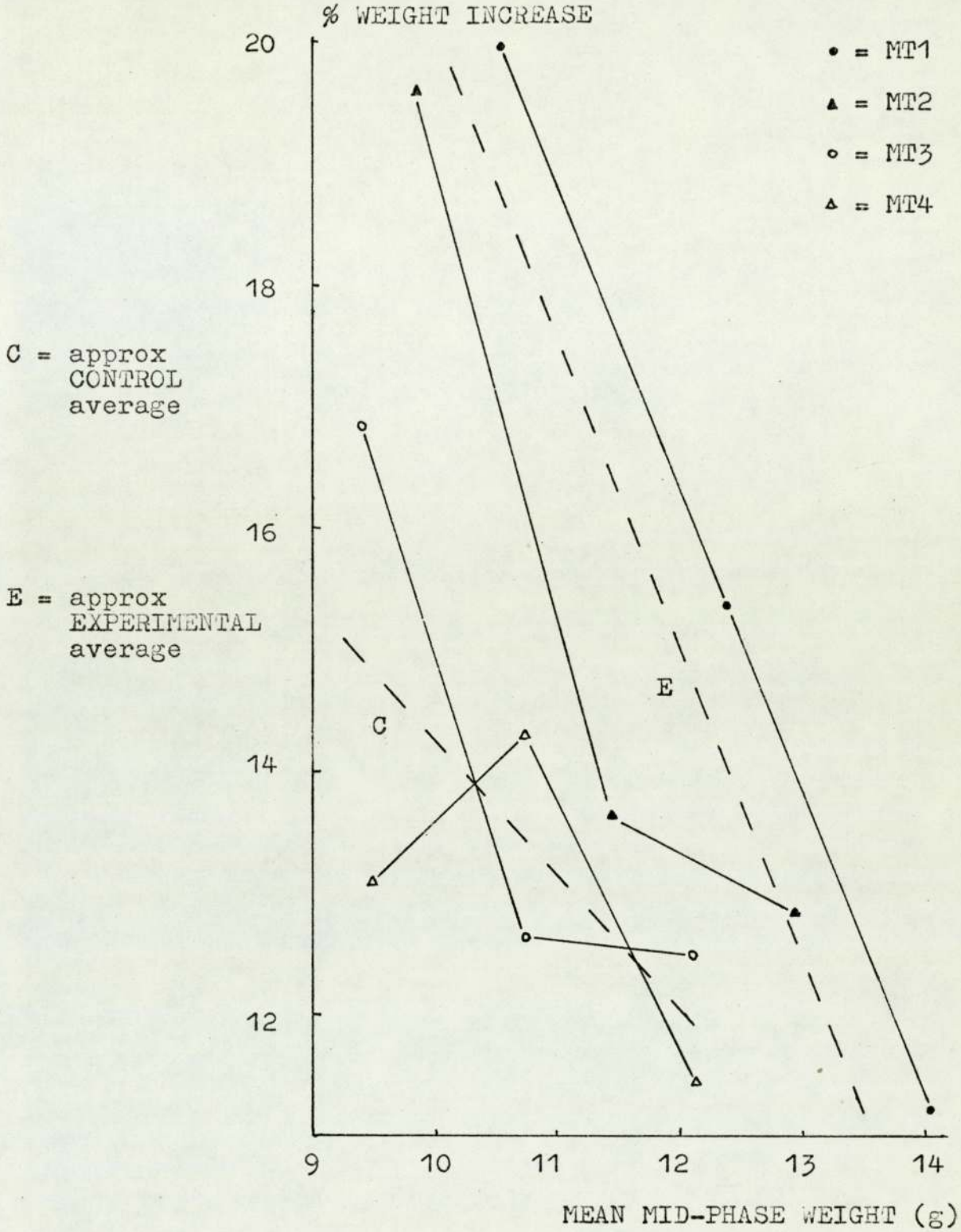
SBDIFF(12) : $s^2_{(b_1-b_2)}$

T(12) : t

} etc., where numbers refer to tanks under comparison

b) I and N are repeater codes for program loops

c) "set" and "compute" used as in Chapter 7, Fig.7.9, note(g)



Averages show increased growth rate in experimentals compared to controls; agrees with Webb & Brett (1973) observations for levels below EC-50

Graph 9.10 T01 % weight increase by size

an increased growth rate (as measured by phase % weight increase) at a given individual fish weight in experimental tanks, although the average lines look as though they are converging as fish become heavier. Since the data are too scanty for statistical analysis, however, this can only remain a suggestion.

Analysis of appetite in TO1

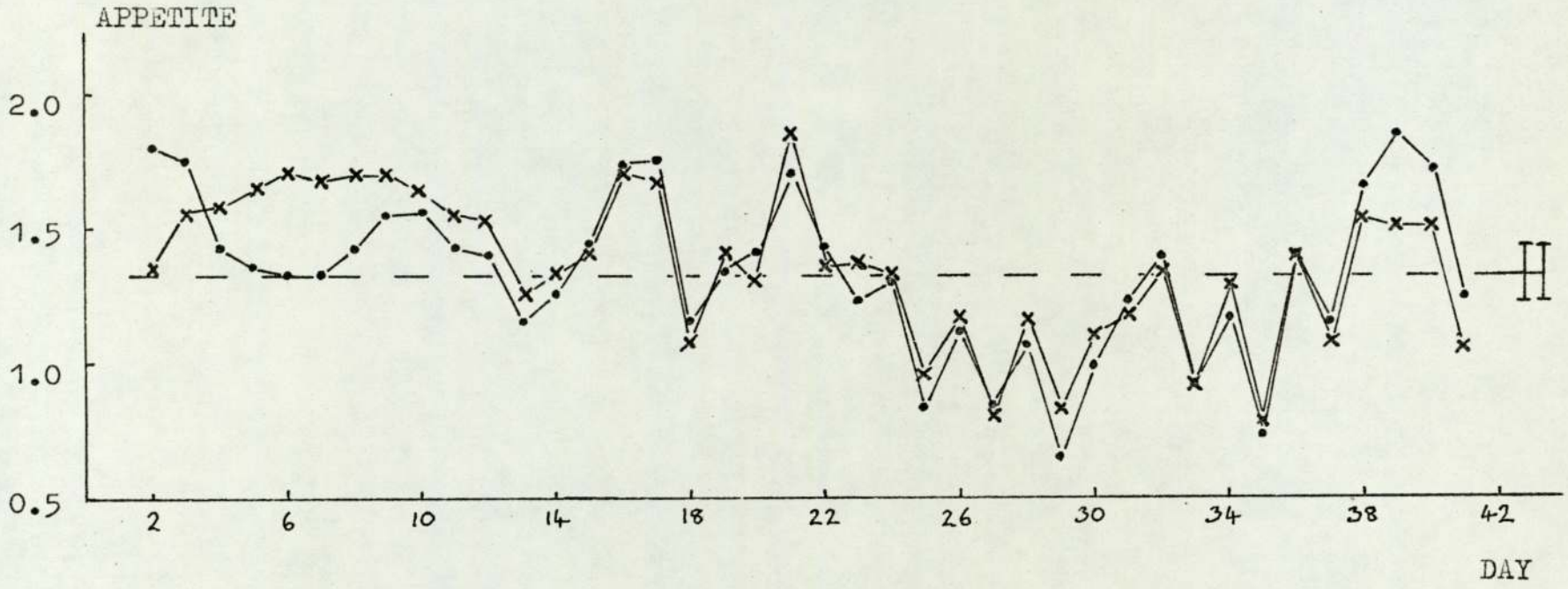
9.56 Food consumption was measured as weight fed per tank per day. In order to compare tanks containing different fish weights, the quantity used for comparison was appetite, arbitrarily defined as:

$$\left(\frac{\text{weight of food fed}}{\text{total weight of fish}}\right) \times 100$$

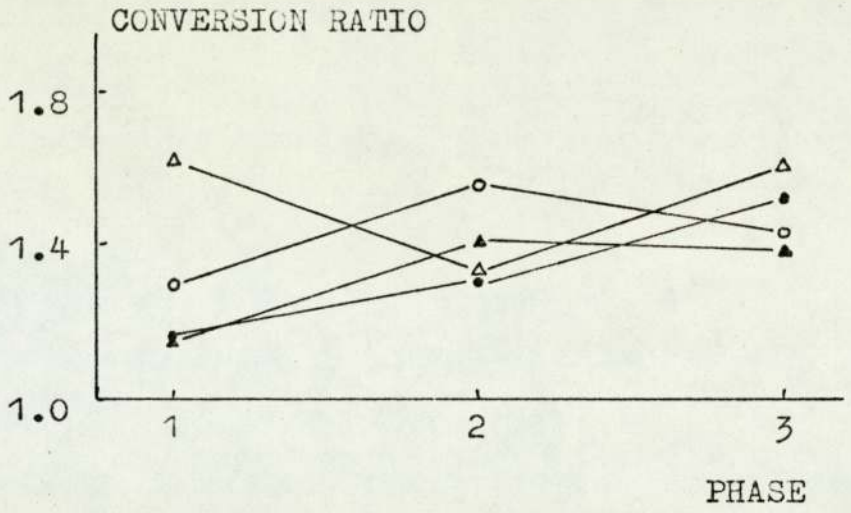
(or food consumed as % of body weight)

This involved intermediate weight estimation as in Chapter 7. As there were no mortalities, the accuracy of this process was at maximum. Due to the fluctuations mentioned in para.9.39, a moving average was used (span 3 days) to provide a plot on which trends could be assessed. Graph 9.11 shows such a plot for one experimental and one control tank. The patterns and values are very similar; the overall trend is downwards, with a rise in the last few days. Differences between the extreme mean values (Table 9.13, MT1 & 2) were found to be statistically insignificant.

9.57 Gross conversion ratio is analysed in Graph 9.12 and Graph 9.13 (plotted against mid-phase mean weight). Overall values are slightly higher (worse) in control tanks; and the tanks display a slight upward trend in phase values (not statistically significant). Graph 9.13 agrees with the impression of Graph 9.10 for % weight increase: the approximate average for experimental tanks appears to be generally superior to that for controls, suggesting that the reason for the suspected superior growth rate in experimentals is that the fish would be converting food at a better rate (appetite is approximately the same in all tanks).

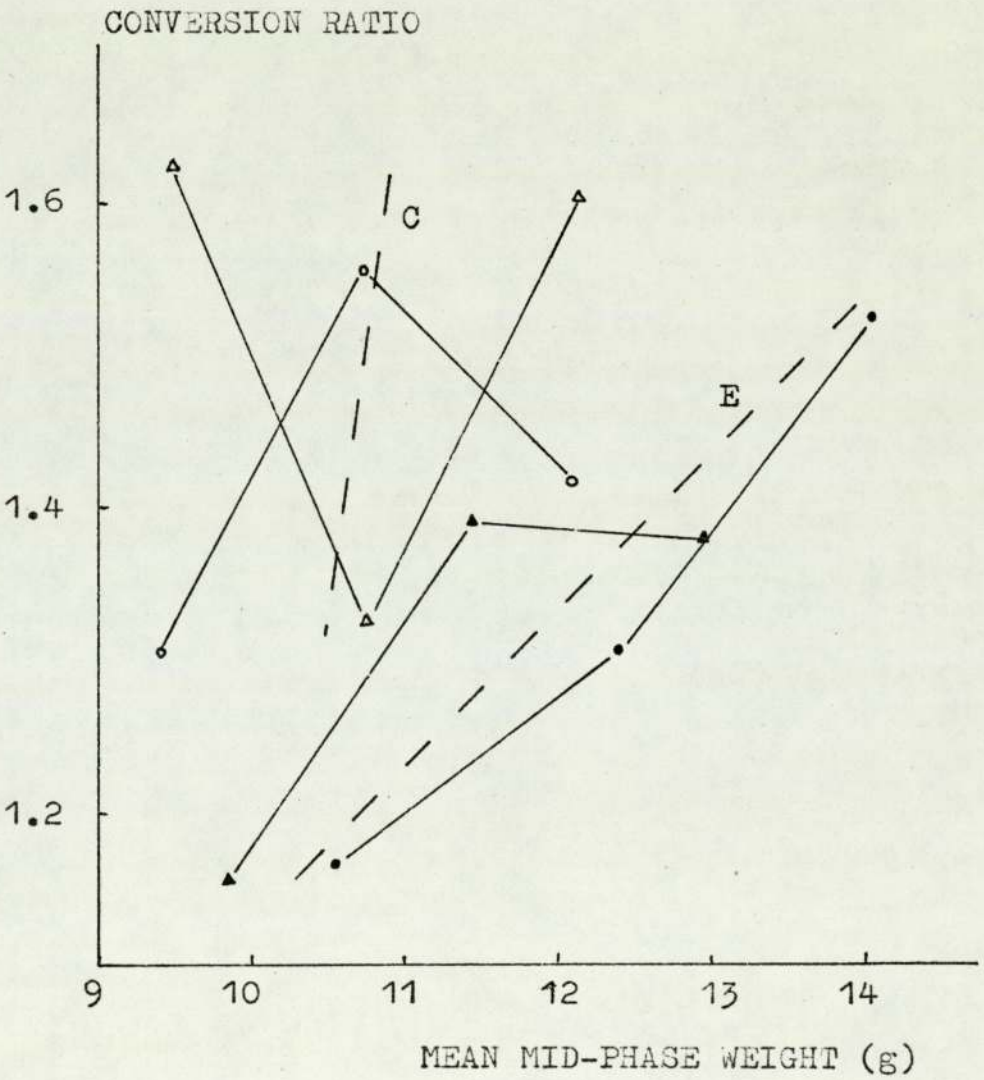


Graph 9.11 T01 appetite: 3-day means (plotted on middle day)



• = MT1 ▲ = MT2 ○ = MT3 △ = MT4

Graph 9.12 T01 conversion ratio by phase



• = MT1 ▲ = MT2 ○ = MT3 △ = MT4; C,E as in Graph 9.10

Graph 9.13 T01 conversion ratio by size

Analysis of growth in TO2

9.58 TO2 growth data were analysed similarly to those of TO1; correlation coefficients for the three weight data presentations are shown in Table 9.14a, and regression data in Table 9.14b. In this case, the log transformation gave the best average fit, and the regression graph of log mean weight against time is plotted in Graph 9.14, the data having been standardised as in Graph 9.7. The only significant slope difference found was between the two experimental tanks. Regression and slope analysis on the mean and cube root weight data showed: no significant differences, and a lower level of significance, respectively, between MT1 and 2. MT4 mean weights showed a correlation coefficient of 0.9999 (± unity) indicating perfect straight line growth (whilst MT1 and 2 displayed accelerating growth).

9.59 Percent weight increase is illustrated in Graph 9.15. Overall values show the control tank between the two experimentals, and the phase values show an interesting difference in pattern. MT4 follows the trend of TO1 results in clearly declining with time, but the MT1 and 2 pattern is one of stability or increase, indicating a possible rise in growth rate over the experimental period. When these results are plotted against phase mean weight, this difference is clearly repeated (Graph 9.16), reinforcing the suggestion of increasing growth rates in experimental tanks.

Analysis of appetite in TO2

9.60 Food consumption data were transformed to appetite. A plot of 3-day span moving average for appetite (Graph 9.17) shows an overall slight increase in all tanks, and a significance test between the mean values showed a significant difference between MT1 and MT4, but not between MT2 and MT4 (Table 9.15). Thus it was not through lack of appetite that MT4 showed poorer growth patterns than the experimental tanks.

9.61 Gross conversion ratio was higher (worse) overall in MT4, and the phase values (Graph 9.18) show this tank's conversion

Table 9.14a Correlation coefficients for TO2 weight and time data

	WEIGHT DATA TRANSFORMATION		
MT	MEAN	log MEAN	$\sqrt[3]{\text{MEAN}}$
1	0.9963	0.9996	0.9989
2	0.9955	0.9995	0.9987
4	0.9999	0.9973	0.9986
MEAN	0.99723	0.99880	0.99873

All values are highly significant ($P<0.001$, df = 3).

Table 9.14b Regression data, TO2: log mean weight and time

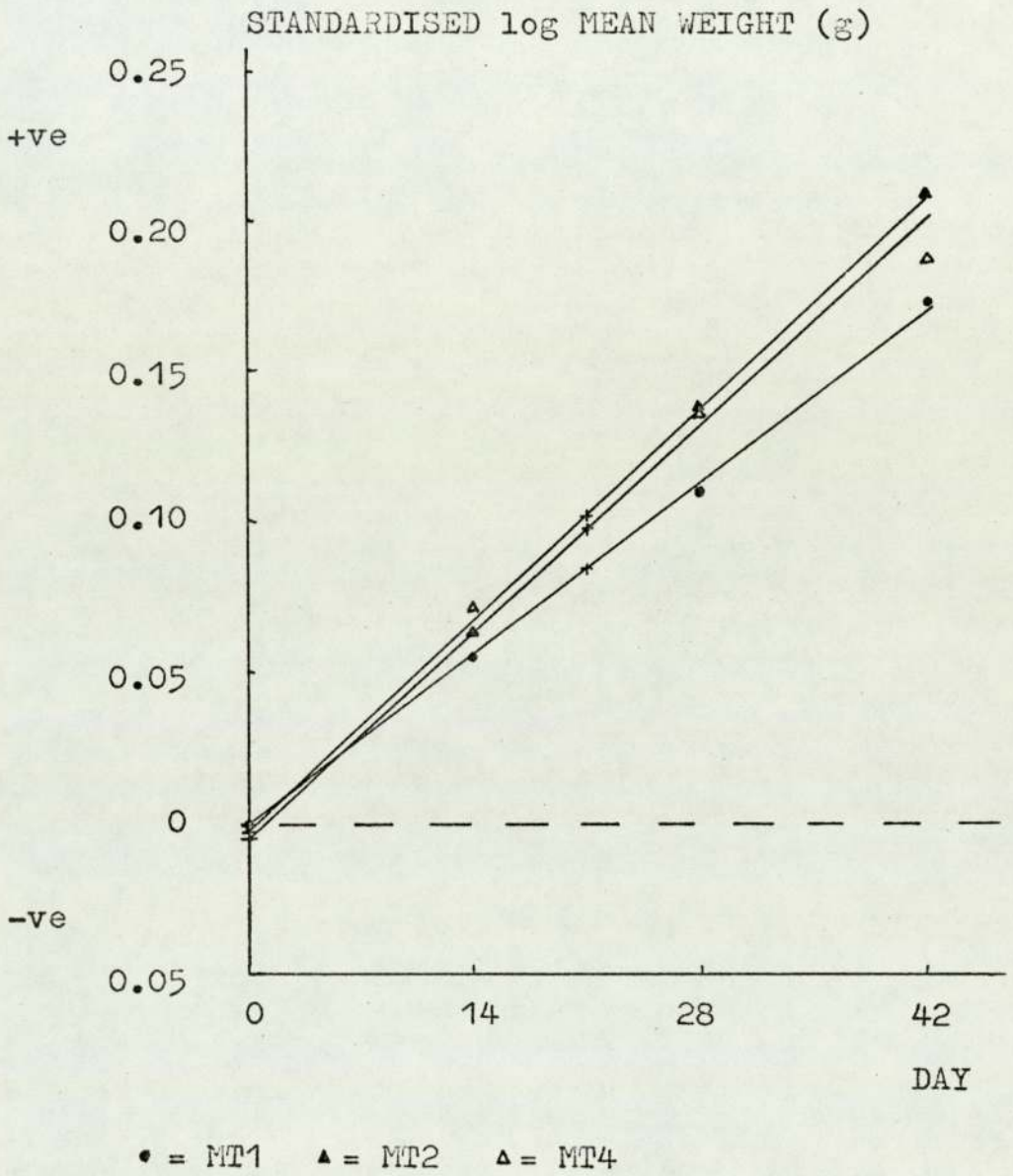
	MT		
QUANTITY	1	2	4
R	0.9996	0.9995	0.9973
a	1.0389	1.0397	1.0288
b	0.0041	0.0050	0.0045
t	6.7316	2.1225	
P	0.001 ⟶ 0.01 **	>0.05 NS	
	t = 1.4570; P>0.05 NS		

Notes:-
a) Notes (a) and (b) from Table 9.12b also apply here.
b) Overall finding:- MT1 < 4 < 2 (1 ≪ 2)

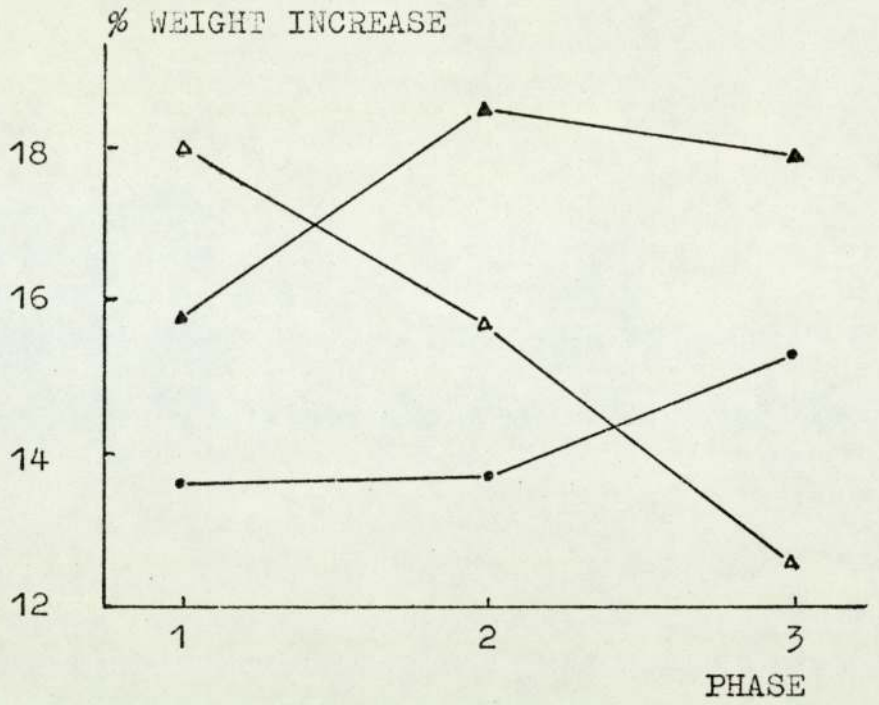
Table 9.15 Appetite in TO2

	MT		
QUANTITY	1	2	4
MEAN	1.2241	1.3581	1.3785
SEM	0.0599	0.0553	0.0472
t	1.6429	0.2808	
P	>0.05 NS	>0.05 NS	
	t = 2.0235; 0.01<P<0.05 *		

(df = n_1+n_2-2 = 82)
Notes:-
a) Values in % of body weight food consumed per day.
b) t and P as in Table 9.14b (df = 82)

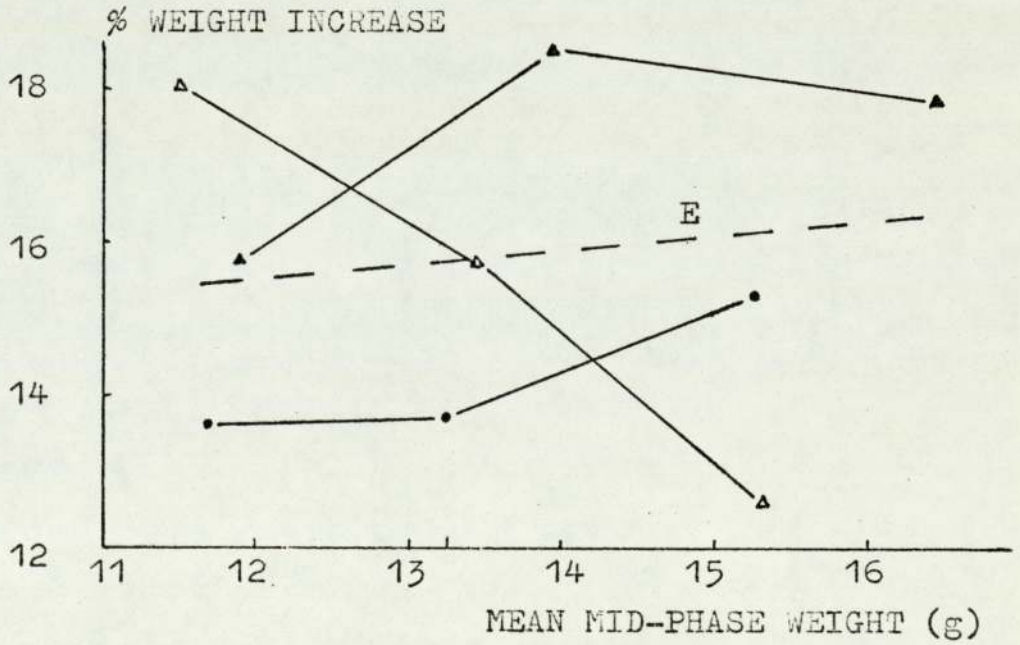


Graph 9.14 Regression of standardised log mean weight with time for T02



Graph 9.15 TO2 % weight increases by phase

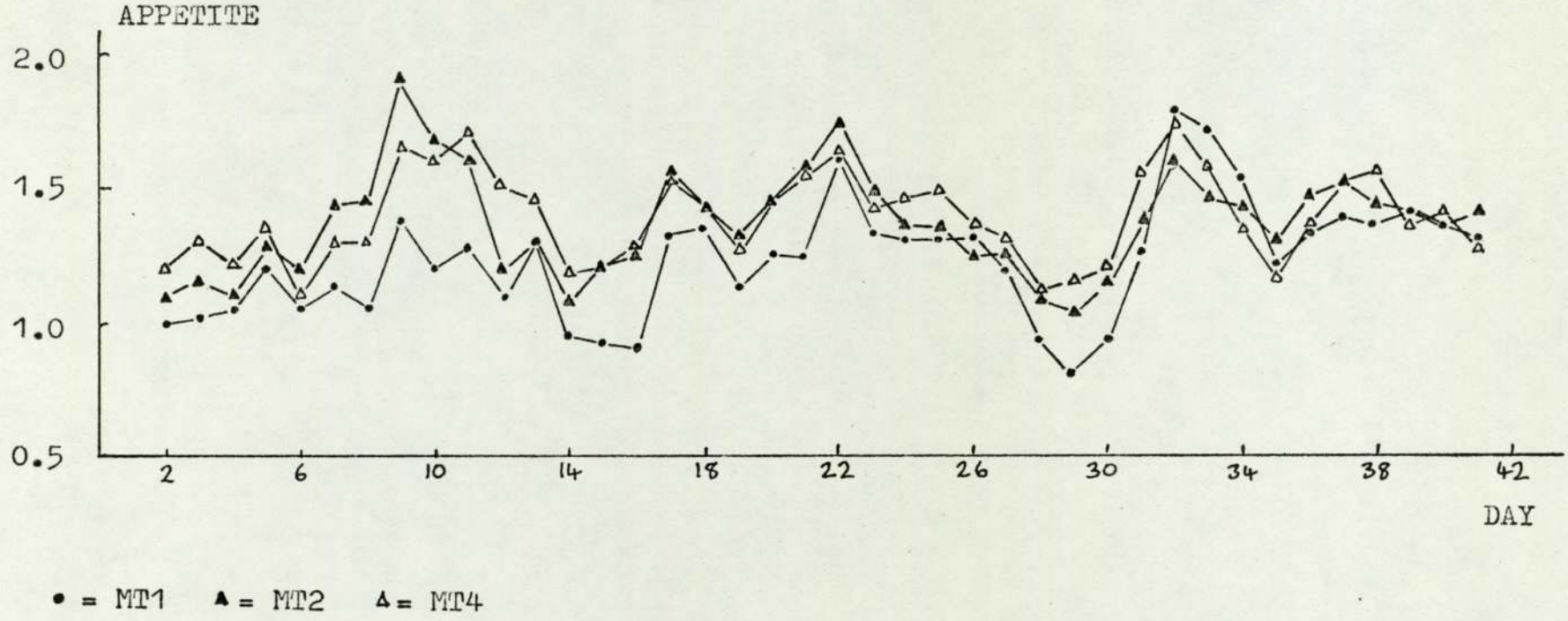
● = MT1 ▲ = MT2 △ = MT4



Average shows increasing growth rate in experimentals compared to fall in control; in general agreement with Graph 9.10

E as in Graph 9.10

Graph 9.16 TO2 % weight increase by size



Graph 9.17 T02 appetite: 3-day means (plotted on middle day)

steadily worsening with time while those of experimental tanks were maintained. This pattern agrees with that of Graph 9.15, and shows that, although having a good appetite, fish in MT4 had a poorer conversion of food. This is emphasised by the plot of conversion against mid-phase mean weight (Graph 9.19); weight for weight, experimental tank fish converted better than controls, in the last two phases of the experiment. In all these graphs, the data for phase 1 show MT4 as superior to the experimentals, but the situation is fully reversed by the end of the experiment.

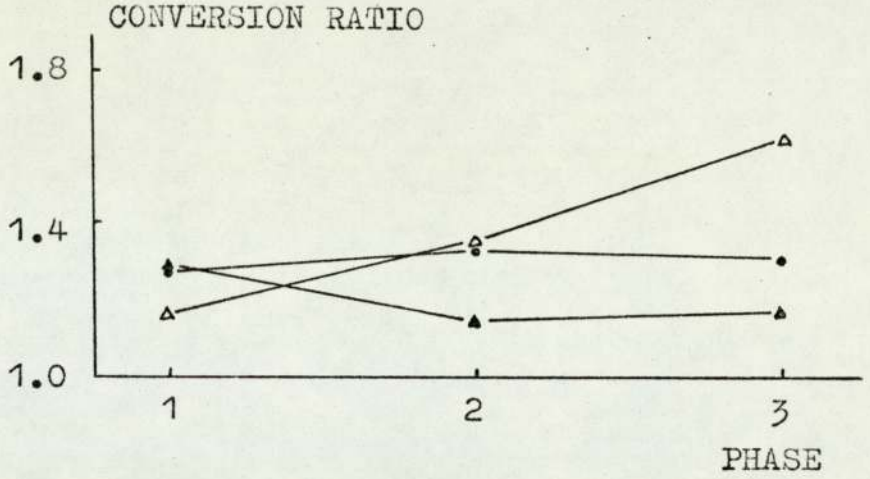
9.62 Table 9.16 gives a full summary of all T-series conditions and measured effects results.

DISCUSSION

9.63 TO1 and TO2 results give rise to several points for comment, of which the most important must be the equivocal results in growth rate differences; it is noteworthy that the log and cube root transformations of TO1 data did not show significant differences. In general growth effects have not been conclusively shown to be different between controls and fish exposed to SREF. In particular, no negative effect has been shown; and a positive effect is possible.

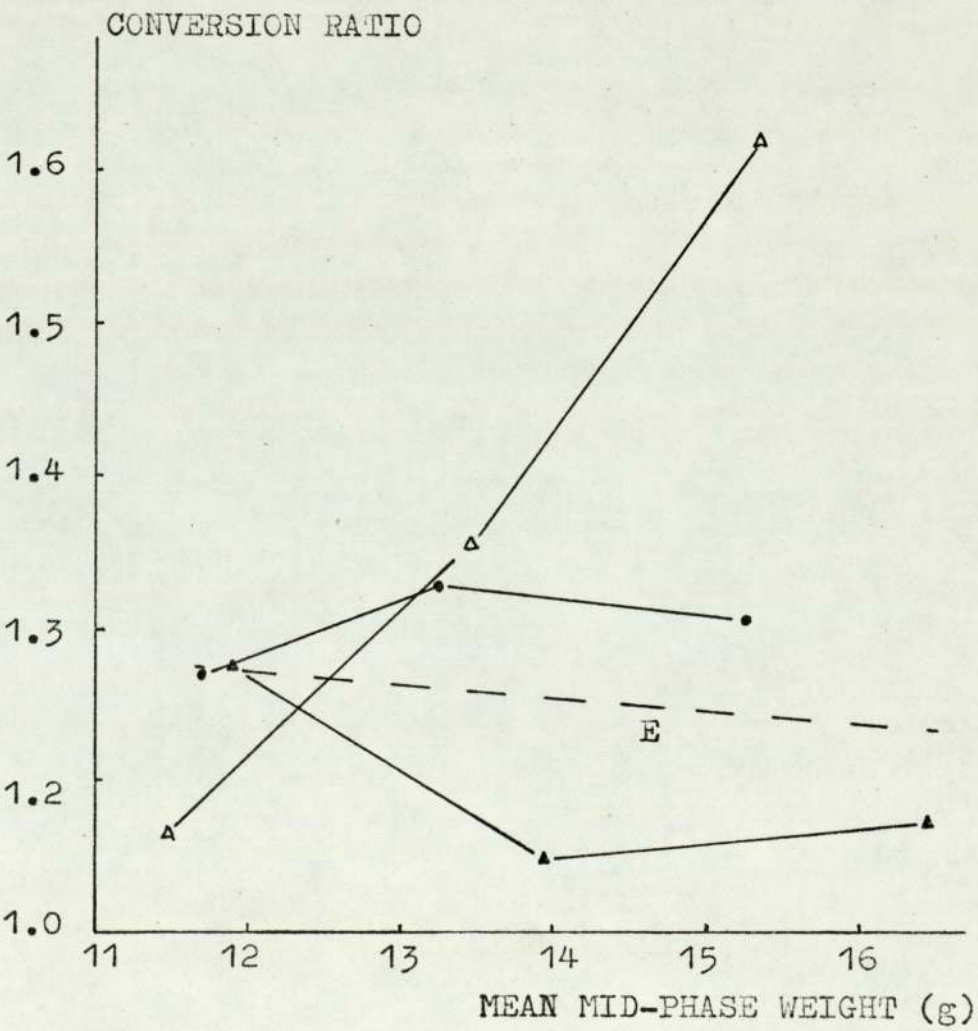
9.64 Differences between conditions in the experiments were mostly insignificant, or did not follow an "experimental v. control" pattern. Temperature must be regarded with some suspicion, as it was demonstrably, though slightly, different between experimentals and controls, and any future work would be better accomplished at a definite fixed temperature. Significant growth differences in TO2 agree with the pattern for volume, and this may explain why there is a significant difference between the two experimental tanks.

9.65 There remain two aspects of SREF which might be responsible for detected differences between experimentals and controls: carbon dioxide and pH changes due to excretion. The latter



Graph 9.18 TO2 conversion ratio by phase

• = MT1 ▲ = MT2 △ = MT4



E as in Graph 9.10

Graph 9.19 TO2 conversion ratio by size

Table 9.16 Summary of TO1 and TO2 results

QUANTITY		TO1	TO2	
Measured effects	Slope of growth regression	4<3≪2<1 *	1<4<2	(1≪2 **)
	% weight increase	4<3<2<1	1<4<2	
	Conversion	2<1<3<4	2<1<4	
	Appetite	2<4<3<1	1<2<4	(1≪4 *)
	Haematocrit	4<3<2≪1 *	4<1<2	
	Oxygen uptake	-	N 1<4<2	
Conditions	Temperature	3<4<1<2	4<2<1	
	Volume	1<4<3≪2 **	1<4<2	(1≪2 **)
	Flow	1<2<4<3	1<2<4	
	Stocking	3<2<4≪1 ***	4<1<2	
	Loading	3<4<2<1	4<1<2	
	Loading factor	-	4<1<2	

Notes:-

a) 1, 2 (experimental) and 3, 4 (control) refer to tanks.

b) Magnitude signs show relationships between tanks for the quantity referred to.

c) Double magnitude sign shows statistically significant difference, followed by rating; underlining indicates no significant difference between the tanks underlined.

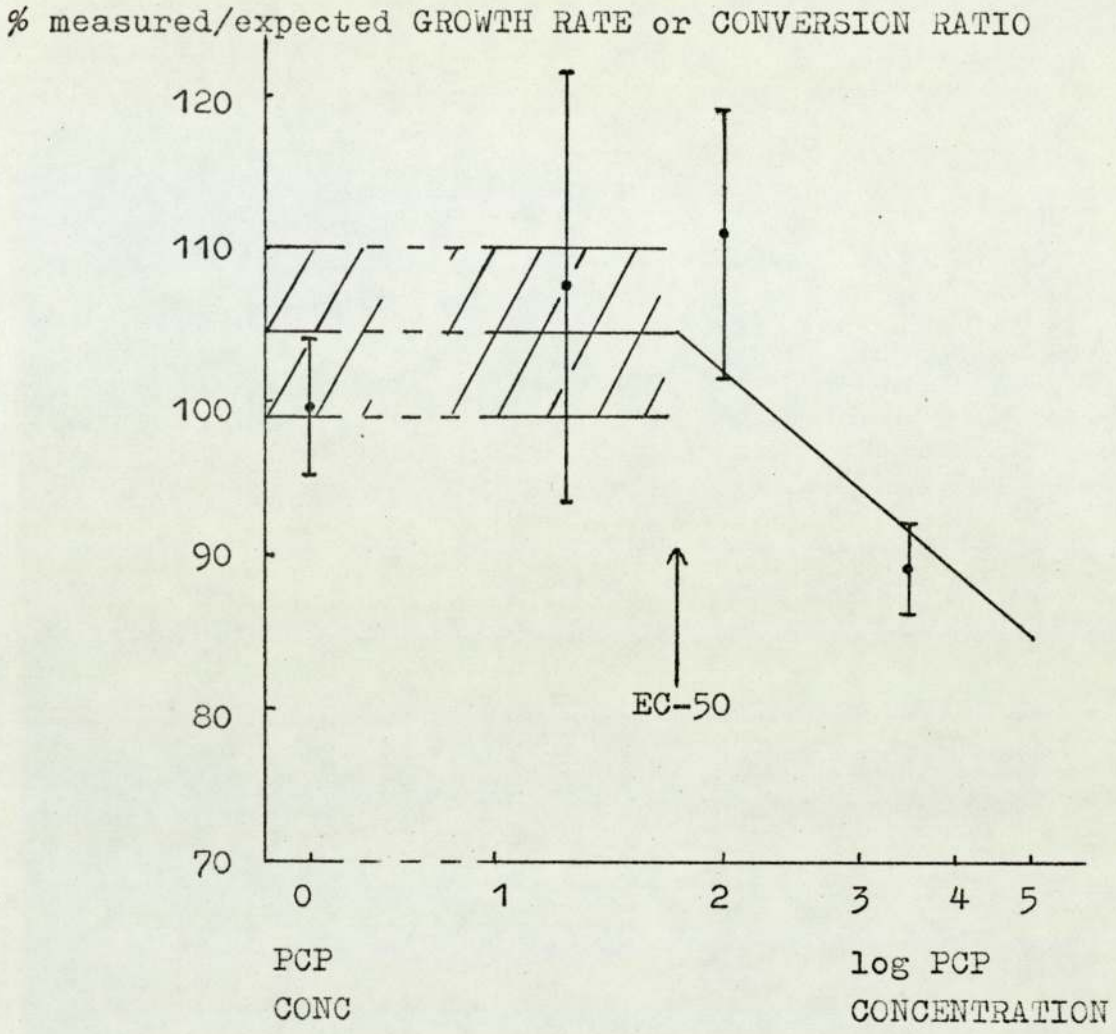
d) Temperature results are subject to proviso as in para.9.36.

e) N indicates not statistically tested.

depends to some extent on the former, but the thorough aeration that took place in the faecal traps probably rules out the former. A direct pH effect on growth rates seems unlikely, there being no evidence to date of such small pH shifts causing growth effects (in contrast to ammonia or excretory solutes), but future work would ideally be better undertaken under buffered conditions in order to remove this doubt.

9.65 Of the major measured effects only growth rate, in TO1, shows a significant difference between experimentals and controls, and this in an unexpected direction, i.e. experimental rates are significantly greater than controls, not less. Although TO2 does not statistically support this, the consideration of % weight increase and conversion ratios by phase shows a striking tendency, in both experiments, for SREF treated fish to be growing faster than controls of the same weight. Thus there is, contrary to expectations, some indication of a positive effect of SREF on growth patterns. In this respect it is interesting to note evidence from Sprague (1971) (quoting Pickering [1968]), that mild exposures to zinc may have a growth-stimulating effect; and further, from Webb & Brett (1973) the observation that this is a "not uncommon feature", which they show again in the context of sodium pentachlorophenate (PCP). Webb & Brett's figure is discussed in Appendix D; here it is presented (Fig.9.8) in order to point out that at concentrations of PCP below the EC-50, the ratio of measured:expected growth rate (treated:control) was definitely found to be greater than 100%. The same effect was found for conversion ratio: i.e. fish at "no stress" concentrations of the chemical ($<$EC-50) were in fact slightly stimulated in their conversion efficiency.

9.67 In the current studies, this slight growth stimulation could agree with the growth rate results of TO1 and the growth pattern differences of TO2, given the limited information available. This would indicate that SREF, as measured at levels of about 0.36 and 0.70 mg ℓ^{-1} TOA, is below the EC-50, and may be having a slight growth stimulation effect.



Shaded area shows ± 2 SEM about the zero response level, which is slightly elevated above 100% to about 104%

Figure 9.8 Elevation of zero response level for PCP effect on growth rate or conversion ratio (modified from Webb & Brett (1973))

9.68 In support of this, both Smith (1972) and Schulze-Wiehenbrauck (1974) were able to note definite positive factors due to ammonia exposure. Smith notes that fish from a treated tank which were subsequently allowed a 48-hour "rinse" in fresh water showed a 22% increase in performance capability, which raised them well above controls. Schulze-Wiehenbrauck (1974), besides noting that ammonia lessened weighing-stress in his fish, also found that a distinct increase in resistance to lethal UIA levels was achieved by culture in mild concentrations (130-170 $\mu g\ \ell^{-1}$). This agrees with Lloyd & Orr's (1969) observations of acclimation to low UIA, and it may be that the processes of acclimation and of slight growth stimulation could be different physiological expressions of the same underlying effect.

9.69 Brett (1974) has recently discussed growth rates of Canadian salmon, and has noted that size and age may be quite distinct in their influences on growth, so that fish weight alone may not determine growth rate. It has frequently been noted that the growth rate slows down with age, and similarly the conversion efficiency gradually falls: for the purposes of these experiments, all fish have been arbitrarily assumed to be on the same portions of these respective graphs since the period involved was short. Brett's observation suggests that it is truly a function of <u>age</u> in determining growth patterns, and not just weight. In this respect the results of TO1 and TO2 could be interpreted as a "rejuvenation" effect, where fish of the same weight are displaying greater growth capabilities when treated with SREF than when not. (The effect is not due to age differences since all fish were sampled from a common population.) If their resistance to lethal toxicity is also thereby increased, this would agree with Schulze-Wiehenbrauck's observations of greater resistance in small trout than in large ones, and of less resistance in fish with high condition factors (condition factor usually increases with age and growth).

9.70 Schulze-Wiehenbrauck (1974) noted that while food consumption fell under the influence of UIA, no deterioration of conversion was caused, and his loss in growth rate of fish exposed to 130-170 $\mu g\, l^{-1}$ UIA can thus be explained in terms of loss of appetite. This finding disagrees with Sprague's (1971) contention that conversion may be a more sensitive indicator than growth rate: it would seem that this is unlikely, since growth rate is primarily a product of the combination of appetite and conversion rate. Webb & Brett's (1973) results show that conversion ratios reacted similarly in response to PCP, but their fish were fed a standard reduced ration. In the current T-series experiments, appetite cannot be said to provide any extra information, but it can be appreciated that in cases where conversion and growth rate indications differ greatly, appetite may well provide an explanation. Thus the concept of appetite as a response is worthy of further assessment when trying to draw up tolerance guidelines: differences in appetite may cause losses in growth rate even when conversion is not affected, and either of these two quantities may be more sensitive in a given situation. An EC-50 for loss of appetite would be an extremely useful quantity for a fish farmer.

9.71 In conclusion, it can be said that on this evidence neither at 0.36 $mg\, l^{-1}$ nor at 0.70 $mg\, l^{-1}$ TOA does SREF exceed its EC-50 for reduction of rainbow trout growth. If fish excretory products are truly responsible for growth losses, two further steps are clearly necessary; (a) the establishing of the EC-50 for SREF, and (b) the definite identification of the exact agent involved (in particular, it would be useful to find out the effects of NH_4^+ alone). Step (b) might provide a basis for simpler filtration systems for recycled water in the future (see Chapter 10).

PART 5

EXCRETORY PROBLEMS

IN FISH CULTURE

10. EXCRETORY PROBLEMS IN CIRCULATING SYSTEMS

DISCUSSION

10.1 This study has been conducted with one specific type of fish-rearing situation, i.e. juvenile rainbow trout in open system circular tanks at fairly high loading. It is therefore important to emphasise that it is not admissible to generalise from this work to other types of situation unless there is appropriate supporting experimental evidence. Further, direct extrapolation to other magnitudes of the same situation (e.g. commercial circular rearing tanks) is also inadvisable. The work is thus presented as a line of approach to the problems rather than as an authoritative statement.

10.2 This said, it would be equally unacceptable to withhold speculation on interpretations of the methods used and the data collected.

Excretory productivity

10.3 The heart of the problem of describing excretory productivity in quantitative terms in a multivariable situation lies in an assessment of the factors determining excretory rate. This study has not been concerned with the internal biochemistry and physiology of excretion as a response to changes within the organism. The approach has been to treat the organism as a 'black box', and to measure external inputs and outputs. The belief underlying this is that with methods available to date, any attempt at internal (intra-organism) assessment of effects would automatically lead to errors simply because of the stresses and disturbance caused by the unavoidable handling, operating techniques, monitoring requirements, etc. involved. In the context of excretion, any such departure from normal culture conditions might provide spurious data. This might seem to invalidate all attempts at intra-organism assessment, but recent advances in miniaturisation of electronic equipment do allow one to conceive of instruments sufficiently small to measure internal parameters with minimal disturbance, the

disturbance limited to the operations required for instrument insertion (which could be followed by recovery and re-adaptation periods). So far, advances in this direction have been made in the context of fish-tagging in ecological work on fish movements (Holliday 1975), but there remains considerable technical progress to be made before devices can be developed which are sufficiently small, disturbance-free and multiple-sensing as to allow adequate internal monitoring for assessment of environmental effects on excretion.

10.4 Given that an extra-organism assessment is thus necessitated, a series of central problems arise relating to measurement of excretory rate:

(a) Is it possible to identify, and then measure, all input variables affecting the system?

(b) Is is possible to measure true excretory rate?

(c) Can the measured excretory rate be satisfactorily and meaningfully related to the input variables, so that future prediction is possible, this providing a firmer basis for fish culture techniques?

10.5 With respect to (a) above, it seems on balance that it is not possible to state that all possible input variables have been identified. Were that so, it would seem likely that (supposing they could be measured), it would be possible to describe and predict excretion in terms of them. With this in mind, the approach of this current work has been to aim at the following objectives: (i) to measure readily identifiable input variables undergoing normal culture-situation fluctuations, (ii) to build a predictive model, and (iii) thus to define the magnitude of effect of the unidentified input variables; i.e. to try to answer question (c), and thereby throw light on question (a). In stating (Chapter 7) that the models generated explain the data "well", the implication is that compared to the input variables measured, the unidentified ones have a small effect on excretion. This may only be true

for the current study - and even then only for populations greater than 100, or for fish of the size ranges studied (in particular, very small fish (<5g) may behave differently, having extremely different feed rates). Clearly, more information is required, but given the limits of applicability, the approach to the problem (i.e. fundamentally a multivariate system attempting to mimic the working fish-culture operation) seems to be justified, and might (in improved form) be worthy of repetition in future studies.

10.6 However, the fact that unexplained variability has been shown to exist, as expected previously, leads to a consideration of whether possible components of that variability can ever be fully identified, or even then whether they are measurable.

10.7 Question (b) above has been partly dealt with in Chapter 5; it is clear that the actual quantity measured is TOA (a concentration), which is related to apparent SER (derived by calculation) thus:-

$$SER = \frac{\text{flow} \times \text{TOA}}{\text{fish mass}}$$

However, TOA itself is governed by a slightly different relationship, thus:-

$$TOA = \frac{TSR \times \text{fish mass}}{\text{flow}} \left(1 - e^{-\frac{\text{flow} \times t}{\text{volume}}}\right)$$

where TSR is the true specific excretory rate (see Chapter 5). The exponential function determines the difference between SER and TSR, and this function in turn will depend mainly on t (time) when flow and volume are steady. If the time required for equilibration is appreciable (see Table 5.2), then TSR may well change before the equilibration has occurred, hence TSR and SER will not be similar. In practice SER is the only useful quantity, but the reasons for TSR change, and the rates of change due to them, are mostly speculation. These reasons would thus conveniently fill the role of unidentified variables mentioned in para.10.6. Some possible reasons are listed below:-

a) diurnal cycles (due to biological clock and/or outside stimulation such as temperature, photoperiod)
b) seasonal cycles (due to similar effects)
c) age (see growth effects quoted from Brett in Chapter 9)
d) feedback from output variables
e) "stress"
f) social effects (e.g. hierarchy).

Clearly, techniques for measuring such possible variables might be lengthy and complex to develop and validate, even supposing that the variable can be quantitatively pinned down (difficult with stress and social effects).

10.8 The possible feedback effect quoted above underlines the fact that ammonia is not the only aspect of excretion (and thus not the only output variable). Other output variables must include production of carbon dioxide and urea (and other excretory compounds), oxygen depletion,and pH shift, besides quantities like production of heat and solid waste, activity, growth and such difficult possibilities as pheromones, if they exist in this situation. It is possible, and even probable, that many of these quantities are related, especially those measurable as chemical effects, but the constancy of the relationships is not necessarily proven. In this respect, the assumption of the current work, that urea is a relatively constant, low proportion of excretory production, would certainly invite thorough investigation, as would the status of CO_2 production.

10.9 In summary, it can be said that the methods used in the current work allow a suitable line of approach to the problem of predicting ammonia excretion in the fish-culture situation used; that improvement and refinement of methods both to identify and to measure input variables would allow a better framework for prediction; and that this kind of multivariate approach would be worthy of application to other fish-culture situations, especially commercial-size circulating systems. Thus a number of future experiments could be envisaged, as detailed later.

10.10 In essence, the ideal approach suggested is (a) to set up a functioning full-size fish culture circulating system, (b) to monitor constantly over an extended period all identifiable and measurable input and output variables, and (c) to perform suitably sophisticated multivariate analysis on the data so produced, in order to generate the shapes and limits of reliable predictive functions for excretory outputs. The methodological problems of this are substantial but not insoluble: suitable equipment for constantly monitoring, multiplexing, logging and analysing a number of data sources (especially if served by electrode probe-type sensors) is already available, although the co-operation of statistics, electronics and programming experts would probably be advisable in building such a system.

10.11 One major experimental problem is the monitoring of weight and length of fish. Theoretically, these quantities should be measured continuously, accurately, and without fish disturbance. In practice, at present, the best that can be done is to disturb the fish periodically by removal for weighing and measuring, and to estimate (interpolate) intermediate values by some method such as that described in Chapter 7. This should undoubtedly be seen as the weakest part of the method used, introducing a further level of error into the system in addition to that of actual measurements. It does seem conceivable that there should exist some method of accurately determining length and mass of moving objects underwater by remote-sensing means, and if this is so it ought to be possible to design such a system to operate at tank scale on a population of fish. If this were possible, the greatest operational problem of scientific fish husbandry would be removed.

10.12 Of the two final models for excretion prediction derived in Chapter 7 (OM1 model (3) and OM2 model (4)), each has interesting features, briefly summarised in the statement: that model (3) (OM1) may be more practically (or commercially) useful in

that prediction is based on envisaging the tank as one large ammonia-producing machine, with individual fish differences unaccounted for (or, strictly averaged out) and with independent variables readily measurable; whereas model (4) (OM2) is perhaps more scientifically interesting in that it includes components which reflect individual differences and possible social effects, and this provides plenty of scope for further definition, elucidation and measurement of contributing input variables. Both types of model would therefore seem worthy of trial application in future experiments.

10.13 In either case, the reduction of the multivariate situation to one descriptive relationship means that it is possible for a fish culturist to fit his own particular values into the equation (e.g. using values of temperature, number, mass and volume for model (4)), and to compute a predicted excretory rate. The process could be made operationally simpler by preparation of suitable tables or graphs for reference, or by a specially prepared slide-rule embodying the equation.

10.14 The kind of constant-monitoring, multivariate approach suggested could provide the basis for a further step: the elucidation of cumulative effects in a closed system. This would be an alternative way, to that used in the current work, of assessing tolerance to excretory products, and would have the advantage of allowing several important input variables (oxygen, pH, temperature) to be held at optimum levels.

10.15 In the context of closed system fish culture, a reliable model for predicting excretion is a fundamental requirement, since characterisation of the effluent governs the treatment facilities that are needed, once a reliable criterion of tolerance/toxicity is available. Current processes of closed system design (e.g. Speece 1973, Liao & Mayo 1972, 1974) thus stand or fall by the accuracy of the expected excretory output for the particular situation under consideration. It is felt

that current knowledge of micro-environmental effects on excretion in fish-culture systems, especially intensive ones, is not as comprehensive as it might be, and so current estimates of excretory productivity are open to doubt; this is not to say that the rules-of-thumb used so far may not turn out to be valid, but simply that they need to be either shown to be valid authoritatively, or discredited and replaced by better-substantiated ones.

10.16 In a wider context still, a reliable model of excretory output would be particularly useful in allowing a fish-farmer to design his operations (whether open- or closed-system) so as to meet local water quality requirements in the final effluent discharged. This consideration becomes more important as effluent control regulations become tighter and quality standards higher, especially when the ability to meet such standards may involve substantial land area, and/or equipment and cost.

Tolerance to dissolved excretory products

10.17 It is important to stress that tolerance as discussed here was only investigated in limited circumstances, as preliminary work aimed at finding an experimental approach to the problem of setting a reliable water quality guide for treatment of fish effluent for recycling. As such, the work used only a limited size range of fish, a limited temperature range, one type of effluent (SREF of soft-water quality with low ammonia dissociation), and only two strengths of SREF. Thus the quantitative results obtained apply only in this situation, and cannot be unduly extrapolated.

10.18 The approach used for this work hinges on the search for a water quality criterion based on tolerance rather than on toxicity; i.e. the objective is a concentration up to which the excretory pollutant can be allowed to accumulate before growth or conversion are affected, not a concentration down to which pollutant must be reduced before physiological effects

are negligible. Secondly, the work has assumed that SREF is worthy of investigation as one total effect. Thus the effluent used to dose experimental tanks was essentially uncharacterised in the terms of any excretion productivity model. It was treated as a homogeneous effector, whose strength could be gauged by measuring TOA as an indicator: thus independent effects due to UIA, TOA, urea or any other component were not separated out. Given however, the same assumptions as were made for the excretory productivity work i.e. that other dissolved excretion products are in a relatively constant, low proportion to ammonia, it is felt that the information generated could ideally be used, in combination with an excretion prediction model, to determine the treatment efficiency required of a filter unit.

10.19 Implied in this is the assumption that some measure of SREF persistence in a closed system might not be incompatible with maintenance of growth and conversion rates (although accumulation, as opposed to persistence, would be a different matter).

10.20 The system of SREF dosage used ruled out possible interference from feedback or hierarchy effects, although interference from pheromones cannot be ruled out (neither can they be positively shown to be present as distinct from excretory output).

10.21 Within these experimental limits, it can be said that both ranges of SREF strength tested were below the EC-50 for growth rate or conversion effect; as the data also tend to agree with previous indications of a slight positive growth effect at $<$EC-50 levels, there may be two aspects of advantage to a less stringent water-treatment policy: (a) possible lower cost for a potential filter, and (b) possible slight beneficial effect on growth.

10.22 Experimentally, the tolerance experiments would benefit in future application from the buffering of temperature and pH:

in practice, this need not necessarily mean constant values, only that at any given instant the same value was true for both experimental and control tanks. An efficient heat exchanger between the two lines might be sufficient to absorb small temperature differences; buffering to a constant value would probably be the simplest solution in the case of pH.

10.23 The chemical nature of recycled effluent is determined by two factors, (a) the chemical nature of the water independent of fish, but affected by such things as deliberate environmental control measures, chemical effects of filtration, etc.; and (b) the excreted material of the fish. Thus recycled effluent is an extremely variable quantity. Hence, any meaningful tests of excretion tolerance in fish-culture situations must take into account the original chemical nature of the water independent of excretion effects. The most reliable way of doing this is to use control and experimental tanks supplied by the same water and to standardise EC-50 measurements individually, for the characteristic water supply.

10.24 This means that in order to establish meaningful local quality standards, the following steps would be logical:-

(a) a series of experimental and control tests to determine an EC-50 under given acclimation conditions;

(b) assuming that the LC-50/EC-50 relationship is roughly constant (see Appendix C), a series of LC-50 tests to allow estimation of EC-50 for other conditions.

10.25 In the long run, it is desirable to attempt to investigate the tolerance/toxicity properties of all excretory products individually, and much work has already been done on ammonia as detailed in Chapter 8. Nevertheless there is still a lack of firm evidence on EC-50 levels for UIA, TOA, urea and other excretory compounds.

10.26 However, it must be remembered that in a fish effluent these agents do not act alone, and ultimately individual recycled effluents are the real criteria, since they represent the

sum of individual effects and interactions. It is rash to assume that growth processes in fish will be affected by recycled effluent in the same way that they are by straightforward ammonia solutions, whether the ammonia is present as TOA or UIA: in other words, interaction effects must be catered for. (Further, if biological filtration is envisaged as a water treatment method in closed fish systems, then the effect of fish effluent on filters may well be different from that of ammonia solutions, due to interaction of simple nitrogenous and other organic compounds, e.g. see Bruce, Merkens & Haynes [1975]).

SUGGESTED FUTURE EXPERIMENTAL POSSIBILITIES

10.27 Excretory productivity

1. The complete instrumentation, by electronic sensing devices with suitable measurement, data logging and analytical equipment, of experimental operating fish-culture tanks in order to continually monitor as many input and output variables of the intra-tank environment as possible, including at least oxygen, pH, temperature, ammonia, volume, flow, illumination, photoperiod, feeding and time. The analysis of such data, in company with all other measurable variables (mass, length, number, chemical quantities which require a sampling approach) in order to build up as complete a picture as possible of the situation in the operational fish-culture system; thus to provide the basis for a truly reliable predictive model for excretory effects due to ammonia, urea and any other excretory compounds.

2. The technical development of remote, accurate, non-stressful measurement methods for estimating at frequent intervals the individual mass and length, and the number, of fish in a culture tank: thus allowing study of other aspects of the situation (environment, growth) without interference.

10.28 Tolerance to recycled water

3. The establishing of EC-50's for loss of growth rate and/or conversion and/or appetite according to strength of recycled

effluent (as measured by an indicator substance such as TOA), for a wide variety of appropriate water supplies; thus to derive working limits for operational closed systems.

4. The investigation of other pertinent criteria of sub-lethal effect e.g. stamina, disease resistance, healthy appearance, as measured by suitably validated rating scales, and the establishment of appropriate EC-50 criteria.

5. The elucidation of the effects of sublethal concentrations of individual components of recycled water e.g. TOA, UIA, urea.

6. The investigation of possible growth or conversion enhancement effects at non-lethal sub-EC-50 concentrations of effluents or effluent components; and the clarification of their modes of action if confirmed.

7. The further analysis of, and formulation of a definitive theoretical basis for, the concept of stress as applicable in fish-culture environments; the investigation of both internal (e.g. endocrinological) and external (e.g. respiratory) aspects of stress in this context.

10.29 General

8. The advancement of intra-organism and extra-organism multiple-channel assessments of environmental effects, including stress situations.

10.30 Overall

9. The truly efficient design of the simplest and cheapest form of closed rearing system which will allow optimal productivity of cultured fish.

NOTE - All of these suggestions have been conceived in the context of rainbow trout work, but clearly all apply equally to any species which is potentially capable of closed system culture, including marine and warm-water types, although these may have additional environmental problems providing further scope for investigation.

CONCLUSION

10.31 This study has attempted to formulate experimental

approaches to two fundamental aspects of fish-culture in circulating systems: the gauging of excretory productivity on an open system, and the investigation of the tolerance of fish to recycled effluent in a closed system. Both are fundamental requirements for informing the efficient design of the treatment equipment so often required in order to recycle safely. During the pursuit of these overall objectives, it has become clear that the fish-culture environment holds a dual challenge for investigators; firstly, because of its immediacy in humanitarian, technological and commercial application, which lends pragmatism and excitement to the endeavour; but secondly, less obviously and yet finally of equal weight to the scientific sense of the investigator, because of its complexity, scope for future co-operation between workers of different disciplines, and sense of being at the beginning of a new field, with much that seems worthy of investigation, and, in particular, the opportunities for new syntheses of information. Whether or not the opportunities are grasped depends on cost, technical advance, and the foresight and inter-disciplinary co-operation of decision-makers; but ultimately on having the will to do it.

APPENDICES

AND BIBLIOGRAPHY

A1. PILOT EXPERIMENT RESULTS

CONTENTS:-

Table A1.1 P01 results: TOA, temperature, pH, UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
2/9/72	1200	X04	20	17.0	7.07	0.075
		BCK	445	17.0	6.26	0.258
	1600	BCK	455	17.5	6.21	0.244
3/9	1150	X02	20	16.5	6.84	0.042
		X04	10	17.0	6.93	0.027
		BCK	450	17.0	6.21	0.233
	1600	X02	30	17.0	7.02	0.100
		X04	30	17.0	7.08	0.115
		BCK	440	17.0	6.26	0.255
4/9	0925	X01	30	17.5	6.81	0.064
		X02	20	17.0	6.88	0.048
		X04	10	16.5	6.87	0.023
		X06	10	16.5	6.88	0.023
	MEAN	X	20	16.9	6.93	0.057
	MEAN	BCK	447	17.1	6.23	0.247
4/9	1715	01	70	16.5	6.80	0.135
		02	70	17.0	6.84	0.154
		04	90	17.0	6.69	0.140
		06	110	17.0	6.55	0.124
5/9	1200	01	60	16.5	6.86	0.133
		02	60	16.5	6.84	0.127
		04	120	16.5	6.71	0.189
		06	110	16.5	6.62	0.141
	1600	01	70	16.5	6.81	0.139
		02	70	16.5	6.91	0.174
		04	120	16.5	6.72	0.193
		06	140	16.5	6.61	0.175
6/9	1000	01	89	16.5	6.80	0.172
		02	72	16.5	6.87	0.164
		04	132	16.5	6.65	0.181
		06	123	16.5	6.60	0.150
	1400	01	80	17.0	6.89	0.198
		02	76	17.0	6.96	0.220
		04	127	17.0	6.74	0.222
		06	132	17.0	6.77	0.247
	1800	01	36	17.0	6.95	0.102
		02	32	17.0	6.94	0.089
		04	87	17.0	6.84	0.192
		06	79	17.0	6.74	0.138
	2200	01	62	17.0	6.98	0.188
		02	47	17.0	7.02	0.156
		04	104	17.0	6.93	0.282
		06	104	17.0	6.81	0.214
7/9	0600	01	22	17.0	6.89	0.054
		02	22	17.0	6.93	0.060
		04	60	16.5	6.81	0.119
		06	67	16.5	6.75	0.116

Table A1.1 continued

DATE	TIME	SAMPLE	TOA	TEMP	pH	UIA
	1010	01	42	17.0	6.88	0.101
		02	31	17.0	6.87	0.073
		04	82	17.0	6.73	0.140
		06	101	17.0	6.66	0.147
	1400	01	50	17.5	6.89	0.128
		02	43	17.5	6.92	0.118
		04	122	17.5	6.71	0.207
		06	109	17.5	6.70	0.181
	1800	01	56	17.5	6.90	0.147
		02	50	17.5	6.96	0.151
		04	112	17.5	6.83	0.250
		06	84	17.5	6.80	0.175
	2200	01	62	17.0	6.96	0.180
		02	56	17.0	7.00	0.178
		04	122	17.0	6.86	0.281
		06	102	17.0	6.85	0.230
8/9	0600	01	40	16.0	6.91	0.096
		02	30	16.0	6.95	0.079
		04	79	16.0	6.82	0.154
		06	86	16.0	6.83	0.171
	1000	01	43	16.5	6.78	0.079
		02	30	16.0	6.92	0.073
		04	99	16.0	6.78	0.176
		06	92	16.0	6.69	0.133
	MEAN	01	53	16.8	6.88	0.131
	MEAN	02	44	16.8	6.92	0.124
	MEAN	04	102	16.8	6.77	0.200
	MEAN	06	100	16.8	6.71	0.171

Notes:-

a) BCK indicates background (holding tank) sample.

b) Sample references are the ST number (preceding X indicates pre-fish sample).

c) TOA and UIA means are calculated from the last 11 samples (Phase 2 of the experiment) except for ST 06 where the last 3 are ignored due to mortality at 2000, 7/9. The values used correspond to spectrophotometer absorbance readings (see Chapter 4).

Table A1.2 P01 results: flow, volume

	DATE	TIME	TANK (ST)			
			01	02	04	06
MEASURED	4/9	AM	33.5	33.0	32.5	32.5
FLOW:	5/9	1200	33.0	32.75	32.75	33.5
time(s)	6/9	1200	33.5	32.5	32.75	33.0
for	7/9	1200	34.0	33.0	32.0	33.0
500 ml	8/9	1200	33.5	33.0	33.5	32.5
	MEAN		33.50	32.85	32.70	32.90
MEAN FLOW	(ℓ min^{-1})		0.896	0.913	0.917	0.912
VOLUME	(ℓ)		2.3	8.2	8.6	2.6

P01 mortality

One fish was lost (no apparent cause) at 2000 on 7/9/72 from ST 06. All discussion of weight, stocking and loading conditions in Chapter 6 is on the basis of the fish weight prior to this loss (i.e. final tank weight plus weight of dead fish).

Table A1.3 PO2 results: TOA, temperature, pH UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ \ell^{-1}$)	TEMP ($^{\circ}C$)	pH	UIA ($\mu g\ \ell^{-1}$)
9/1/73	1200	X09	46	8.8	7.21	0.126
		X10	35	8.8	7.25	0.105
		X11	10	8.8	7.17	0.025
		X12	18	8.8	7.19	0.047
		BCK	410	8.0	6.44	0.180
	1600	X09	21	-	-	-
		X10	18	-	-	-
		X11	28	-	-	-
		X12	24	-	-	-
		BCK	640	-	-	-
10/1	1000	X09	46	8.8	7.21	0.126
		X10	36	8.8	7.28	0.116
		X11	37	8.9	7.19	0.098
		X12	36	8.8	7.20	0.097
		BCK	1150	8.0	6.50	0.579
	MEAN	X	30	8.8	7.21	0.092
	MEAN	BCK	733	8.0	6.47	0.379
10/1	2200	09	100	8.9	6.51	0.055
		10	76	8.9	6.57	0.048
		11	135	8.9	6.42	0.061
		12	109	8.9	6.33	0.040
		IS	20	8.8	7.29	0.066
11/1	0200	09	66	8.9	6.63	0.048
		10	84	8.9	6.68	0.069
		11	94	8.9	6.54	0.056
		12	87	8.9	6.47	0.044
		IS	15	8.8	7.12	0.033
	0600	09	35	8.8	6.63	0.025
		10	34	8.8	6.70	0.029
		11	56	8.8	6.49	0.029
		12	77	8.8	6.52	0.043
		IS	24	8.8	7.18	0.062
	1000	09	37	8.7	6.64	0.027
		10	34	8.7	6.50	0.018
		11	40	8.7	6.46	0.019
		12	57	8.7	6.33	0.020
		IS	-	8.6	7.10	-
	1400	09	92	8.6	6.73	0.082
		10	76	8.6	6.67	0.059
		11	145	8.6	6.36	0.055
		12	75	8.6	6.42	0.033
		IS	23	8.5	7.15	0.054
	1800	09	96	8.6	6.72	0.084
		10	54	8.6	6.75	0.051
		11	127	8.6	6.57	0.079
		12	91	8.6	6.40	0.038
		IS	26	8.5	7.12	0.057

Table A1.3 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
12/1	1000	09	35	8.6	6.83	0.040
		10	46	8.6	6.73	0.041
		11	65	8.6	6.62	0.045
		12	56	8.6	6.48	0.028
		IS	20	8.3	7.29	0.063
	1400	09	76	8.4	6.43	0.034
		10	72	8.4	6.57	0.044
		11	96	8.4	6.36	0.036
		12	83	8.4	6.31	0.028
		IS	27	7.9	7.21	0.069
	1800	09	31	8.8	6.78	0.032
		10	30	8.8	6.71	0.026
		11	49	8.8	6.62	0.035
		12	42	8.8	6.65	0.032
		IS	15	8.2	7.19	0.037
	2200	09	36	8.9	6.87	0.046
		10	41	8.9	6.88	0.053
		11	59	8.9	6.64	0.044
		12	57	8.9	6.79	0.051
		IS	21	8.4	7.18	0.052
13/1	0200	09	38	8.5	6.96	0.057
		10	36	8.5	6.87	0.044
		11	54	8.5	6.74	0.049
		12	60	8.5	6.71	0.051
		IS	19	8.4	7.20	0.049
	0600	09	34	8.5	6.92	0.047
		10	34	8.5	6.83	0.038
		11	67	8.5	6.81	0.072
		12	103	8.5	6.77	0.100
		IS	10	8.4	7.17	0.024
	1000	09	42	9.0	6.95	0.064
		10	55	9.0	6.93	0.081
		11	109	9.0	6.51	0.061
		12	82	9.0	6.74	0.078
		IS	-	8.2	7.25	-
	1400	09	75	9.0	6.48	0.039
		10	70	9.0	6.73	0.065
		11	140	9.0	6.59	0.094
		12	111	9.0	6.40	0.048
		IS	25	8.2	7.31	0.082
14/1	1000	09	33	9.0	6.94	0.049
		10	39	9.0	6.83	0.045
		11	67	9.0	6.58	0.044
		12	62	9.0	6.54	0.037
		IS	19	8.2	7.23	0.052
	1400	09	38	9.0	6.93	0.056
		10	51	9.0	6.60	0.035
		11	94	9.0	6.60	0.064
		12	95	9.0	6.49	0.051
		IS	23	8.4	7.24	0.066

Table A1.3 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
14/1	1800	09	37	9.0	6.83	0.043
		10	52	9.0	6.78	0.054
		11	84	9.0	6.64	0.063
		12	74	9.0	6.68	0.061
		IS	24	8.3	7.14	0.054
15/1	1000	09	65	9.0	6.91	0.091
		10	82	9.0	6.75	0.079
		11	96	8.9	6.70	0.082
		12	94	8.8	6.77	0.094
		IS	35	8.1	7.28	0.107
	1400	09	52	8.9	6.85	0.063
		10	50	8.9	6.76	0.049
		11	106	8.9	6.60	0.072
		12	133	8.9	6.63	0.097
		IS	32	8.0	7.20	0.081
	2200	09	54	9.1	6.80	0.059
		10	34	9.1	6.97	0.055
		11	110	9.1	6.75	0.107
		12	121	9.1	6.49	0.065
		IS	24	8.5	7.20	0.063
16/1	0200	09	52	9.1	6.90	0.072
		10	43	9.1	6.83	0.050
		11	110	9.1	6.78	0.115
		12	75	9.1	6.61	0.053
		IS	29	8.7	7.17	0.072
	0600	09	58	9.1	6.90	0.080
		10	55	9.1	6.84	0.066
		11	102	9.1	6.80	0.112
		12	102	9.1	6.75	0.100
		IS	35	8.5	7.22	0.096
	1000	09	58	9.0	6.90	0.079
		10	67	8.9	6.88	0.087
		11	120	9.0	6.50	0.065
		12	100	8.9	6.55	0.061
		IS	32	8.1	7.23	0.087
	MEAN	09	54	8.8	6.78	0.055
	MEAN	10	53	8.8	6.75	0.052
	MEAN	11	92	8.8	6.59	0.063
	MEAN	12	85	8.8	6.56	0.054
	MEAN	IS	24	8.4	7.20	0.063

Notes:-

a) Sample references as in Table A1.1; IS indicates inlet sample.

b) Means are calculated from all tabulated data after fish-introduction.

Table A1.4 PO2 results: flow, volume

	DATE	TIME	TANK (ST) 09	10	11	12
FLOW (s ℓ^{-1})	10/1	1730	72	71	73.5	72
		2300	74	72	74	73.5
	11/1	1200	73	71.5	73.5	70.5
	12/1	1100	73	73	73.5	71.5
		2400	72	72	73.5	73.5
	13/1	1430	73.5	73.5	72.5	72.5
	14/1	1100	73.5	73.5	73	71
	15/1	1145	74.5	73	72	74.5
	16/1	1100	73.5	73.5	72	73.5
	MEAN		73.2	72.6	73.1	72.5
MEAN FLOW (ℓ min^{-1})			0.819	0.827	0.821	0.828
VOLUME (ℓ)			3.6	7.8	7.7	3.8

Table A1.5 PO3 results: TOA, temperature, pH, UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ \ell^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ \ell^{-1}$)
5/2/73	1000	09	100	9.4	6.21	0.029
		10	74	9.3	6.37	0.031
		11	98	9.2	6.39	0.042
		12	115	9.1	6.27	0.037
		IS	15	8.6	7.35	0.056
	1200	09	83	9.2	6.22	0.024
		10	101	9.2	6.41	0.045
		11	93	9.1	6.29	0.031
		12	146	9.1	6.26	0.046
		IS	14	8.7	7.27	0.044
	1400	09	88	9.2	6.29	0.030
		10	143	9.2	6.44	0.069
		11	122	9.2	6.34	0.047
		12	171	9.2	6.38	0.072
		IS	-	8.7	7.39	-
	1600	09	93	9.2	6.34	0.036
		10	163	9.2	6.42	0.075
		11	141	9.2	6.37	0.058
		12	152	9.1	6.39	0.065
		IS	15	8.4	7.33	0.053
6/2	1000	09	131	9.3	6.42	0.061
		10	157	9.2	6.45	0.077
		11	201	9.2	6.37	0.082
		12	233	9.2	6.33	0.087
		IS	22	8.6	7.36	0.084
	1200	09	144	9.3	6.31	0.052
		10	167	9.2	6.46	0.084
		11	233	9.2	6.30	0.081
		12	305	9.3	6.31	0.110
		IS	21	8.7	7.51	0.114
	1400	09	162	9.3	6.39	0.070
		10	202	9.2	6.48	0.107
		11	265	9.2	6.34	0.106
		12	335	9.2	6.39	0.144
		IS	22	8.4	7.35	0.081
	1600	09	143	9.3	6.44	0.069
		10	217	9.0	6.59	0.145
		11	269	9.2	6.35	0.105
		12	385	9.3	6.29	0.132
		IS	22	8.6	7.40	0.092
7/2	1000	09	150	9.5	6.48	0.081
		10	202	9.4	6.45	0.101
		11	212	9.4	6.30	0.075
		12	258	9.4	6.31	0.094
		IS	24	8.7	7.35	0.090

Table A1.5 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
7/2	1200	09	175	9.5	6.35	0.070
		10	236	9.4	6.41	0.108
		11	187	9.4	6.33	0.071
		12	297	9.5	6.31	0.109
		IS	-	8.6	7.35	-
	1400	09	196	9.4	6.44	0.096
		10	285	9.3	6.38	0.121
		11	207	9.3	6.27	0.068
		12	305	9.2	6.28	0.102
		IS	25	8.0	7.32	0.083
	1600	09	183	8.7	6.43	0.083
		10	295	8.7	6.53	0.168
		11	199	8.7	6.27	0.062
		12	340	8.8	6.30	0.115
		IS	21	8.0	7.39	0.082
8/2	1000	09	182	9.1	6.49	0.098
		10	272	9.1	6.50	0.149
		11	265	9.0	6.39	0.112
		12	323	9.1	6.35	0.126
		IS	34	8.1	7.37	0.127
	1200	09	210	9.0	6.39	0.089
		10	323	8.8	6.41	0.141
		11	292	8.9	6.33	0.107
		12	303	8.9	6.27	0.096
		IS	26	8.2	7.37	0.098
	1400	09	228	8.8	6.47	0.114
		10	405	8.8	6.41	0.176
		11	285	8.8	6.33	0.103
		12	365	8.8	6.39	0.152
		IS	22	8.1	7.34	0.077
	1600	09	252	8.8	6.46	0.123
		10	435	8.8	6.47	0.218
		11	278	8.8	6.39	0.116
		12	365	8.8	6.43	0.167
		IS	20	8.0	7.34	0.069
	MEAN	09	137	9.3	6.36	0.058
	MEAN	10	230	9.1	6.45	0.113
	MEAN	11	200	9.1	6.33	0.079
	MEAN	12	275	9.1	6.33	0.103
	MEAN	IS	22	8.4	7.36	0.082

Notes:-

a) Sample references as in Table A1.3.

b) Means are calculated from all tabulated data except ST 09; for this tank values subsequent to mortality at 0845, 8/2 are ignored.

Table A1.6 PO3 results: food consumption

DATE	TIME	TANK (ST) 09 NETX	09 %	10 NETX	10 %	11 NETX	11 %	12 NETX	12 %
4/2/73	1100	29	79.4	61	56.7	76	56.6	63	64.0
	1300	55	61.0	56	60.3	52	70.3	52	70.3
	1500	40	71.6	70	50.4	59	66.3	57	67.4
	1700	64	54.6	68	51.8	37	78.9	67	61.7
5/2	0900	28	80.1	0	100.0	22	87.4	0	100.0
	1100	68	51.8	32	77.3	45	74.3	58	66.9
	1300	56	60.3	18	87.2	66	62.3	36	79.4
	1500	61	56.7	1	99.3	52	70.3	97	44.6
	1700	69	51.1	5	96.5	29	83.4	31	82.3
	1900	78	44.7	17	87.9	53	69.7	0	100.0
6/2	0945	44	68.8	0	100.0	14	92.0	0	100.0
	1100	58	58.9	3	97.9	38	78.3	12	93.1
	1330	49	65.2	0	100.0	26	85.1	24	86.3
	1500	74	47.5	0	100.0	81	53.7	77	56.0
	1700	21	85.1	0	100.0	76	56.6	97	44.6
7/2	0915	7	95.0	0	100.0	50	71.4	0	100.0
	1100	30	78.7	0	100.0	56	68.0	20	88.6
	1300	54	61.7	0	100.0	93	46.9	56	68.0
	1500	40	71.6	0	100.0	101	42.3	45	74.3
	1700	43	69.5	0	100.0	87	50.3	60	65.7
	1900	52	63.1	0	100.0	80	54.3	84	52.0
8/2	0855	2	98.6	0	100.0	123	29.7	68	61.1
	1110	51	63.8	0	100.0	92	47.4	53	69.7
	1300	43	69.5	0	100.0	70	60.0	84	52.0
	1515	45	68.1	0	100.0	76	56.6	40	77.1
	1725	67	52.5	0	100.0	63	64.0	0	100.0
	MEAN	47	66.5	13	91.0	62	64.5	45	74.0

Notes:-

a) NETX = number of pellets uneaten;*

b) % = percentage of offered food eaten.

c) Means are calculated from all tabulated data; ignoring 4/2 (on which no ammonia measurements were made) has little effect on the means except in ST 10 where the mean NETX drops to 3 and the mean % rises to 97.6.

*rations are ST 09, 10: 141 pellets and ST 11, 12: 175 pellets.

Table A1.7 PO3 results: flow, volume

	DATE	TIME	TANK (ST) 09	10	11	12
FLOW (s ℓ^{-1})	5/2	1130	94.5	89	92	93
		1915	90.5	104.5	92	92.5
	6/2	1120	94	85	94.5	101
		1910	-	75	-	-
	7/2	0900	90	119	89	98.5
		1145	95.5	85.5	95	97.5
		1540	96	88.5	95	96.5
	8/2	1125	96.5	99	93.5	96
		1705	95	99.5	96.5	97
	MEAN		94	94	93.4	96.5
MEAN FLOW (ℓ min $^{-1}$)			0.638	0.638	0.642	0.622
VOLUME (ℓ)			6.0	7.6	5.7	8.0

PO3 mortality

One fish was removed from ST 09 at 0845 on 8/2/73. The fish displayed flank and tail wounds and abrasions of the kind associated with cannibalistic bullying by other fish, and died soon afterwards. All weight, stocking and loading conditions discussed in Chapter 6 imply the fish weight prior to this loss (i.e. final tank weight plus weight of dead fish) as the final weight.

A2. E-SERIES PRELIMINARY WORK RESULTS

CONTENTS:-

Table A2.1 Summary of BMP results

BMP	FCAT	No.of FISH		TOTAL WEIGHT (g)		MEAN LENGTH (cm)		CONDITION FACTOR		FOOD FED (g)	FINAL CR	VOL (ℓ)	FINAL STOK (g ℓ$^{-1}$)	MEAN TEMP (°C)
		INT	FIN	INT	FIN	INT	FIN	INT	FIN					
1	8	34	34	4503	5909	-	22.9	-	-	769	0.55	126	46.9	
	9	54	49	9634	10794	-	24.9	-	-	1555	0.74	134	80.6	12.0
	10	18	18	4373	5125	-	27.1	-	-	625	0.83	105	48.8	
2	8	13	13	2043	2301	22.1	22.8	1.46	1.48	322	1.25	92	25.0	
	9A	22	22	4042	4560	23.4	24.2	1.43	1.46	596	1.15	100	45.6	
	9B	32	31	6680	7324	24.2	25.2	1.43	1.48	968	1.14	147	49.8	11.7
	10	35	35	9239	10156	26.4	27.1	1.43	1.46	1265	1.38	155	65.5	
3	8	6	6	963	1049	22.3	22.9	1.44	1.45	148	1.72	84	12.5	
	9A	21	21	4136	4479	22.8	24.2	1.65	1.50	587	1.71	117	38.3	
	9B	25	25	5558	6095	24.7	25.4	1.47	1.48	789	1.47	126	48.4	10.7
	10A	26	26	6766	7221	26.1	27.8	1.47	1.45	886	1.95	147	49.1	
	10B	23	23	6918	7436	27.5	28.0	1.44	1.48	894	1.73	142	52.4	
4	9A	16	16	3098	3113	23.4	23.8	1.50	1.45	312	20.81	84	37.1	
	9B	19	19	4211	4310	24.7	25.2	1.46	1.43	425	4.29	84	51.3	
	10A	28	27	6853	7031	25.0	26.2	1.46	1.44	619	3.48	142	49.5	7.3
	10B	28	28	8364	8691	27.2	27.5	1.48	1.50	752	2.30	147	59.1	
	10C	10	10	3478	3557	28.9	29.2	1.45	1.44	314	3.97	105	33.9	

Notes:-

a) BMP: 1-4 represent the four two-week periods.

b) INT = Initial, FIN = final.

c) CR = Conversion rate = food fed per weight gain.

d) STOK = Stocking = weight per volume.

e) Conversions for BMP period 1 are so low as to be particularly suspicious; given that conversions below 1.0 are possible (water uptake can account for excess weight gain) nevertheless a value of 0.55 seems highly unlikely. The most probable error is in the feed record.

f) In BMP 4 conversions are at the opposite extreme; fish were growing very large (although stocking was no higher than in earlier periods) and this combined with a sharp fall in temperature probably accounts for a lack in feeding and a great fall off in growth, indicated by poor conversion (high values) and a deterioration in condition factor.

g) BMP lasted from 10/10/73 to 5/12/73.

Table A2.2 Summary of PO4 results

Number of fish		initial		16
		final		10
Weight	total	initial	(g)	886
		final	(g)	591
	mean	initial	(g)	55.375
		final	(g)	59.100
		increase	(g)	3.725
Length;	mean	initial	(cm)	16.469
		final	(cm)	16.600
		increase	(cm)	0.131
Condition factor		initial		1.24
		final		1.29
Total food fed			(g)	205.8
Estimated overall conversion*				5.525
Temperature		initial	(^{o}C)	16.3
		final	(^{o}C)	14.4
		mean	(^{o}C)	14.9

* estimated from:-

$$\frac{\text{total food fed}}{(\text{mean weight increase}) \times (\text{final number of fish})}$$

(This assumes that the 6 fish which died consumed no food.)

Table A2.3 Summary of EOO results

				MT 7	MT 8
FCAT				4	3
Number of fish		initial		30	30
		final		27	30
Weight	total	initial	(g)	489	278
		final	(g)	458	289
	mean	initial	(g)	16.30	9.27
		final	(g)	16.96	9.63
		increase	(g)	0.66	0.36
Length;	mean	initial	(cm)	10.93	8.90
		final	(cm)	10.95	9.05
		increase	(cm)	0.02	0.15
Condition factor		initial		1.25	1.31
		final		1.29	1.30
Total food fed			(g)	30.1	21.5
Conversion ratio*				1.479	1.955
				1.523	
				1.507	
				1.900	
Temperature		initial	(^{o}C)	5.5	5.5
		final	(^{o}C)	6.1	6.1
		mean	(^{o}C)	6.0	6.0
Average TOA			($\mu g\ \ell^{-1}$)	107	55
Average SER			(mg $kg^{-1}\ h^{-1}$)	9.57	5.42
Average flow			($\ell\ min^{-1}$)	0.972	0.945
Average loading			(kg $min\ \ell^{-1}$)	0.495	0.299
Estimated average stocking			(g ℓ^{-1})	40	24

* The conversions quoted were calculated by the standard weight estimation routine used in the E-series (see DATA ANALYSIS section) and those in MT 7 represent phases of the experiment before, between, and after mortalities.

Notes:-

a) The stocking values given in the table assume a volume of 12 ℓ in each tank; this was approximately correct by eye observation

b) Poor growth in MT 8 (FCAT 3) is indicated by the fall in condition factor.

A3. E-SERIES EXPERIMENT RAW RESULTS

CONTENTS:-

BASIC DATA

A3.1 The data are computer-listed in Table A3.1 and follow a particular convention in 8 columns. The first column consists of experimental data string codings, made up of 5 sections, thus:-

E / W / X / YY / Z

where W, X, Y, Z represent digits. The last 7 columns each bear a heading which indicated the quantity (as described in Chapter 7 para. 7.75).

A3.2 Codings:-

E = experiment type; E indicates environment/excretion experiment (as opposed to T, used in tolerance experiments).

W = series of experiments; this takes values 0,1 or 2 where 0 represents a pilot experiment, 1 an experiment using MTS, and 2 an experiment using LTS.

X = experiment number; this takes values 0,1,2,3,4,5,6, 7,8,9 according to the number of experiments in the particular series. A value of 0 was found only in series 1 (MTS experiments) and corresponds to the run-up experiment E00. X is the last digit of experiment titles as in Chapters 6 and 7.

YY = experimental DAY; values were from 01 to 14 (see Chapter 7).

Z = experimental tank; this takes values from 1 to 8 (since the MTS, the largest system, held 8 tanks). For pilot experiments P02 and P03, St Nos. 09,10,11,12 were designated tanks 1,2,3,4.

A3.3 For example, the coding

E03042

indicates:-

E - environment/excretion

O - pilot series experiment
3 - pilot experiment 3 (PO3)
O4 - DAY O4
2 - tank 2 (ST lO in this case)

Similarly, the coding

E1OO38

indicates:-

E1 - E-series experiment, MTS
O - run-up experiment (EOO)
O3 - DAY O3
8 - MT8

A3.4 Values of zero in the columns headed VØLUME, SIZELN and AVERLN correspond to situations where these values were not measured. The full matrix comprises 264 data strings.

A3.5 Units are as follows:-

TØTAMM - $\mu g\ \ell^{-1}$
ENUMBR - (number)
FLØWRT - $\ell\ min^{-1}$
VØLUME - ℓ
WEIGHT - g
SIZELN - cm
AVERLN - cm

A3.6 All PO3 data except for ST lO are omitted due to the difficulties in food consumption mentioned in Chapter 6.

Table A3.1/see over

XX

	TOTAMM	ENUMBR	FLOWRT	VOLUMF	WEIGHT	SIZELN	AVERLN
XX							
E01011	80.	4.	0.896	2.3	61.00	0.00	0.000
E01012	76.	5.	0.909	8.2	57.00	0.00	0.000
E01014	127.	13.	0.916	8.6	170.00	0.00	0.000
E01016	132.	18.	0.909	2.6	211.00	0.00	0.000
E01021	50.	4.	0.896	2.3	61.00	0.00	0.000
E01022	43.	5.	0.909	8.2	57.00	0.00	0.000
E01024	122.	13.	0.916	8.6	170.00	0.00	0.000
E01026	109.	18.	0.909	2.6	211.00	0.00	0.000
E02021	92.	7.	0.822	3.6	306.00	0.00	0.000
E02022	76.	6.	0.839	7.8	325.00	0.00	0.000
E02023	145.	13.	0.816	7.7	609.00	0.00	0.000
E02024	75.	13.	0.851	3.8	591.00	0.00	0.000
E02031	76.	7.	0.822	3.6	306.00	0.00	0.000
E02032	72.	6.	0.822	7.8	325.00	0.00	0.000
E02033	96.	13.	0.816	7.7	609.00	0.00	0.000
E02034	83.	13.	0.839	3.8	591.00	0.00	0.000
E02041	75.	7.	0.816	3.6	306.00	0.00	0.000
E02043	140.	13.	0.828	7.7	609.00	0.00	0.000
E02044	111.	13.	0.828	3.8	591.00	0.00	0.000
E02051	38.	7.	0.816	3.6	306.00	0.00	0.000
E02052	51.	6.	0.816	7.8	325.00	0.00	0.000
E02053	94.	13.	0.822	7.7	609.00	0.00	0.000
E02054	95.	13.	0.845	3.8	591.00	0.00	0.000
E02061	52.	7.	0.805	3.6	306.00	0.00	0.000
E02062	50.	6.	0.822	7.8	325.00	0.00	0.000
E02063	106.	13.	0.833	7.7	609.00	0.00	0.000
E02064	133.	13.	0.805	3.8	591.00	0.00	0.000
E03032	202.	18.	0.706	7.6	1035.00	0.00	0.000
E03042	285.	18.	0.702	7.6	1035.00	0.00	0.000
E03052	405.	18.	0.606	7.6	1035.00	0.00	0.000
E04047	119.	16.	3.243	14.8	900.93	264.03	16.502
E04057	122.	15.	3.333	12.9	837.69	247.22	16.481
E04067	107.	15.	3.333	14.1	843.66	247.44	16.496
E04077	116.	14.	3.429	14.3	795.85	231.58	16.542
E04127	89.	11.	3.429	14.9	645.77	182.70	16.609

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E10037	107.	29.	0.972	0.0	481.41	317.21	10.938
E10038	55.	30.	0.945	0.0	283.08	269.08	8.969
E11051	282.	30.	0.193	0.0	294.67	271.60	9.053
E11052	246.	20.	0.348	0.0	385.88	228.42	11.421
E11054	328.	29.	0.171	0.0	301.08	265.05	9.140
E11055	280.	20.	0.297	0.0	368.54	226.42	11.321
E11057	294.	30.	0.212	0.0	297.00	272.37	9.079
E11058	378.	20.	0.277	0.0	410.84	231.16	11.558
E11081	104.	30.	0.452	0.0	296.83	272.90	9.097
E11082	191.	19.	0.282	0.0	371.05	217.71	11.458
E11084	82.	29.	0.513	0.0	303.21	265.65	9.160
E11085	164.	19.	0.344	0.0	356.40	216.07	11.372
E11087	165.	30.	0.257	0.0	297.00	273.15	9.105
E11088	165.	20.	0.392	0.0	414.49	232.42	11.621
E11131	257.	30.	0.205	0.0	301.04	275.42	9.181
E11132	189.	19.	0.299	0.0	374.42	218.81	11.516
E11134	335.	29.	0.195	0.0	307.17	266.77	9.199
E11135	263.	19.	0.267	0.0	367.07	217.19	11.431
E11137	272.	30.	0.193	0.0	297.00	274.27	9.142
E11138	415.	20.	0.230	0.0	420.55	234.50	11.725
E12031	172.	30.	0.284	8.8	288.49	272.76	9.092
E12032	237.	20.	0.330	9.5	327.79	218.71	10.935
E12033	307.	30.	0.191	9.4	285.08	270.60	9.020
E12034	259.	20.	0.464	9.3	377.25	217.58	11.452
E12035	201.	29.	0.346	9.3	265.02	258.35	8.909
E12036	255.	20.	0.421	9.9	363.70	227.46	11.373
E12037	247.	29.	0.323	10.0	252.99	256.04	8.829
E12038	367.	20.	0.438	10.6	393.21	231.22	11.561
E12071	410.	30.	0.237	8.4	303.27	276.15	9.205
E12072	292.	20.	0.248	9.5	340.82	221.02	11.051
E12073	429.	30.	0.176	9.3	296.60	274.23	9.141
E12074	344.	20.	0.364	9.2	394.01	220.65	11.613
E12075	384.	29.	0.292	9.2	277.17	262.18	9.041
E12076	640.	20.	0.208	9.6	378.62	229.12	11.456
E12077	477.	29.	0.225	10.0	260.13	258.88	8.927

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E12078	837.	20.	0.249	10.6	399.67	233.68	11.684
E12091	330.	30.	0.241	8.4	310.99	277.91	9.264
E12092	325.	20.	0.230	9.4	347.59	222.23	11.111
E12093	397.	30.	0.186	9.1	303.03	276.26	9.209
E12094	292.	20.	0.370	9.3	402.54	222.21	11.695
E12095	377.	29.	0.293	9.2	283.71	264.24	9.112
E12096	565.	20.	0.208	9.4	386.00	229.93	11.497
E12097	497.	29.	0.233	10.0	263.62	260.27	8.975
E12098	495.	20.	0.216	10.5	403.07	234.98	11.749
E12101	445.	30.	0.228	8.3	317.09	279.31	9.310
E12102	590.	20.	0.241	9.4	352.73	223.14	11.157
E12103	535.	30.	0.214	9.1	307.95	277.82	9.261
E12104	472.	20.	0.360	9.3	409.56	223.49	11.763
E12105	447.	29.	0.293	9.2	289.09	265.93	9.170
E12106	795.	19.	0.230	9.4	370.84	219.10	11.532
E12107	580.	29.	0.214	10.0	266.27	261.32	9.011
E12108	502.	20.	0.200	10.4	405.34	235.85	11.792
E13031	245.	27.	0.214	8.8	131.27	200.32	7.419
E13032	146.	40.	0.870	10.1	425.06	382.06	9.551
E13033	137.	30.	1.000	9.9	445.05	321.69	10.723
E13034	233.	40.	0.571	9.5	422.11	380.54	9.513
E13035	226.	30.	0.732	11.0	439.83	323.94	10.798
E13036	215.	40.	0.811	11.8	426.24	385.83	9.646
E13037	248.	29.	0.755	10.5	588.61	351.41	12.117
E13038	307.	20.	0.529	11.6	553.79	267.81	13.391
E13041	225.	27.	0.207	8.9	135.36	201.85	7.476
E13042	138.	40.	0.889	10.4	435.02	384.21	9.605
E13043	117.	30.	1.000	9.9	454.24	322.88	10.763
E13044	197.	40.	0.612	9.6	430.62	382.30	9.557
E13045	202.	30.	0.741	11.2	448.40	326.08	10.869
E13046	176.	40.	0.779	11.8	435.70	387.72	9.693
E13047	233.	29.	0.694	10.5	601.27	352.64	12.160
E13048	224.	20.	0.494	12.1	557.04	268.20	13.410
E13051	141.	27.	0.217	8.9	137.09	202.49	7.500
E13052	111.	39.	1.043	10.7	432.61	376.58	9.656

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E13053	126.	29.	1.000	10.0	446.90	313.37	10.806
E13054	199.	40.	0.591	9.6	438.69	383.97	9.599
E13055	163.	30.	0.750	11.1	455.82	327.92	10.931
E13056	154.	40.	0.800	11.8	443.97	389.36	9.734
E13057	195.	29.	0.632	10.5	611.84	353.68	12.196
E13058	246.	20.	0.476	11.9	559.83	268.54	13.427
E13061	110.	27.	0.209	8.8	138.11	202.87	7.514
E13062	93.	39.	1.008	10.8	439.44	378.05	9.693
E13063	108.	29.	1.000	10.2	455.22	314.41	10.842
E13064	168.	40.	0.563	11.2	445.27	385.33	9.633
E13065	149.	30.	0.719	11.1	461.97	329.46	10.982
E13066	133.	40.	0.774	11.8	450.12	390.59	9.765
E13067	192.	29.	0.642	10.5	620.42	354.52	12.225
E13068	209.	20.	0.460	11.9	562.11	268.81	13.440
E13071	151.	27.	0.208	8.6	139.57	203.41	7.534
E13072	142.	38.	0.960	12.2	438.80	370.90	9.761
E13073	141.	28.	0.992	13.1	454.58	305.53	10.912
E13074	229.	40.	0.585	11.2	453.89	387.11	9.678
E13075	203.	29.	0.779	12.2	455.01	320.78	11.061
E13076	163.	40.	0.795	11.8	458.20	392.20	9.805
E13077	236.	26.	0.625	11.7	567.45	319.41	12.285
E13078	253.	20.	0.476	12.0	565.07	269.16	13.458
E13111	181.	27.	0.375	9.1	156.05	209.55	7.761
E13112	154.	37.	0.902	13.6	464.89	369.13	9.976
E13113	146.	27.	0.976	13.2	467.92	298.30	11.048
E13114	227.	38.	0.606	11.4	465.82	375.00	9.869
E13115	152.	24.	0.759	13.3	397.26	272.03	11.334
E13116	143.	39.	0.811	11.8	480.67	389.13	9.978
E13117	202.	25.	0.566	11.6	584.29	311.07	12.443
E13118	217.	19.	0.426	11.9	548.30	257.34	13.544
E13121	225.	27.	0.374	9.2	160.70	211.28	7.825
E13122	175.	37.	0.896	14.0	472.76	370.74	10.020
E13123	150.	26.	0.984	13.3	456.37	288.29	11.088
E13124	262.	38.	0.553	11.3	474.47	376.76	9.915
E13125	167.	24.	0.784	13.6	405.11	273.37	11.391

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E13126	207.	39.	0.800	11.8	487.96	390.59	10.015
E13127	304.	25.	0.558	11.4	592.55	311.63	12.465
E13128	367.	19.	0.403	11.8	551.23	257.59	13.557
E14064	119.	29.	1.250	10.5	357.80	287.21	9.904
E14065	161.	19.	0.706	12.6	295.95	205.74	10.829
E14066	120.	30.	1.304	10.8	365.82	296.06	9.869
E14067	97.	19.	1.379	12.4	295.26	204.83	10.781
E14068	86.	19.	1.739	12.8	349.35	218.10	11.479
E14074	125.	29.	1.538	11.2	366.39	289.27	9.975
E14075	185.	19.	0.857	13.0	302.72	206.67	10.877
E14076	180.	30.	1.212	11.2	375.68	298.05	9.935
E14077	191.	19.	0.889	11.8	303.03	205.82	10.832
E14078	153.	19.	1.250	12.4	356.86	219.11	11.532
E14084	112.	29.	1.538	11.1	374.96	291.33	10.046
E14085	145.	19.	0.863	13.2	309.06	207.53	10.923
E14086	123.	30.	1.154	11.2	384.76	299.88	9.996
E14087	156.	19.	0.851	12.1	310.18	206.72	10.880
E14088	102.	18.	1.081	12.3	347.94	208.87	11.604
E14094	185.	29.	0.800	10.1	382.13	293.06	10.105
E14095	145.	19.	0.833	13.2	314.29	208.24	10.960
E14096	126.	30.	1.154	11.2	393.80	301.70	10.057
E14097	137.	19.	0.952	12.2	315.63	207.41	10.916
E14098	124.	16.	0.938	12.1	320.02	187.18	11.699
E14104	204.	29.	0.816	10.3	388.31	294.54	10.157
E14105	163.	19.	0.839	13.3	319.46	208.95	10.997
E14106	163.	30.	1.165	11.4	402.68	303.49	10.116
E14107	116.	19.	0.960	12.2	320.49	208.03	10.949
E14108	142.	16.	0.976	12.1	321.93	187.42	11.714
E15036	177.	31.	1.611	14.1	898.26	431.66	13.925
E15046	170.	31.	1.589	14.1	914.91	432.52	13.952
E15076	109.	31.	1.875	15.5	950.01	434.33	14.011
E15086	99.	31.	1.529	14.5	962.36	434.97	14.031
E15096	149.	30.	1.538	14.5	948.60	421.28	14.043
E15106	184.	30.	1.538	14.7	961.48	422.07	14.069
E15037	173.	30.	1.529	12.8	919.32	418.07	13.936

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E15047	189.	30.	1.644	13.0	940.00	419.36	13.979
E15077	141.	30.	1.860	13.7	980.49	421.88	14.063
E15087	135.	30.	1.500	12.8	994.93	422.78	14.093
E15097	175.	30.	1.548	12.8	1011.72	423.83	14.128
E15107	165.	30.	1.500	12.9	1028.01	424.84	14.161
E15038	257.	26.	1.622	13.7	1607.50	442.80	17.031
E15048	237.	26.	1.558	13.7	1618.24	443.66	17.064
E15078	190.	26.	1.765	14.5	1645.66	445.85	17.148
E15088	239.	26.	1.319	13.7	1655.54	446.64	17.179
E15098	276.	26.	1.326	13.7	1666.32	447.51	17.212
E15108	261.	26.	1.364	13.7	1676.25	448.30	17.242
E16025	140.	30.	0.427	12.5	541.43	347.43	11.581
E16035	162.	30.	0.492	12.5	548.26	347.99	11.600
E16085	136.	30.	0.594	12.8	586.18	351.09	11.703
E16095	232.	30.	0.471	12.3	594.89	351.80	11.727
E16135	190.	30.	0.449	12.3	623.54	354.14	11.805
E16026	201.	30.	0.492	9.2	585.29	356.48	11.883
E16036	152.	30.	0.462	9.2	593.38	357.13	11.904
E16086	151.	30.	0.600	9.4	631.13	360.15	12.005
E16096	242.	29.	0.515	9.9	616.28	348.14	12.005
E16136	189.	29.	0.533	9.6	646.91	351.02	12.104
E16027	217.	30.	0.548	11.2	584.06	352.67	11.756
E16037	157.	30.	0.531	11.6	590.95	353.28	11.776
E16087	111.	29.	0.800	12.0	615.36	345.59	11.917
E16097	225.	29.	0.649	11.7	623.86	346.32	11.942
E16137	159.	29.	0.682	11.9	650.95	348.65	12.022
E16028	198.	30.	0.536	11.2	558.96	348.97	11.632
E16038	134.	30.	0.682	11.6	565.41	349.50	11.650
E16088	161.	30.	0.469	11.2	592.30	351.69	11.723
E16098	285.	30.	0.504	11.2	601.85	352.46	11.749
E16138	228.	29.	0.407	11.0	611.57	343.14	11.832
E27062	365.	63.	1.579	84.0	3351.38	1027.76	16.314
E27072	550.	63.	1.702	84.0	3383.96	1030.56	16.358
E27102	447.	63.	1.690	89.0	3462.81	1037.33	16.466
E27112	440.	63.	1.404	84.0	3490.06	1039.67	16.503

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E27122	390.	63.	1.644	84.0	3517.15	1041.99	16.540
E27132	330.	63.	1.297	84.0	3542.20	1044.14	16.574
E27064	427.	212.	1.905	97.0	7129.01	3001.37	14.157
E27074	630.	212.	2.202	93.0	7200.37	3006.49	14.182
E27104	397.	212.	2.637	97.0	7377.09	3019.16	14.241
E27114	452.	212.	2.051	101.0	7445.35	3024.05	14.264
E27124	380.	212.	2.143	97.0	7513.60	3028.95	14.287
E27134	332.	212.	2.069	101.0	7571.95	3033.13	14.307
E27065	347.	147.	2.069	84.0	3170.35	1762.59	11.990
E27075	870.	147.	1.231	76.0	3226.06	1768.37	12.030
E27105	565.	147.	1.600	72.0	3362.02	1782.49	12.126
E27115	650.	147.	1.081	72.0	3407.34	1787.20	12.158
E27125	480.	147.	1.148	76.0	3452.26	1791.86	12.190
E27135	470.	147.	1.021	76.0	3495.27	1796.33	12.220
E27066	505.	350.	2.143	55.0	3183.53	3102.36	8.864
E27076	1190.	350.	1.379	50.0	3243.06	3114.52	8.899
E27106	690.	350.	1.569	50.0	3393.31	3145.20	8.986
E27116	800.	350.	1.096	50.0	3445.25	3155.80	9.017
E27126	620.	350.	1.159	50.0	3496.39	3166.24	9.046
E27136	610.	350.	1.096	50.0	3545.93	3176.36	9.075
E18065	139.	40.	0.676	12.5	856.64	472.56	11.814
E18075	156.	40.	0.623	12.5	864.20	473.22	11.831
E18115	128.	40.	0.608	12.5	894.44	475.87	11.897
E18125	143.	40.	0.611	12.5	901.41	476.48	11.912
E18135	172.	40.	0.606	12.5	908.45	477.10	11.928
E18066	158.	40.	0.637	9.8	923.16	481.94	12.049
E18076	224.	40.	0.562	9.7	929.96	482.73	12.068
E18116	149.	38.	0.569	9.7	907.00	460.90	12.129
E18126	183.	38.	0.580	9.8	914.72	461.95	12.157
E18136	290.	38.	0.557	9.8	922.29	462.99	12.184
E18067	137.	40.	0.614	11.5	815.04	467.60	11.690
E18077	240.	40.	0.526	11.5	821.30	468.36	11.709
E18117	167.	40.	0.460	11.7	846.18	471.35	11.784
E18127	238.	40.	0.545	11.7	852.98	472.17	11.804
E18137	287.	40.	0.485	11.7	860.29	473.05	11.826

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E18068	106.	40.	0.787	11.3	863.19	476.64	11.916
E18078	185.	39.	0.605	11.2	844.15	464.64	11.914
E18118	164.	38.	0.600	11.3	845.64	454.49	11.960
E18128	203.	38.	0.620	11.1	852.17	455.09	11.976
E18138	382.	35.	0.585	11.4	792.56	419.48	11.985
E29042	313.	92.	1.967	89.0	4737.95	1490.31	16.199
E29052	305.	92.	1.765	89.0	4766.39	1491.95	16.217
E29062	257.	92.	1.633	89.0	4794.68	1493.58	16.235
E29072	284.	92.	1.805	89.0	4823.76	1495.26	16.253
E29102	274.	92.	1.818	89.0	4915.52	1500.56	16.310
E29112	246.	92.	1.778	89.0	4946.85	1502.37	16.330
E29132	325.	92.	1.860	93.0	5008.48	1505.93	16.369
E29044	260.	234.	2.474	109.0	7798.81	3278.82	14.012
E29054	245.	234.	2.500	109.0	7846.23	3283.53	14.032
E29064	229.	234.	2.727	114.0	7896.50	3288.53	14.054
E29074	244.	234.	2.581	114.0	7950.41	3293.90	14.076
E29104	245.	233.	3.000	114.0	8069.83	3294.77	14.141
E29114	190.	233.	2.727	114.0	8119.94	3299.84	14.162
E29134	276.	233.	2.727	109.0	8219.69	3309.93	14.206

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CALCULATED DATA

A3.5 Calculated data are listed in Table A3.2 in a similar way to Table A3.1, under the same data string codings. Values of zero indicate absence of measurement, and the units are as follows:-

SPEXRT - mg kg^{-1} h^{-1}

TEMPRT - ^{o}C

DENSTY - (number) ℓ^{-1}

DNLØAD - kg min ℓ^{-1}

DNSTØK - g ℓ^{-1}

FACTØR - kg min ℓ^{-1} m^{-1}

GRUBFD - g

Table A3.2/see over

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	SPEXRT	TEMPRT	DENSTY	DNLOAD	DNSTOK	FACTOR	GRUBFD	FDMEAL
XX								
E01011	70,464	17,0	1,739	0,06812	26,52	0,000	0,00	0,00
E01012	72,727	17,0	0,610	0,06270	6,95	0,000	0,00	0,00
E01014	41,060	17,0	1,512	0,18558	19,77	0,000	0,00	0,00
E01016	34,123	17,0	6,923	0,23210	81,15	0,000	0,00	0,00
E01021	44,040	17,5	1,739	0,06812	26,52	0,000	0,00	0,00
E01022	41,148	17,5	0,610	0,06270	6,95	0,000	0,00	0,00
E01024	39,444	17,5	1,512	0,18558	19,77	0,000	0,00	0,00
E01026	28,178	17,5	6,923	0,23210	81,15	0,000	0,00	0,00
E02021	14,827	8,6	1,944	0,37230	85,00	0,000	0,00	0,00
E02022	11,774	8,6	0,769	0,38729	41,67	0,000	0,00	0,00
E02023	11,662	8,6	1,688	0,74602	79,09	0,000	0,00	0,00
E02024	6,480	8,6	3,421	0,69443	155,53	0,000	0,00	0,00
E02031	12,248	8,4	1,944	0,37230	85,00	0,000	0,00	0,00
E02032	10,925	8,4	0,769	0,39542	41,67	0,000	0,00	0,00
E02033	7,721	8,4	1,688	0,74602	79,09	0,000	0,00	0,00
E02034	7,071	8,4	3,421	0,70428	155,53	0,000	0,00	0,00
E02041	12,005	9,0	1,944	0,37485	85,00	0,000	0,00	0,00
E02043	11,415	9,0	1,688	0,73587	79,09	0,000	0,00	0,00
E02044	9,326	9,0	3,421	0,71412	155,53	0,000	0,00	0,00
E02051	6,082	9,0	1,944	0,37485	85,00	0,000	0,00	0,00
E02052	7,686	9,0	0,769	0,39812	41,67	0,000	0,00	0,00
E02053	7,612	9,0	1,688	0,74095	79,09	0,000	0,00	0,00
E02054	8,150	9,0	3,421	0,69935	155,53	0,000	0,00	0,00
E02061	8,212	8,9	1,944	0,37995	85,00	0,000	0,00	0,00
E02062	7,587	8,9	0,769	0,39542	41,67	0,000	0,00	0,00
E02063	8,703	8,9	1,688	0,75080	79,09	0,000	0,00	0,00
E02064	10,875	8,9	3,421	0,73382	155,53	0,000	0,00	0,00
E03032	8,266	9,2	2,368	1,46625	136,18	0,000	0,00	0,00
E03042	11,594	9,3	2,368	1,47488	136,18	0,000	0,00	0,00
E03052	14,229	8,8	2,368	1,70775	136,18	0,000	0,00	0,00
E04047	25,703	15,7	1,081	0,27779	60,87	1,683	5,76	14,40
E04057	29,127	15,5	1,163	0,25131	64,94	1,525	6,36	15,00
E04067	25,365	15,2	1,064	0,25310	59,83	1,534	7,48	17,02
E04077	29,984	15,0	0,979	0,23212	55,65	1,403	7,48	18,70
E04127	28,351	14,1	0,738	0,18835	43,34	1,134	6,88	16,12

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E10037	12,958	6.1	0.000	0.49545	0.00	4,530	2.64	5.64
E10038	11,015	6.1	0.000	0.29959	0.00	3,340	1.64	4.10
E11051	11,096	7.0	0.000	1.52491	0.00	16,844	1.35	3.70
E11052	13,305	6.6	0.000	1.10939	0.00	9,714	1.60	4.60
E11054	11,189	7.0	0.000	1.75882	0.00	19,244	1.30	3.02
E11055	13,557	6.7	0.000	1.23920	0.00	10,946	1.50	4.28
E11057	12,581	6.9	0.000	1.40209	0.00	15,443	1.75	3.93
E11058	15,299	6.9	0.000	1.48243	0.00	12,826	1.50	4.28
E11081	9,501	7.0	0.000	0.65674	0.00	7,220	1.84	3.44
E11082	8,700	7.1	0.000	1.31723	0.00	11,496	1.68	3.43
E11084	8,321	7.0	0.000	0.59125	0.00	6,454	1.96	3.26
E11085	9,493	7.0	0.000	1.03652	0.00	9,115	1.92	3.22
E11087	8,574	7.2	0.000	1.15459	0.00	12,681	1.56	3.16
E11088	9,366	7.1	0.000	1.05696	0.00	9,095	1.72	3.67
E11131	10,480	7.1	0.000	1.47134	0.00	16,026	2.04	4.28
E11132	9,052	6.9	0.000	1.25274	0.00	10,878	1.40	3.70
E11134	12,768	7.1	0.000	1.57426	0.00	17,114	1.94	4.13
E11135	11,489	7.0	0.000	1.37345	0.00	12,015	2.00	3.91
E11137	10,593	7.2	0.000	1.54069	0.00	16,852	1.64	4.21
E11138	13,611	7.1	0.000	1.82940	0.00	15,602	2.14	4.95
E12031	10,148	10.0	3,409	1.01694	32,78	11,185	2.04	3.96
E12032	14,321	10.0	2,105	0.99294	34,50	9,080	2.08	5.14
E12033	12,366	10.0	3,191	1.48954	30,33	16,514	1.64	4.10
E12034	19,123	9.9	2,151	0.81265	40,56	7,096	2.88	7.14
E12035	15,737	9.9	3,118	0.76636	28,50	8,602	2.24	5.42
E12036	17,712	9.9	2,020	0.86380	36,74	7,595	2.44	5.92
E12037	18,922	10.0	2,900	0.78321	25,30	8,871	2.32	5.86
E12038	24,526	10.0	1,887	0.89783	37,10	7,766	2.60	5.54
E12071	19,199	9.9	3,571	1.28132	36,10	13,920	2.64	6.60
E12072	12,758	9.9	2,105	1.37321	35,88	12,426	2.36	5.90
E12073	15,315	10.0	3,226	1.68071	31,89	18,386	2.20	5.56
E12074	19,049	9.9	2,174	1.08354	42,83	9,330	2.32	5.86
E12075	24,271	9.9	3,152	0.94930	30,13	10,500	2.12	5.36
E12076	21,056	9.9	2,083	1.82367	39,44	15,919	2.12	5.36
E12077	24,795	9.9	2,900	1.15434	26,01	12,931	2.24	5.60

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E12078	31,349	9.9	1,887	1.60199	37,70	13,711	2,52	6.30
E12091	15,373	10.5	3,571	1.28800	37,02	13,904	4,20	7.26
E12092	12,897	10.5	2,128	1.51201	36,98	13,608	3,60	6.42
E12093	14,647	10.5	3,297	1.62628	33,30	17,660	3,72	6.84
E12094	16,120	10.4	2,151	1.08685	43,28	9,295	4,36	7.60
E12095	23,393	10.3	3,152	0.96697	30,84	10,613	4,04	7.10
E12096	18,265	10.5	2,128	1.85602	41,06	16,144	4,24	7.24
E12097	26,383	10.5	2,900	1.13027	26,36	12,594	3,56	6.44
E12098	15,946	10.6	1,905	1.86253	38,39	15,853	3,72	7.62
E12101	19,173	10.6	3,614	1.39257	38,20	14,957	4,20	10.50
E12102	24,159	10.5	2,128	1.46532	37,52	13,134	3,60	9.00
E12103	22,336	10.7	3,297	1.43712	33,84	15,519	3,76	9.34
E12104	24,918	10.5	2,151	1.13652	44,04	9,662	4,36	10.90
E12105	27,220	10.5	3,152	0.98532	31,42	10,745	4,08	10.14
E12106	29,626	10.6	2,021	1.61005	39,45	13,962	4,28	10.64
E12107	27,931	10.7	2,900	1.24591	26,63	13,826	3,56	8.90
E12108	14,861	10.9	1,923	2.02672	38,98	17,187	3,76	9.34
E13031	23,997	15.6	3,068	0.61257	14,92	8,256	2,64	5.16
E13032	17,921	15.4	3,960	0.48882	42,09	5,118	5,28	11.76
E13033	18,470	15.4	3,030	0.44505	44,95	4,150	5,68	11.50
E13034	18,925	15.4	4,211	0.73869	44,43	7,765	5,44	11.92
E13035	22,559	15.4	2,727	0.60110	39,98	5,567	5,64	12.12
E13036	24,539	15.4	3,390	0.52569	36,12	5,450	6,04	13.36
E13037	19,079	15.4	2,762	0.77990	56,06	6,436	7,28	16.34
E13038	17,583	15.4	1,724	1.04758	47,74	7,823	5,84	12.32
E13041	20,671	15.6	3,034	0.65310	15,21	8,736	0,96	4.92
E13042	16,919	15.4	3,846	0.48939	41,83	5,095	4,60	12.52
E13043	15,454	15.3	3,030	0.45424	45,88	4,221	4,88	13.40
E13044	16,806	15.4	4,167	0.70334	44,86	7,359	5,04	13.20
E13045	20,022	15.3	2,679	0.60534	40,04	5,569	4,40	12.86
E13046	18,886	15.4	3,390	0.55915	36,92	5,769	5,28	14.34
E13047	16,128	15.4	2,762	0.86683	57,26	7,128	5,60	16.52
E13048	11,915	15.4	1,653	1.12800	46,04	8,412	4,56	13.32
E13051	13,367	15.1	3,034	0.63289	15,40	8,439	0,64	2.08
E13052	16,064	15.1	3,645	0.41458	40,43	4,294	4,28	11.18

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E13053	16,917	15.1	2.900	0.44690	44.69	4.136	4.68	12.00
E13054	16,089	15.1	4.167	0.74211	45.70	7.731	4.96	12.52
E13055	16,092	15.1	2.703	0.60775	41.06	5.560	4.52	11.12
E13056	16,650	15.1	3.390	0.55496	37.62	5.701	4.60	12.52
E13057	12,077	15.1	2.762	0.96875	58.27	7.943	5.40	13.80
E13058	12.555	15.1	1.681	1.17564	47.04	8.756	4.60	11.44
E13061	9,990	15.2	5.068	0.66063	15.69	8.792	0.27	1.23
E13062	12,805	15.1	3.611	0.43578	40.69	4.496	2.37	8.79
E13063	14.235	15.0	2.843	0.45522	44.63	4.199	5.50	12.52
E13064	12,754	15.1	3.571	0.79035	39.76	8.205	2.77	10.21
E13065	13,906	15.0	2.703	0.64291	41.62	5.854	2.45	9.23
E13066	13,725	15.0	3.390	0.58141	38.15	5.954	2.42	9.32
E13067	11,915	15.1	2.762	0.96683	59.09	7.909	5.10	11.20
E13068	10,257	15.1	1.681	1.22259	47.24	9.096	2.47	9.37
E13071	13,477	15.3	5.140	0.67225	16.23	8.923	0.92	1.75
E13072	18,640	15.0	3.115	0.45708	35.97	4.683	4.88	12.01
E13073	18,457	15.0	2.137	0.45837	34.70	4.201	4.56	21.06
E13074	17,720	15.0	3.571	0.77539	40.53	8.012	5.04	13.37
E13075	20,859	15.0	2.377	0.58393	37.30	5.279	4.40	11.75
E13076	16,962	15.0	3.390	0.57657	38.83	5.880	4.96	12.24
E13077	15,596	15.0	2.222	0.90792	48.50	7.390	5.40	14.70
E13078	12,792	15.0	1.667	1.18664	47.09	8.817	4.68	12.11
E13111	26,096	15.9	2.967	0.41615	17.15	5.362	2.04	5.52
E13112	17,933	15.8	2.721	0.51526	34.18	5.165	5.00	11.96
E13113	18,264	15.7	2.045	0.47962	35.45	4.341	4.20	11.10
E13114	17,721	15.8	3.333	0.76860	40.86	7.788	5.64	13.92
E13115	17,436	15.8	1.805	0.52306	29.87	4.615	4.44	11.46
E13116	14,473	15.8	3.305	0.59283	40.73	5.942	4.72	12.10
E13117	11,741	15.8	2.155	1.03224	50.37	8.296	5.40	13.32
E13118	10,105	15.8	1.597	1.28850	46.08	9.513	4.76	11.78
E13121	31,406	16.0	2.935	0.42986	17.47	5.493	2.52	5.58
E13122	19,889	16.0	2.643	0.52792	33.77	5.269	4.88	12.38
E13123	19,397	15.9	1.955	0.46398	34.31	4.184	4.40	10.70
E13124	18,322	16.0	3.363	0.85799	41.99	8.654	5.44	13.90
E13125	19,399	15.9	1.765	0.51652	29.79	4.535	4.44	11.10

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E13126	20,362	15.9	3.305	0.60995	41.35	6.090	4.12	11.20
E13127	17,181	16.0	2.193	1.06164	51.98	8.517	5.44	13.54
E13128	16,086	16.0	1.610	1.36889	46.71	10.097	4.80	11.94
E14064	24,944	16.6	2.762	0.28624	34.08	2.890	4.28	10.58
E14065	23,041	16.5	1.508	0.41926	23.49	3.872	3.76	8.44
E14066	25,671	16.5	2.778	0.28047	33.87	2.842	5.16	10.92
E14067	27,187	16.5	1.532	0.21407	23.81	1.986	3.84	8.94
E14068	25,687	16.5	1.484	0.20088	27.29	1.750	4.72	10.24
E14074	31,493	16.4	2.589	0.23815	32.71	2.388	4.64	11.06
E14075	31,430	16.4	1.462	0.35317	23.29	3.247	3.84	9.48
E14076	34,845	16.4	2.679	0.30994	33.54	3.120	4.36	12.10
E14077	33,616	16.4	1.610	0.34091	25.68	3.147	4.04	9.80
E14078	32,156	16.5	1.532	0.28548	28.78	2.476	4.36	11.44
E14084	27,573	16.6	2.613	0.24372	33.78	2.426	4.08	11.04
E14085	24,302	16.6	1.439	0.35799	23.41	3.278	3.12	8.88
E14086	22,132	16.6	2.679	0.33346	34.35	3.336	4.60	11.14
E14087	25,682	16.6	1.570	0.36446	25.63	3.350	2.96	9.02
E14088	19,016	16.6	1.463	0.32184	28.29	2.773	4.00	10.54
E14094	23,238	16.9	2.871	0.47767	37.84	4.727	3.12	9.24
E14095	23,068	16.8	1.439	0.37714	23.81	3.441	2.64	7.32
E14096	22,151	16.8	2.679	0.34129	35.16	3.394	4.20	11.10
E14097	24,803	16.8	1.557	0.33141	25.87	3.036	2.44	6.88
E14098	21,795	16.8	1.322	0.34136	26.45	2.918	2.80	8.80
E14104	25,731	17.0	2.816	0.47569	37.70	4.683	3.28	7.96
E14105	25,690	17.0	1.429	0.38069	24.02	3.462	3.28	7.24
E14106	28,296	17.0	2.632	0.34563	35.32	3.417	4.60	10.90
E14107	20,848	17.0	1.557	0.33385	26.27	3.049	2.48	6.14
E14108	25,820	17.0	1.322	0.32998	26.61	2.817	2.84	7.04
E15036	19,044	12.7	2.199	0.55767	63.71	4.005	7.40	16.82
E15046	17,720	12.5	2.199	0.57563	64.89	4.126	5.88	16.98
E15076	12,908	11.8	2.000	0.50667	61.29	3.616	4.48	11.20
E15086	9,435	11.8	2.138	0.62954	66.37	4.487	5.88	12.60
E15096	14,499	11.7	2.069	0.61659	65.42	4.391	5.96	14.78
E15106	17,665	11.5	2.041	0.62496	65.41	4.442	5.44	14.38
E15037	17,260	12.7	2.344	0.60139	71.82	4.315	9.12	17.70

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E15047	19,831	12,5	2,308	0,57184	72,31	4,091	5,60	19,28
E15077	16,053	11,8	2,190	0,52701	71,57	3,748	4,84	12,16
E15087	12,212	11,7	2,344	0,66328	77,73	4,707	6,20	13,46
E15097	16,070	11,7	2,344	0,65341	79,04	4,625	6,36	15,66
E15107	14,445	11,5	2,326	0,68534	79,69	4,839	5,64	15,18
E15038	15,555	12,7	1,898	0,99129	117,34	5,821	7,40	16,16
E15048	13,695	12,5	1,898	1,03837	118,12	6,085	5,32	16,42
E15078	12,225	11,8	1,793	0,93254	113,49	5,438	5,64	14,10
E15088	11,422	11,8	1,898	1,25545	120,84	7,308	6,64	15,10
E15098	13,178	11,7	1,898	1,25668	121,63	7,301	6,52	16,48
E15108	12,739	11,5	1,898	1,22925	122,35	7,129	5,40	15,18
E16025	6,625	8,3	2,400	1,26785	43,31	10,948	2,68	7,72
E16035	8,719	8,0	2,400	1,11479	43,86	9,611	2,60	6,62
E16085	8,270	7,3	2,344	0,98674	45,80	8,432	1,46	5,66
E16095	11,012	7,4	2,439	1,26413	48,36	10,780	2,60	8,44
E16135	8,217	7,1	2,439	1,38738	50,69	11,753	2,88	6,78
E16026	10,134	8,3	3,261	1,19009	63,62	10,015	2,84	7,82
E16036	7,094	8,0	3,261	1,28567	64,50	10,800	3,08	7,34
E16086	8,613	7,3	3,191	1,05189	67,14	8,762	1,50	6,30
E16096	12,134	7,3	2,929	1,19661	62,25	9,968	2,96	8,96
E16136	9,349	7,1	3,021	1,21296	67,39	10,021	2,88	7,26
E16027	12,215	8,3	2,679	1,06590	52,15	9,067	2,24	7,76
E16037	8,464	8,0	2,586	1,11295	50,94	9,451	2,68	6,04
E16087	8,658	7,3	2,417	0,76920	51,28	6,455	1,48	6,04
E16097	14,036	7,3	2,479	0,96179	53,32	8,054	3,16	9,08
E16137	9,992	7,1	2,437	0,95473	54,70	7,941	2,88	6,96
E16028	11,386	8,2	2,679	1,04340	49,91	8,970	2,28	7,26
E16038	9,695	8,0	2,586	0,82927	48,74	7,118	2,28	5,70
E16088	7,645	7,3	2,679	1,26358	52,88	10,779	1,46	4,76
E16098	14,326	7,3	2,679	1,19366	53,74	10,160	2,60	8,44
E16138	9,099	7,1	2,636	1,50343	55,60	12,706	2,96	6,62
E27062	10,318	7,3	0,750	2,12254	39,90	13,011	6,16	23,26
E27072	16,599	7,2	0,750	1,98807	40,29	12,153	11,72	36,36
E27102	13,090	7,4	0,708	2,04883	38,91	12,443	11,64	28,38
E27112	10,617	7,1	0,750	2,48667	41,55	15,068	12,96	30,42

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E27122	10,937	6,9	0,750	2,13960	41,87	12,936	10,80	50,24
E27132	7,252	7,0	0,750	2,73044	42,17	16,475	11,76	27,96
E27064	6,845	7,3	2,186	3,74273	73,49	26,437	12,40	48,88
E27074	11,559	7,2	2,280	3,27017	77,42	23,059	23,60	73,20
E27104	8,516	7,2	2,186	2,79715	76,05	19,641	26,60	67,04
E27114	7,472	7,0	2,099	3,62961	73,72	25,445	30,12	70,02
E27124	6,502	6,9	2,186	3,50635	77,46	24,541	24,84	70,02
E27134	5,443	6,8	2,099	3,65978	74,97	25,580	22,60	59,86
E27065	13,587	7,3	1,750	1,53233	37,74	12,780	8,40	32,70
E27075	19,915	7,2	1,934	2,62117	42,45	21,789	16,16	49,76
E27105	16,133	7,3	2,042	2,10126	46,69	17,329	16,00	39,70
E27115	12,374	7,0	2,042	3,15179	47,32	25,924	16,48	40,48
E27125	9,580	6,9	1,934	3,00634	45,42	24,663	15,40	40,12
E27135	8,240	7,0	1,934	3,42245	45,99	28,007	15,32	38,42
E27066	20,395	7,1	6,364	1,48565	57,88	16,761	10,04	40,64
E27076	30,367	7,2	7,000	2,35122	64,86	26,422	20,88	61,04
E27106	19,138	7,3	7,000	2,16324	67,87	24,073	21,16	51,10
E27116	15,268	7,0	7,000	3,14379	68,90	34,867	21,52	53,26
E27126	12,336	6,9	7,000	3,01563	69,93	33,355	20,16	52,44
E27136	11,311	7,0	7,000	3,23566	70,92	35,653	20,56	50,80
E18065	6,582	7,5	3,200	1,26711	68,53	10,725	5,68	8,90
E18075	6,752	7,5	3,200	1,38631	69,14	11,718	5,44	8,96
E18115	5,217	7,5	3,200	1,47210	71,56	12,374	5,40	8,74
E18125	5,813	7,0	3,200	1,47606	72,11	12,391	5,16	8,26
E18135	6,885	6,9	3,200	1,49893	72,68	12,567	5,60	8,54
E18066	6,537	7,5	4,082	1,45014	94,20	12,036	5,76	9,16
E18076	8,123	7,5	4,124	1,65455	95,87	13,710	5,64	9,28
E18116	5,606	7,5	3,918	1,59481	93,51	13,149	4,00	8,14
E18126	6,959	7,0	3,878	1,57789	93,34	12,980	4,72	10,72
E18136	10,506	6,9	3,878	1,65627	94,11	13,594	5,44	10,52
E18067	6,191	7,5	3,478	1,32784	70,87	11,359	5,16	8,20
E18077	9,228	7,5	3,478	1,56047	71,42	13,327	5,56	8,30
E18117	5,444	7,5	3,419	1,84044	72,32	15,618	5,16	7,96
E18127	9,132	7,0	3,419	1,56379	72,90	13,248	4,28	9,02
E18137	9,705	6,9	3,419	1,77435	73,53	15,003	5,28	9,70

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E18068	5,798	7.5	3.540	1.09697	76,39	9,206	3,36	7.74
E18078	7,949	7.5	3.482	1.39637	75,37	11,720	3,08	8.12
E18118	6,982	7.5	3.363	1.40940	74,84	11,784	3,24	8.52
E18128	8,864	7.0	3.423	1.37412	76,77	11,474	3,12	7.98
E18138	16,928	6.9	3.070	1.35395	69,52	11,297	2,96	7.64
E29042	7,798	7.5	1.034	2.40846	53,24	14,868	12,88	30.16
E29052	6,775	7.0	1.034	2.70095	53,55	16,655	12,56	31.88
E29062	5,251	6.9	1.034	2.93674	53,87	18,089	12,88	31.72
E29072	6,374	6.5	1.034	2.67317	54,20	16,447	13,28	32.60
E29102	6,081	6.5	1.034	2.70354	55,23	16,575	13,84	34.54
E29112	5,304	6.4	1.034	2.78261	55,58	17,040	14,36	35.12
E29132	7,244	6.3	0.989	2.69206	53,85	16,446	13,84	40.80
E29044	4,949	7.4	2.147	3.15202	71,55	22,495	22,16	55.46
E29054	4,684	7.0	2.147	3.13849	71,98	22,366	21,84	55.08
E29064	4,745	6.8	2.053	2.89538	69,27	20,602	23,64	58.40
E29074	4,752	6.5	2.053	3.08078	69,74	21,886	24,16	62.62
E29104	5,465	6.5	2.044	2.68994	70,79	19,023	22,92	58.68
E29114	3,829	6.3	2.044	2.97731	71,23	21,023	24,24	58.62
E29134	5,495	6.1	2.138	3.01389	75,41	21,216	23,64	69.00

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A4. E-SERIES EXPERIMENT SCREENED RESULTS

CONTENTS:-

SCREENED DATA

A4.1 The data which satisfied the criteria of Chapter 7 (para.7.58) were selected for multiple regression analysis, and are listed in table A4.1. The matrix comprised 122 data strings.

A4.2 The coding prefacing each data string is explained in Appendix A3; only experiments of series 1 (MTS) and 2 (LTS) were eligible for selection. Not all basic or calculated values were required for the analysis; space limits prevented more than the 11 quantities shown from being entered into the analysis; any others required were generated from these during the course of the analysis.

A4.3 Units were as follows:-

SPEXRT	-	mg kg^{-1} h^{-1}
TEMPRT	-	^{o}C
ENUMBR	-	(number)
FLØWRT	-	ℓ min^{-1}
VØLUME	-	ℓ
AVERLN	-	cm
DENSTY	-	(number) ℓ^{-1}
DNLØAD	-	kg min ℓ^{-1}
DNSTØK	-	g ℓ^{-1}
GRUBFD	-	g
FDMEAL	-	g

Table A4.1/see over

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	SPEXRT	ENUMBR	VOLUME	DENSTY	DNSTOK	FDMEAL
	TEMPRT	FLOWRT	AVERLN	DNLOAD	GRUBFD	
XX						
E12031	10,148	10,0	30, 0.284	8,8 9,09	3,409 1.017	32,78 2,04 3,96
E12032	14,321	10,0	20, 0.330	9,5 10,94	2,105 0.993	34,50 2,08 5,14
E12071	19,199	9,9	30, 0.237	8,4 9,20	3,571 1.281	36,10 2,64 6,60
E12072	12,758	9,9	20, 0.248	9,5 11,05	2,105 1.373	35,88 2,36 5,90
E12091	15,373	10,5	30, 0.241	8,4 9,26	3,571 1.288	37,02 4,20 7,26
E12092	12,897	10,5	20, 0.230	9,4 11,11	2,128 1.312	36,98 3,60 6,42
E12101	19,173	10,6	30, 0.228	8,5 9,31	3,614 1.593	38,20 4,20 10,50
E12102	24,159	10,5	20, 0.241	9,4 11,16	2,128 1.465	37,52 3,60 9,00
E13034	18,925	15,4	40, 0.571	9,5 9,51	4,211 0.739	44,43 5,44 11,92
E13036	24,539	15,4	40, 0.811	11,8 9,65	3,390 0.526	36,12 6,04 13,36
E13044	16,806	15,4	40, 0.612	9,6 9,56	4,167 0.703	44,86 5,04 13,20
E13046	18,886	15,4	40, 0.779	11,8 9,69	3,390 0.559	36,92 5,28 14,34
E13054	16,089	15,1	40, 0.591	9,6 9,60	4,167 0.742	45,70 4,96 12,52
E13056	16,650	15,1	40, 0.800	11,8 9,73	3,390 0.555	37,62 4,60 12,52
E13064	12,754	15,1	40, 0.563	11,2 9,63	3,571 0.790	39,76 2,77 10,21
E13066	13,725	15,0	40, 0.774	11,8 9,77	3,390 0.581	38,15 2,42 9,32
E13074	17,720	15,0	40, 0.585	11,2 9,68	3,571 0.775	40,53 5,04 13,37
E13076	16,962	15,0	40, 0.795	11,8 9,81	3,390 0.577	38,83 4,96 12,24
E13114	17,721	15,8	38, 0.606	11,4 9,87	3,333 0.769	40,86 5,64 13,92
E13116	14,473	15,8	39, 0.811	11,8 9,98	3,305 0.593	40,73 4,72 12,10
E13124	18,322	16,0	38, 0.553	11,5 9,92	3,363 0.858	41,99 5,44 13,90
E13126	20,362	15,9	39, 0.800	11,8 10,02	3,305 0.610	41,35 4,12 11,20
E14064	24,944	16,6	29, 1.250	10,5 9,90	2,762 0.486	34,08 4,28 10,58
E14066	25,671	16,5	30, 1.304	10,8 9,87	2,778 0.480	33,87 5,16 10,92
E14067	27,187	16,5	19, 1.379	12,4 10,78	1,532 0.414	23,81 3,84 8,94
E14074	31,493	16,4	29, 1.538	11,2 9,97	2,589 0.438	32,71 4,64 11,06
E14076	34,845	16,4	30, 1.212	11,2 9,94	2,679 0.510	33,54 4,36 12,10
E14077	33,616	16,4	19, 0.889	11,8 10,83	1,610 0.541	25,68 4,04 9,80
E14084	27,373	16,6	29, 1.538	11,1 10,05	2,613 0.444	35,78 4,08 11,04
E14086	22,132	16,6	30, 1.154	11,2 10,00	2,679 0.533	34,35 4,60 11,14
E14087	25,682	16,6	19, 0.851	12,1 10,88	1,570 0.564	25,63 2,96 9,02
E14094	23,238	16,9	29, 0.800	10,1 10,10	2,871 0.478	37,83 3,12 9,24
E14096	22,151	16,8	30, 1.154	11,2 10,06	2,679 0.541	35,16 4,20 11,10
E14097	24,803	16,8	19, 0.952	12,2 10,92	1,557 0.531	25,87 2,44 6,88
E14104	25,731	17,0	29, 0.816	10,5 10,16	2,816 0.476	37,70 3,28 7,96
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E14106	28,496	17,0	30,	1.165	11,4	10,12	2,632	0.546	35,32	4,60	10,90
E14107	20,648	17,0	19,	0.960	12,2	10,95	1,557	0.334	26,27	2,48	6,14
E15037	17,260	12,7	30,	1.529	12,8	13,94	2,344	0.601	71,82	9,12	17,70
E15047	19,851	12,5	30,	1.644	13,0	13,98	2,308	0.572	72,31	5,60	19,28
E15077	16,053	11,8	30,	1.860	13,7	14,06	2,190	0.527	71,57	4,84	12,16
E15087	12,212	11,7	30,	1.500	12,8	14,09	2,344	0.663	77,73	6,20	13,46
E15097	16,070	11,7	30,	1.548	12,8	14,13	2,344	0.653	79,04	6,36	15,66
E15107	14,445	11,5	30,	1.500	12,9	14,16	2,326	0.685	79,69	5,64	15,18
E15038	15,355	12,7	26,	1.622	13,7	17,03	1,898	0.991	117,34	7,40	16,16
E15048	13,695	12,5	26,	1.558	13,7	17,06	1,898	1.038	118,12	5,32	16,42
E15078	12,225	11,8	26,	1.765	14,5	17,15	1,793	0.933	113,49	5,64	14,10
E15088	11,422	11,8	26,	1.319	13,7	17,18	1,898	1.255	120,84	6,64	15,10
E15098	13,178	11,7	26,	1.326	13,7	17,21	1,898	1.257	121,63	6,52	16,48
E15108	12,739	11,5	26,	1.364	13,7	17,24	1,898	1.229	122,35	5,40	15,18
E16025	6,625	8,3	30,	0.427	12,5	11,58	2,400	1.268	43,31	2,68	7,72
E16035	8,719	8,0	30,	0.492	12,5	11,60	2,400	1.115	43,86	2,60	6,62
E16085	8,270	7,3	30,	0.594	12,8	11,70	2,344	0.987	45,80	1,46	5,66
E16095	11,012	7,4	30,	0.471	12,3	11,73	2,439	1.264	48,37	2,60	8,44
E16135	8,217	7,1	30,	0.449	12,3	11,81	2,439	1.387	50,69	2,88	6,78
E16026	10,134	8,3	30,	0.492	9,2	11,88	3,261	1.190	63,62	2,84	7,82
E16036	7,094	8,0	30,	0.462	9,2	11,90	3,261	1.286	64,50	3,08	7,34
E16086	8,613	7,3	30,	0.600	9,4	12,01	3,191	1.052	67,14	1,50	6,30
E16096	12,134	7,3	29,	0.515	9,9	12,01	2,929	1.197	62,25	2,96	8,96
E16136	9,349	7,1	29,	0.533	9,6	12,10	3,021	1.213	67,39	2,88	7,26
E16027	12,215	8,3	30,	0.548	11,2	11,76	2,679	1.066	52,15	2,24	7,76
E16037	8,464	8,0	30,	0.531	11,6	11,78	2,586	1.113	50,94	2,68	6,04
E16087	8,658	7,3	29,	0.800	12,0	11,92	2,417	0.769	51,28	1,48	6,04
E16097	14,036	7,3	29,	0.649	11,7	11,94	2,479	0.962	53,32	3,16	9,08
E16137	9,992	7,1	29,	0.682	11,9	12,02	2,437	0.955	54,70	2,88	6,96
E16028	11,386	8,2	30,	0.536	11,2	11,63	2,679	1.043	49,91	2,28	7,26
E16038	9,695	8,0	30,	0.682	11,6	11,65	2,586	0.829	48,74	2,28	5,70
E16088	7,645	7,3	30,	0.469	11,2	11,72	2,679	1.264	52,88	1,46	4,76
E16098	14,326	7,3	30,	0.504	11,2	11,75	2,679	1.194	53,74	2,60	8,44
E16138	9,099	7,1	29,	0.407	11,0	11,83	2,636	1.303	55,60	2,96	6,62
E27062	10,318	7,3	63,	1.579	84,0	16,31	0,750	2.123	39,90	6,16	23,26

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E27072	16.599	7.2	63.	1.702	84.0	16.36	0.750	1.988	40.29	11.72	36.56
E27102	13.090	7.4	63.	1.690	89.0	16.47	0.708	2.049	38.91	11.64	28.38
E27112	10.617	7.1	63.	1.404	84.0	16.50	0.750	2.487	41.55	12.96	30.42
E27122	10.937	6.9	63.	1.644	84.0	16.54	0.750	2.140	41.87	10.80	30.24
E27132	7.252	7.0	63.	1.297	84.0	16.57	0.750	2.730	42.17	11.76	27.96
E27064	6.845	7.3	212.	1.905	97.0	14.16	2.186	3.743	73.49	12.40	48.88
E27074	11.359	7.2	212.	2.202	93.0	14.18	2.280	3.270	77.42	23.60	73.20
E27104	8.516	7.2	212.	2.637	97.0	14.24	2.186	2.797	76.05	26.60	67.04
E27114	7.472	7.0	212.	2.051	101.0	14.26	2.099	3.630	73.72	30.12	70.02
E27124	6.502	6.9	212.	2.143	97.0	14.29	2.186	3.506	77.46	24.84	70.02
E27134	5.443	6.8	212.	2.069	101.0	14.31	2.099	3.660	74.97	22.60	59.86
E27065	13.587	7.3	147.	2.069	84.0	11.99	1.750	1.532	37.74	8.40	32.70
E27075	19.915	7.2	147.	1.231	76.0	12.03	1.934	2.621	42.45	16.16	49.76
E27105	16.133	7.3	147.	1.600	72.0	12.13	2.042	2.101	46.69	16.00	39.70
E27115	12.374	7.0	147.	1.081	72.0	12.16	2.042	3.152	47.32	16.48	40.48
E27125	9.580	6.9	147.	1.148	76.0	12.19	1.934	3.006	45.42	15.40	40.12
E27135	8.240	7.0	147.	1.021	76.0	12.22	1.934	3.422	45.99	15.32	38.42
E27066	20.395	7.1	350.	2.143	55.0	8.86	6.364	1.486	57.88	10.04	40.64
E27076	30.367	7.2	350.	1.379	50.0	8.90	7.000	2.351	64.86	20.88	61.04
E27106	19.138	7.3	350.	1.569	50.0	8.99	7.000	2.163	67.87	21.16	51.10
E27116	15.268	7.0	350.	1.096	50.0	9.02	7.000	3.144	68.91	21.52	53.26
E27126	12.356	6.9	350.	1.159	50.0	9.05	7.000	3.016	69.93	20.16	52.44
E27136	11.311	7.0	350.	1.096	50.0	9.07	7.000	3.236	70.92	20.56	50.80
E18065	6.582	7.5	40.	0.676	12.5	11.81	3.200	1.267	68.53	3.68	8.90
E18075	6.752	7.5	40.	0.623	12.5	11.83	3.200	1.386	69.14	3.44	8.96
E18115	5.217	7.5	40.	0.608	12.5	11.90	3.200	1.472	71.56	3.40	8.74
E18125	5.813	7.0	40.	0.611	12.5	11.91	3.200	1.476	72.11	3.16	8.26
E18135	6.885	6.9	40.	0.606	12.5	11.93	3.200	1.499	72.68	3.60	8.34
E18066	6.537	7.5	40.	0.637	9.8	12.05	4.082	1.450	94.20	3.76	9.16
E18076	8.123	7.5	40.	0.562	9.7	12.07	4.124	1.655	95.87	3.64	9.28
E18116	5.606	7.5	38.	0.569	9.7	12.13	3.918	1.595	93.51	4.00	8.14
E18126	6.959	7.0	38.	0.580	9.8	12.16	3.878	1.578	93.34	4.72	10.72
E18136	10.506	6.9	38.	0.557	9.8	12.18	3.878	1.656	94.11	3.44	10.52
E18067	6.191	7.5	40.	0.614	11.5	11.69	3.478	1.528	70.87	3.16	8.20
E18077	9.228	7.5	40.	0.526	11.5	11.71	3.478	1.360	71.42	3.56	8.30

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E18117	5,444	7,5	40,	0.460	11,7	11,78	3,419	1.840	72,32	3,16	7,96
E18127	9,132	7,0	40,	0.545	11,7	11,80	3,419	1.564	72,90	4,28	9,02
E18137	9,705	6,9	40,	0.485	11,7	11,83	3,419	1.774	73,53	3,28	9,70
E29042	7,798	7,5	92,	1.967	89,0	16,20	1,034	2.408	53,24	12,88	30,16
E29052	6,775	7,0	92,	1.765	89,0	16,22	1,034	2.701	53,55	12,56	31,88
E29062	5,251	6,9	92,	1.633	89,0	16,24	1,034	2.937	53,87	12,88	31,72
E29072	6,374	6,5	92,	1.805	89,0	16,25	1,034	2.673	54.20	13,28	32,60
E29102	6,081	6,5	92,	1.818	89,0	16,31	1,034	2.704	55,23	13,84	34,54
E29112	5,304	6,4	92,	1.778	89,0	16,33	1,034	2.783	55,58	14,36	35,12
E29132	7,244	6,3	92,	1.860	93,0	16,37	0,989	2.692	53,85	13,84	40,80
E29044	4,949	7,4	234,	2.474	109,0	14,01	2,147	3.152	71,55	22,16	55,46
E29054	4,684	7,0	234,	2.500	109,0	14,03	2,147	3.138	71,98	21,84	55,08
E29064	4,745	6,8	234,	2.727	114,0	14,05	2,053	2.895	69,27	25,64	58,40
E29074	4,752	6,5	234,	2.581	114,0	14,08	2,053	3.081	69,74	24,16	62,62
E29104	5,465	6,5	233,	3.000	114,0	14,14	2,044	2.690	70,79	22,92	58,68
E29114	3,829	6,3	233,	2.727	114,0	14,16	2,044	2.977	71,23	24,24	58,62
E29134	5,495	6,1	233,	2.727	109,0	14,21	2,138	3.014	75,41	23,64	69,00

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B. FISH LABORATORY CONVERSION PLANS

CONTENTS:-

NOTES

B.1 Plans are shown approximately as drawn, but with additions made for extra electric points and taps which were added after the original draughting.

B.2 Figure B.1 shows the space available for building experimental rigs; the space along the long wall adjacent to the ramp and ladder was used for the work described.

B.3 The parts of Figure B.2 overlap at point X and show the proportions of the header room and upper laboratory.

B.4 The position of the section of Figure B.3 is shown as A-A in Fig. B.1 and B.2.

B.5 All plans are to an approximate scale of 1 cm rep. 50 cm.

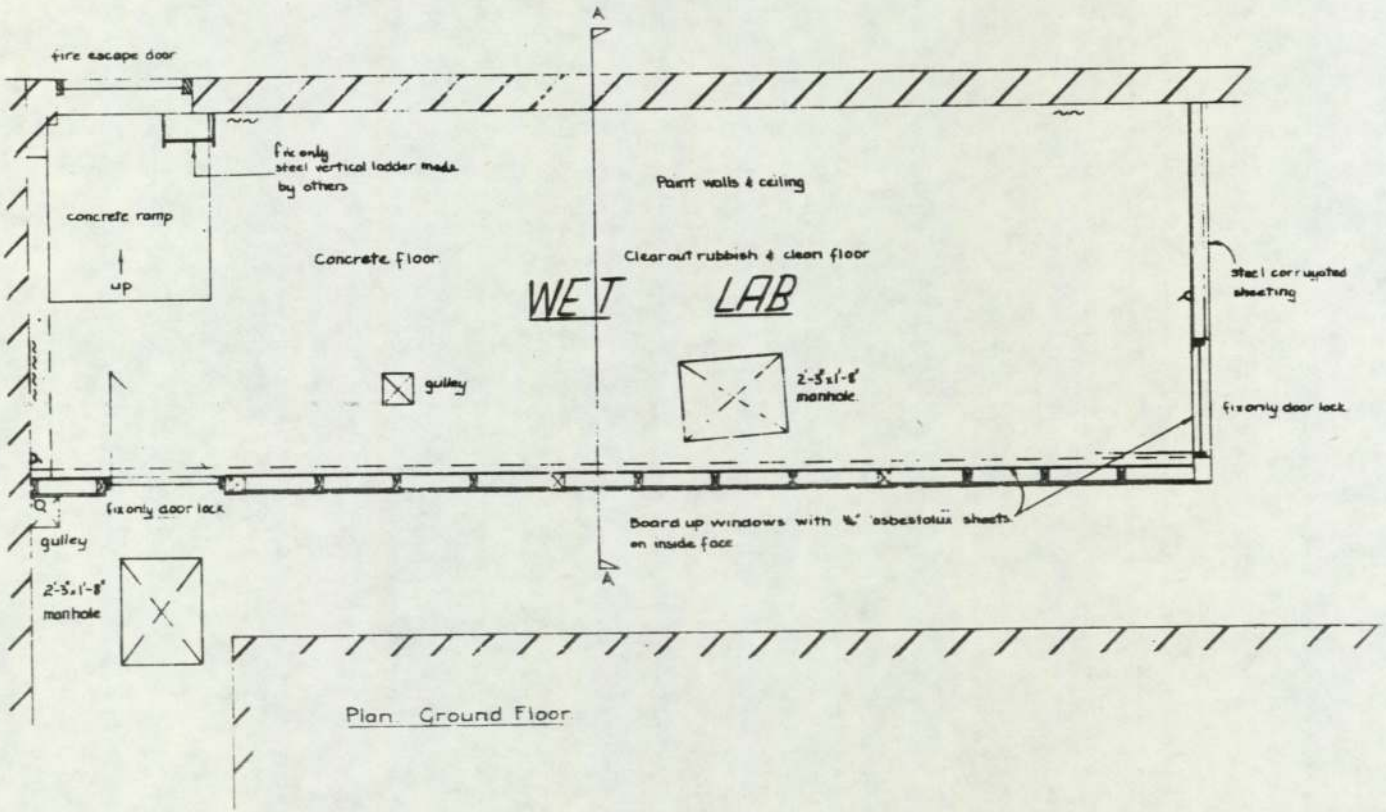

fire escape door
fix only steel vertical ladder made by others
concrete ramp
up
Concrete floor
Paint walls & ceiling
Clear out rubbish & clean floor
WET LAB
steel corrugated sheeting
gulley
2'-3"x1'-8" manhole
fix only door lock
fix only door lock
Board up windows with ¼" asbestolux sheets on inside face
gulley
2'-3"x1'-8" manhole
A
A
Plan Ground Floor

Figure B.1

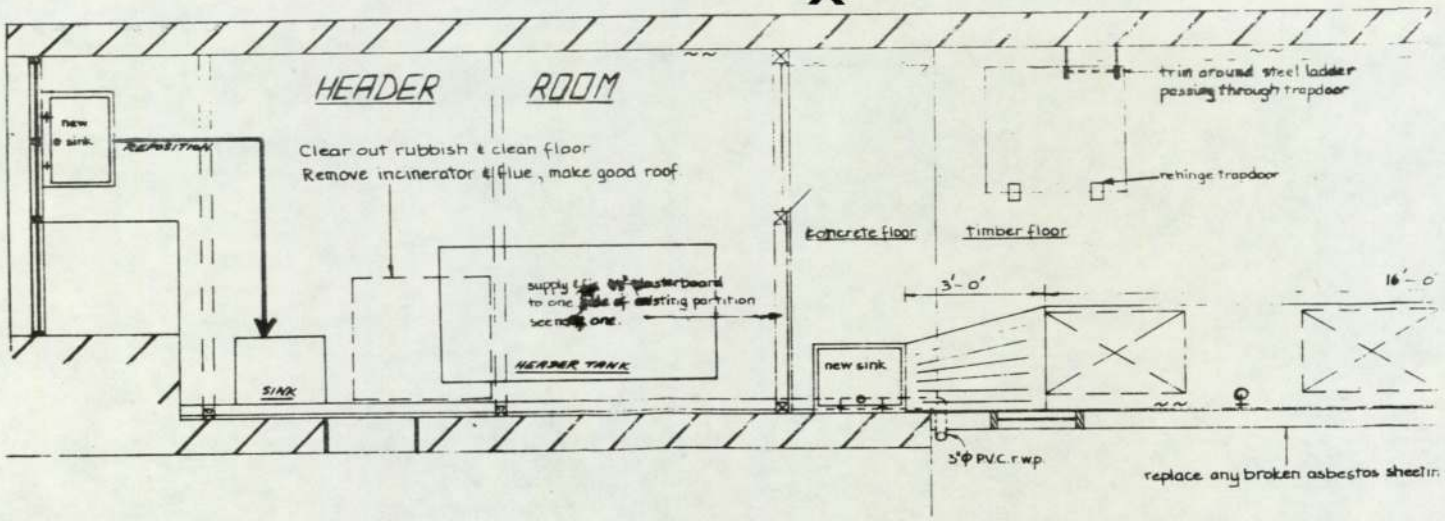


Figure B.2a

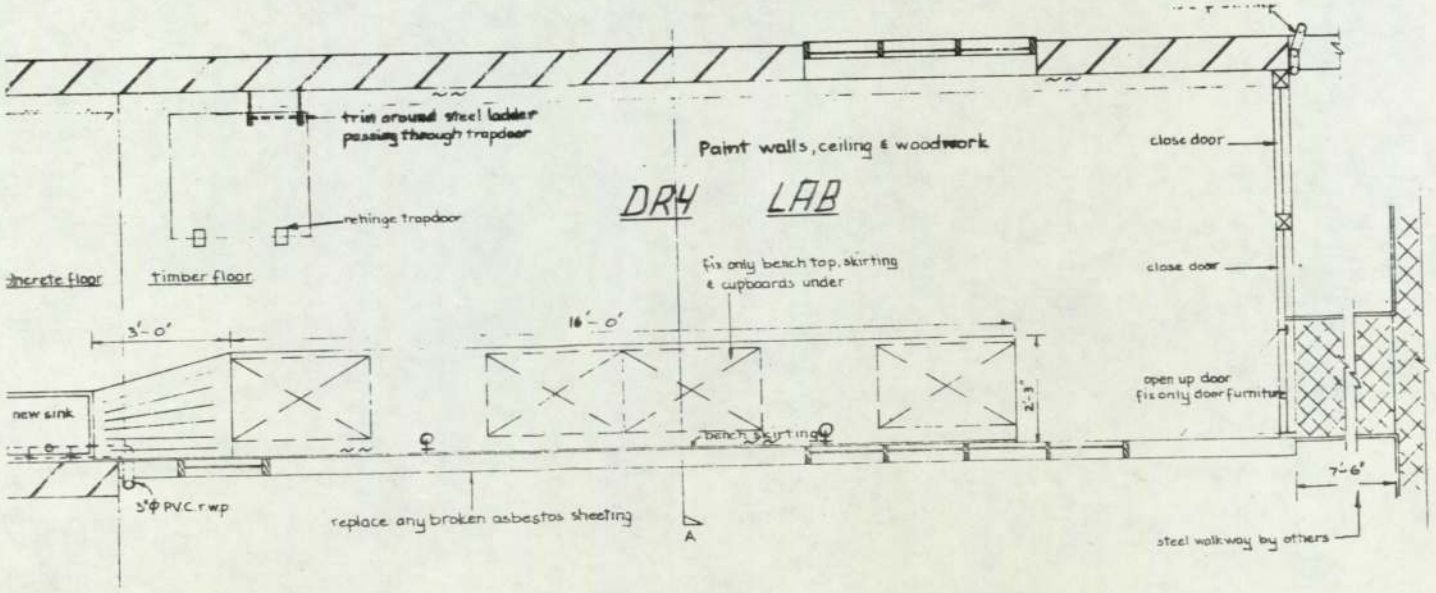
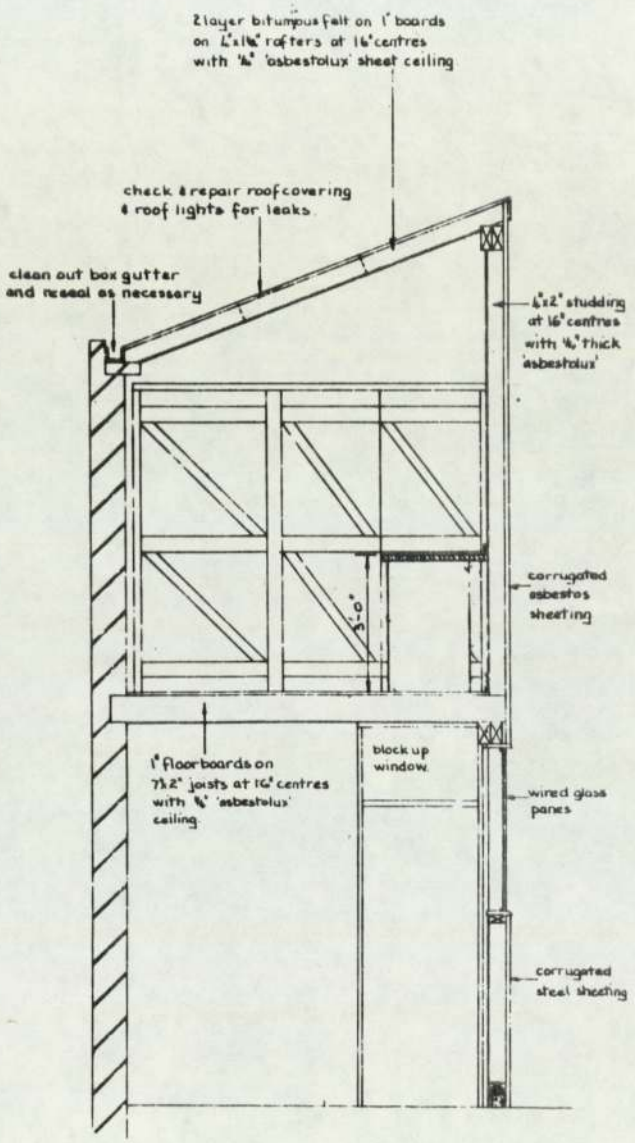


Figure B.2b

Figure B.3



Section A-A

C. MULTIPLE REGRESSION INTERPRETATION

METHOD

C1 Promising models of the relationship between SER and various X-quantities could be computer-graphed to enable examination of individual quantities, in context, as described in Chapter 7. The method used is given in more detail here, first for a dependent variable (Y), related to three independent variables (X_1, X_2, X_3) thus:-

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3$$

C2 If an observed value of Y is y, and X_1, X_2, X_3 have "typical" values of t_1, t_2, t_3; then a fully corrected estimate of Y, denoted by $\hat{y}$, is given by:-

$$\hat{y} = y - b_1(x_1 - t_1) - b_2(x_2 - t_2) - b_3(x_3 - t_3).$$

The "typical" value is an arbitrary value, taken for convenience in the middle of the range of each X, so that when all X quantities except one are set to their typical value, that one can be represented in a meaningful way in a graph plot against the resulting range of values of the dependent variable (with the other X quantities taken into account). Thus partially-corrected values of Y can be calculated, e.g. for a plot against X_2, using typical values of X_1 and X_3:-

$$\hat{y}_{\text{(partially corrected for } X_2)} = y - b_1(x_1 - t_1) - b_3(x_3 - t_3)$$

Using this equation, a set of partially-corrected Y values can be calculated, and plotted against values of X_2.

C3 The series of three graphs (for each of X_1, X_2, X_3) which are thus generated can be examined for further interpretation of the factors in the multiple regression equation (see para. 7.73).

COMPUTER PROCEDURES

C4 The preparation of the graphs was by means of Aston applications program UA13, prepared by Dr. John Aston of the Department of Metallurgy at the University. This package used the same

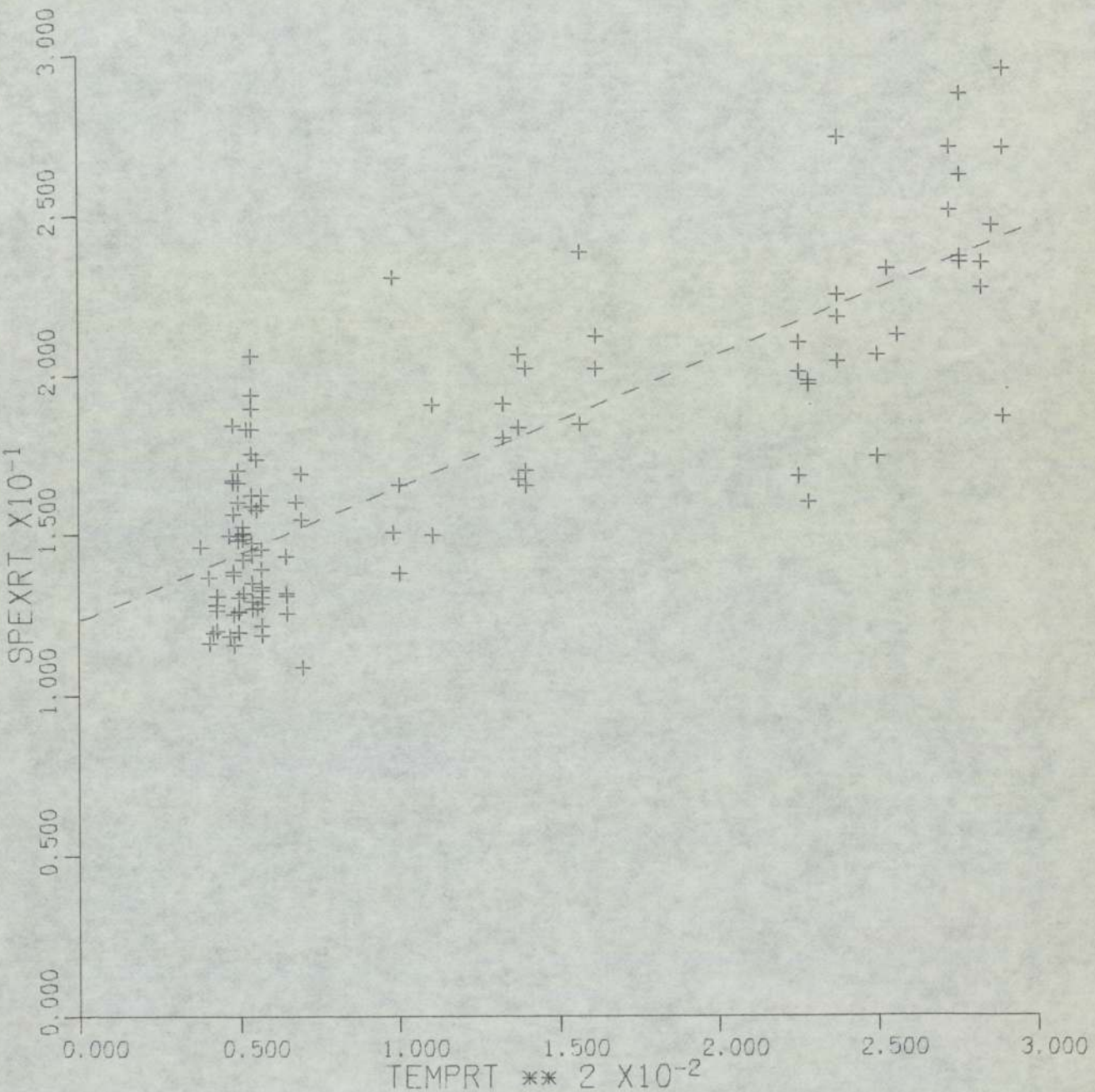
data matrix as the Statistical Package (see Chapter 7), and required input of the key quantities of the particular multiple regression model under examination. Output was in the form of lists of partially-corrected Y values, error values of observed values as compared to the model, and theoretical (model) values for Y. Graphs were also produced, of observed against calculated (model) values of Y, and the partially-corrected Y values against each one of the independent variables.

RESULTS

C5 The final model (4) for explanation of SER was the one most fully examined using the graphical interpretation procedure; this model (see para. 7.106, Chapter 7) predicts SER (computer code SPEXRT) in terms of temperature (TEMPRT), fish number (ENUMBR), stocking (DNSTØK), and mean free path (FREPAT).

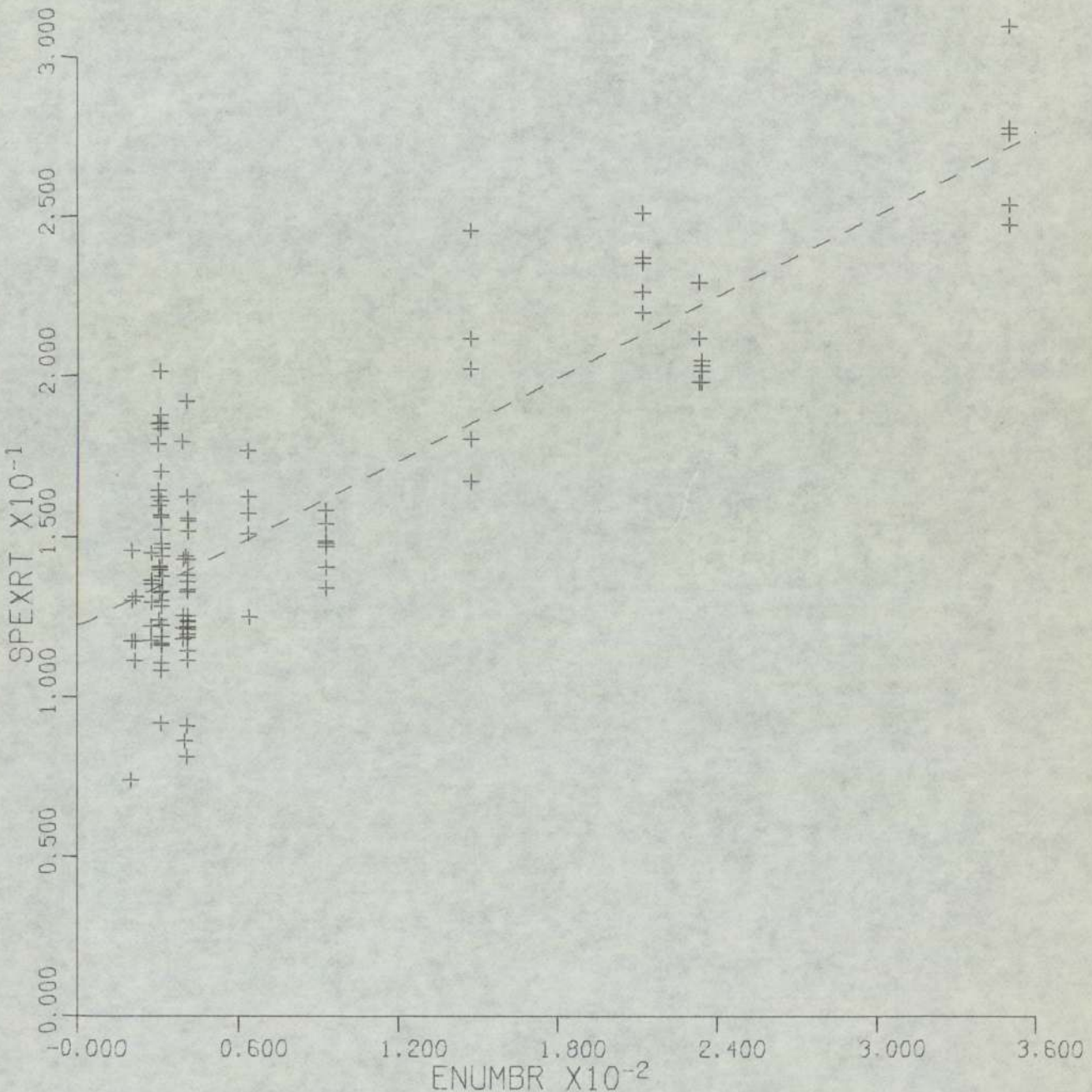
C6 The first stage of interpretation took the significant independent variables as listed in the model. At this point the true independence of these quantities should be confirmed; although DNSTØK is defined as mass/volume, it can be distinguished both intuitively and mathematically from FREPAT ($\sqrt[3]{\text{volume}}$/number), since it is possible to vary number and mass independently; further, FREPAT can be varied independently of ENUMBR by manipulation of volume, while DNSTØK can still remain independent by manipulation of mass.

C7 The graphs which follow show the individual effects of the significant independent variables; TEMPRT^2, ENUMBR, ENUMBR^2, (ENUMBR x DNSTOK) and (TEMPRT x FREPAT). In all cases, there is evident a fairly wide range of values for SPEXRT for small ranges of the independent variable, or even for the same value of the independent, and this tendency is particularly marked for the low-magnitude values in Graphs C.2, C.3 and C.4. Only Graph C.4 (showing the number/stocking interaction) displays a negative effect on specific excretory rate, and this is consistent with speculation that increased stocking results in a decrease in excretory rate.



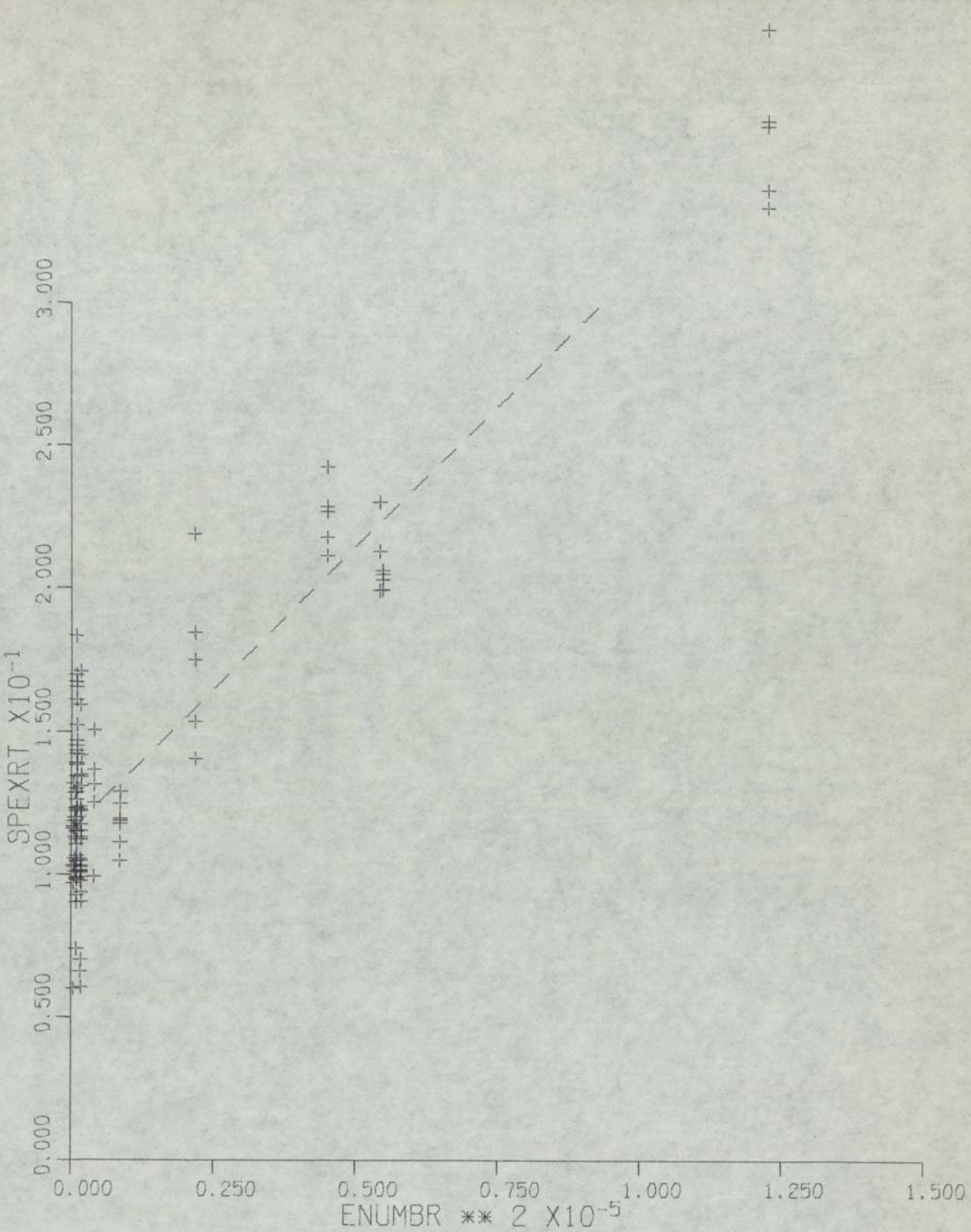
Graph C.1

SPEXRT V. TEMPRT ** 2



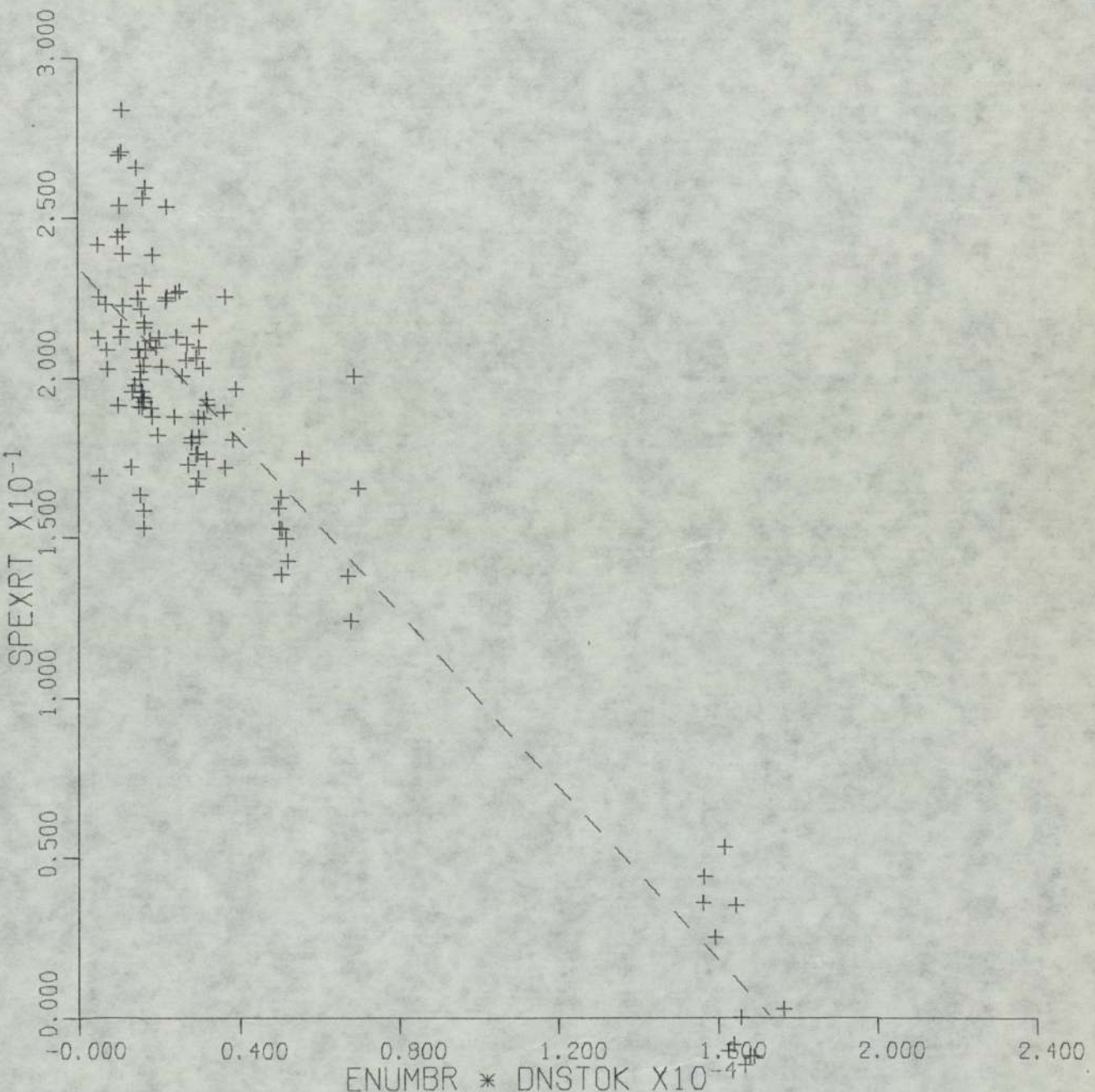
Graph C.2

SPEXRT V. ENUMBR



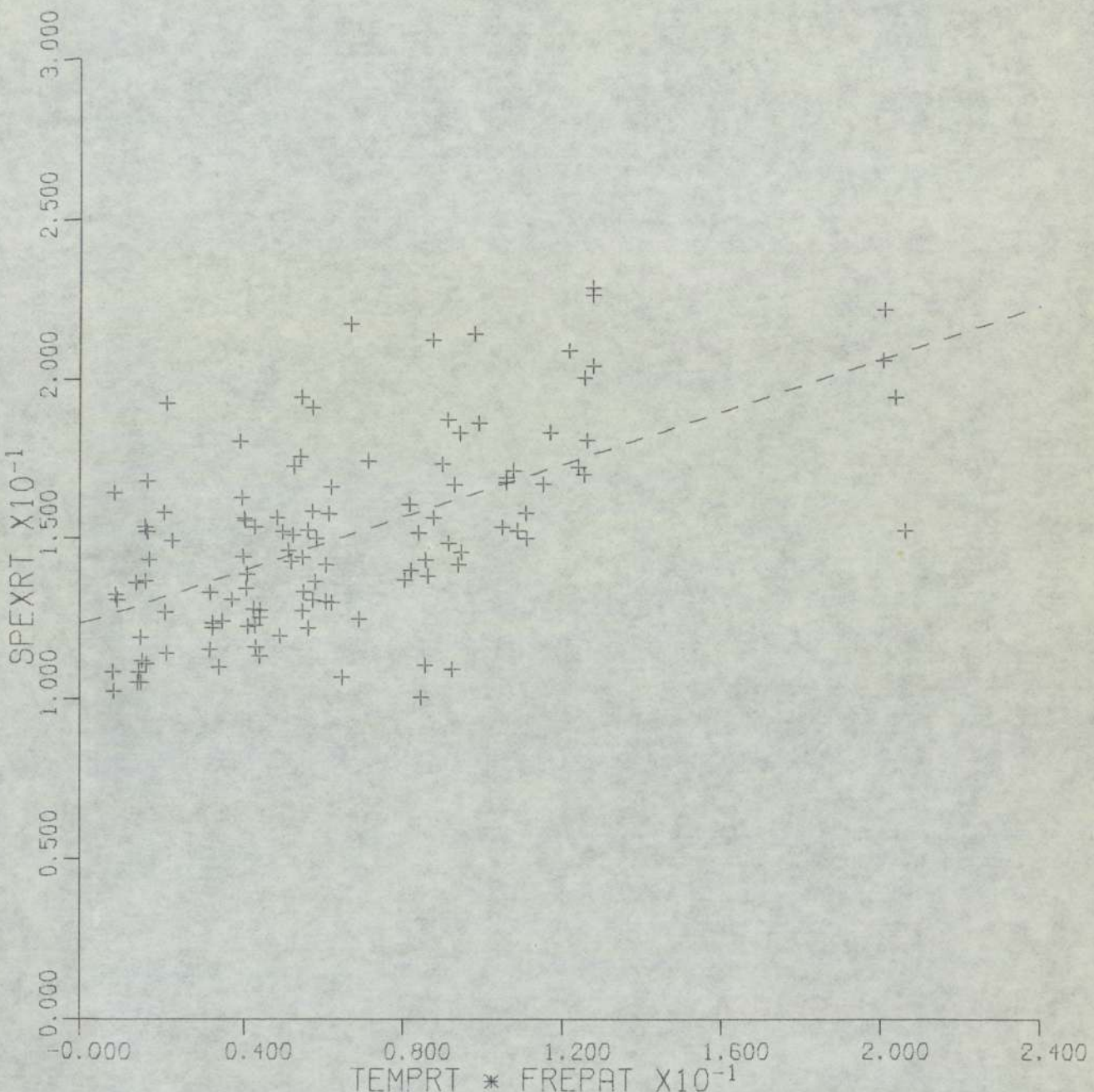
Graph C.3

SPEXRT V. ENUMBR ** 2



Graph C.4

SPEXRT V. ENUMBR * DNSTOK



Graph C.5

SPEXRT V. TEMPRT * FREPAT

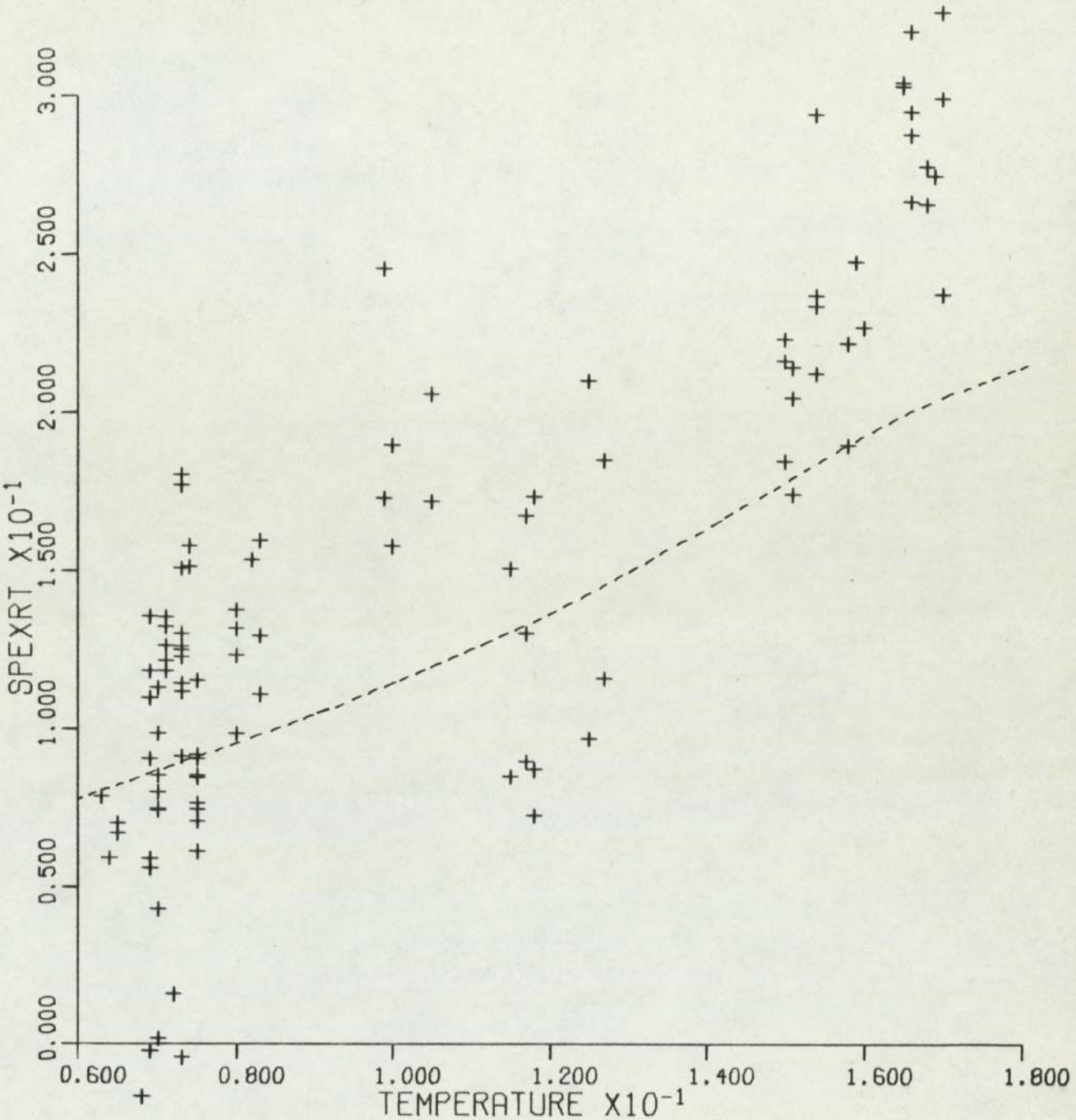
C8 Graph C.1 shows that readings fell approximately (though not by design) into three temperature classes. These classes correspond to winter (low readings), summer (high readings) and spring and autumn (middle range). There are fewer of the latter, since water temperature tended to change quickly at these seasons, while remaining steadier for longer periods during summer and winter. This difference in circumstances does not seem to have affected the specific excretory rate, so that temperature value (rather than rate of change of temperature) is the correct factor to include. Variability in SPEXRT seems to be unaffected by the magnitude of TEMPRT.

C9 Although it is very formal to regard ENUMBR and ENUMBR^2 as independent (see below), they are plotted on separate Graphs (C.2 and C.3), and show similar effects which differ in degree. Up to about 100 fish, the correlation between number and SPEXRT is very poor, and it is only with the larger numbers of fish that the line seems reliably valid.

C10 Graph C.4 shows an unfortunate distribution of readings, with an uncomfortably wide gap in the middle of the independent variable range. In all of Graphs C.2, C.3 and C.4, the greater scatter at low independent variable values is probably due to the larger number of observations.

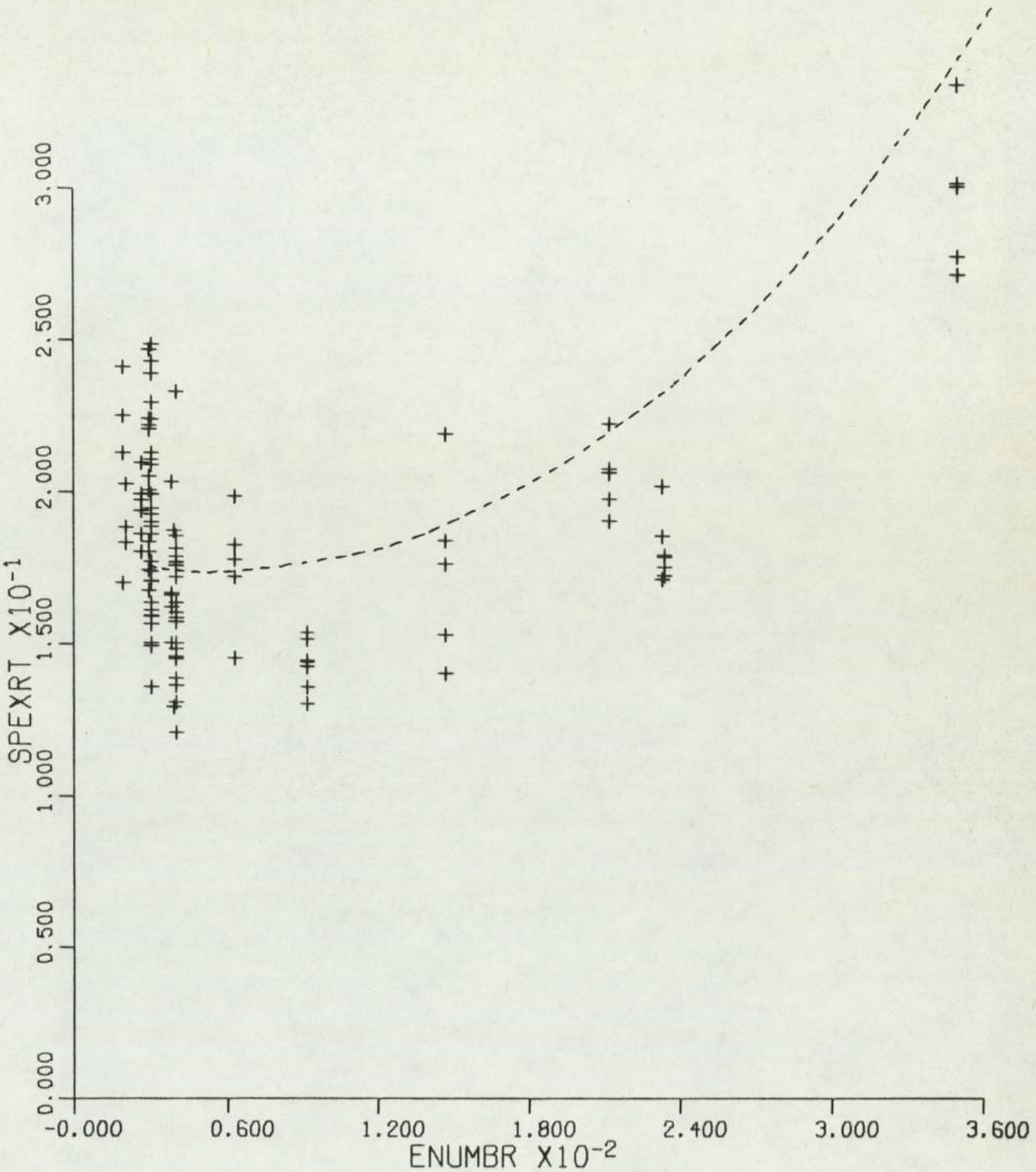
C11 The points of Graph C.5 also show a gap; clearly, further information in the upper range of this plot would be useful.

C12 Due to the interpretation difficulties of the quantities (ENUMBR x DNSTØK) and (TEMPRT x FREPAT)(see para.7.67), a further refinement of the graph presentation program was employed to "unscramble" these quantities. The final set of graphs (Graphs C.6, C.7, C.8, C.9) correspond to partially-corrected plots against the simple quantities TEMPRT, ENUMBR, DNSTØK and FREPAT. In these cases, the square factors (see para.C9) and interactions are taken into account, and the plotted lines are derived from substituting some new "typical" values into the multiple regression equation, in each case varying one of the



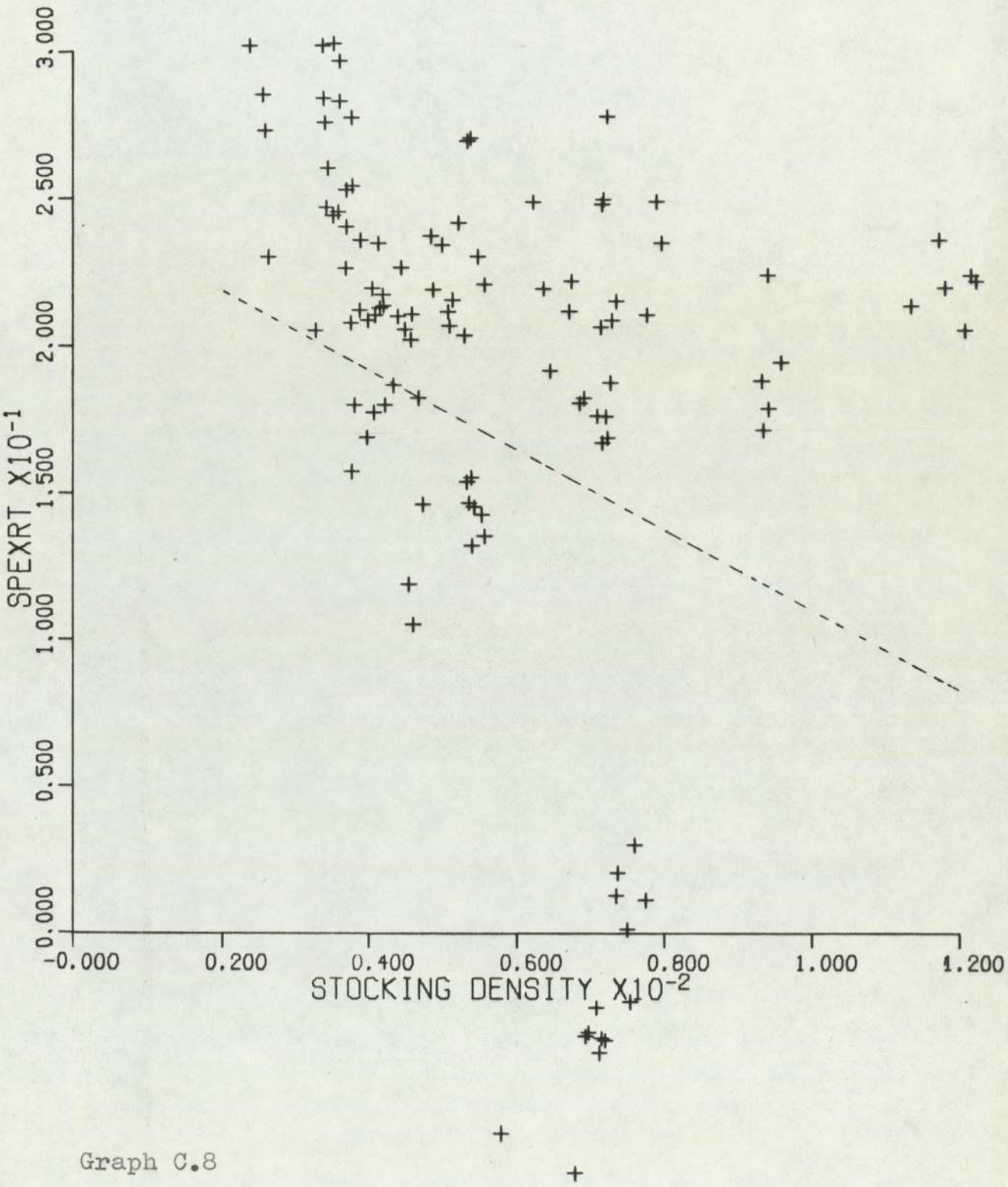
Graph C.6

SPEXRT V. TEMPERATURE



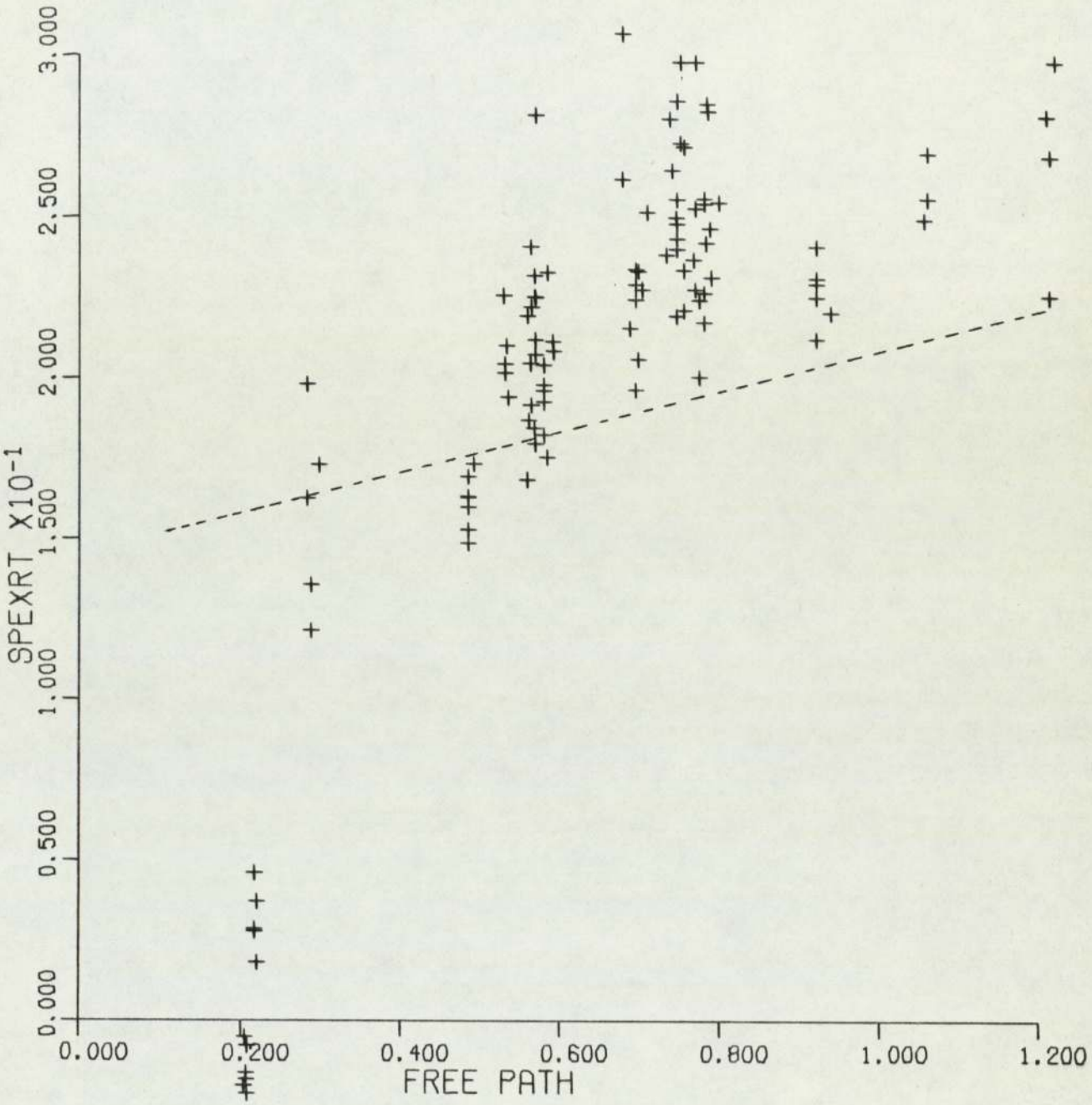
Graph C.7

SPEXRT V. ENUMBR



Graph C.8

SPEXRT V. STOCKING DENSITY



Graph C.9

SPEXRT V. FREE PATH

above simple quantities and holding the other three steady. The typical values used are given in Table C.1, with the resulting partially-corrected values of SPEXRT.

C13 Graph C.6 shows the curved plot relating temperature to partially-corrected Y values, and Graph C.7 the much more pronounced curve due to fish number. The straight-line Graphs C.8 and C.9, for stocking and mean free path, respectively, show particularly large amounts of scatter, and raise doubts as to the truly linear nature of the relationships.

DISCUSSION

C14 A closer examination of the 'observed' against 'calculated' data (Graph 7.5), for the final model selected by multiple regression analysis, provides some interesting information. Fig. C.1 is a semi-graphical treatment of this data, plotting the % deviation of observed from calculated values of SER, for individual experiments. It is noticeable that some points are extremely removed from the ideal-fit position (0%). For experiment E09 in particular, the observed values are often considerably higher than the calculated values, in percentage terms. Reference to Graph 7.5 shows that these values are nevertheless adequately explained by the model, as they fall in the lower left-hand region of the plot (where a small absolute difference of value will have a large percentage effect) and inside the CL_{95} lines. However, it is disturbing that most deviations are in one direction (i.e. model underestimates, or observational overestimates), and they do not correlate with the area of doubt for numbers of fish below 100.

C15 If this tendency in E09 is due to observational overestimate, then several sources of error are possible: poor calibration of ammonia-measurement (due to possible deterioration of standards) or differences in feeds seem the most likely ones which have not been accounted for in some measure by the methods used. However, the same feed batch was used for E06, E07 and E08 without such extreme effects, which indicates that feed differences can be ruled out. (All four

Table C.1 Typical values used to plot best-fit lines for Graphs C.6 - C.9

ENUMBR	TEMPRT (^{o}C)	FREPAT ($\ell^{1/3}$)	DNSTØK ($g\,\ell^{-1}$)	SPEXRT ($mg\ kg^{-1}\ h^{-1}$)
30	15	0.5	50	17.6
60				17.4
90				17.6
120				18.1
150				19.0
180				20.2
210				21.8
240				23.8
270				26.1
300				28.7
330				31.8
360				35.2
100	6	0.5	50	8.0
	8			9.6
	10			11.5
	12			13.7
	14			16.3
	16			19.2
	18			22.4
100	15	0.1	50	15.2
		0.2		15.8
		0.3		16.5
		0.4		17.1
		0.5		17.7
		0.6		18.3
		0.7		19.0
		0.8		19.6
		0.9		20.2
		1.0		20.8
		1.1		21.4
		1.2		22.1
100	15	0.5	20	21.8
			40	19.1
			60	16.4
			80	13.7
			100	11.0
			120	8.3

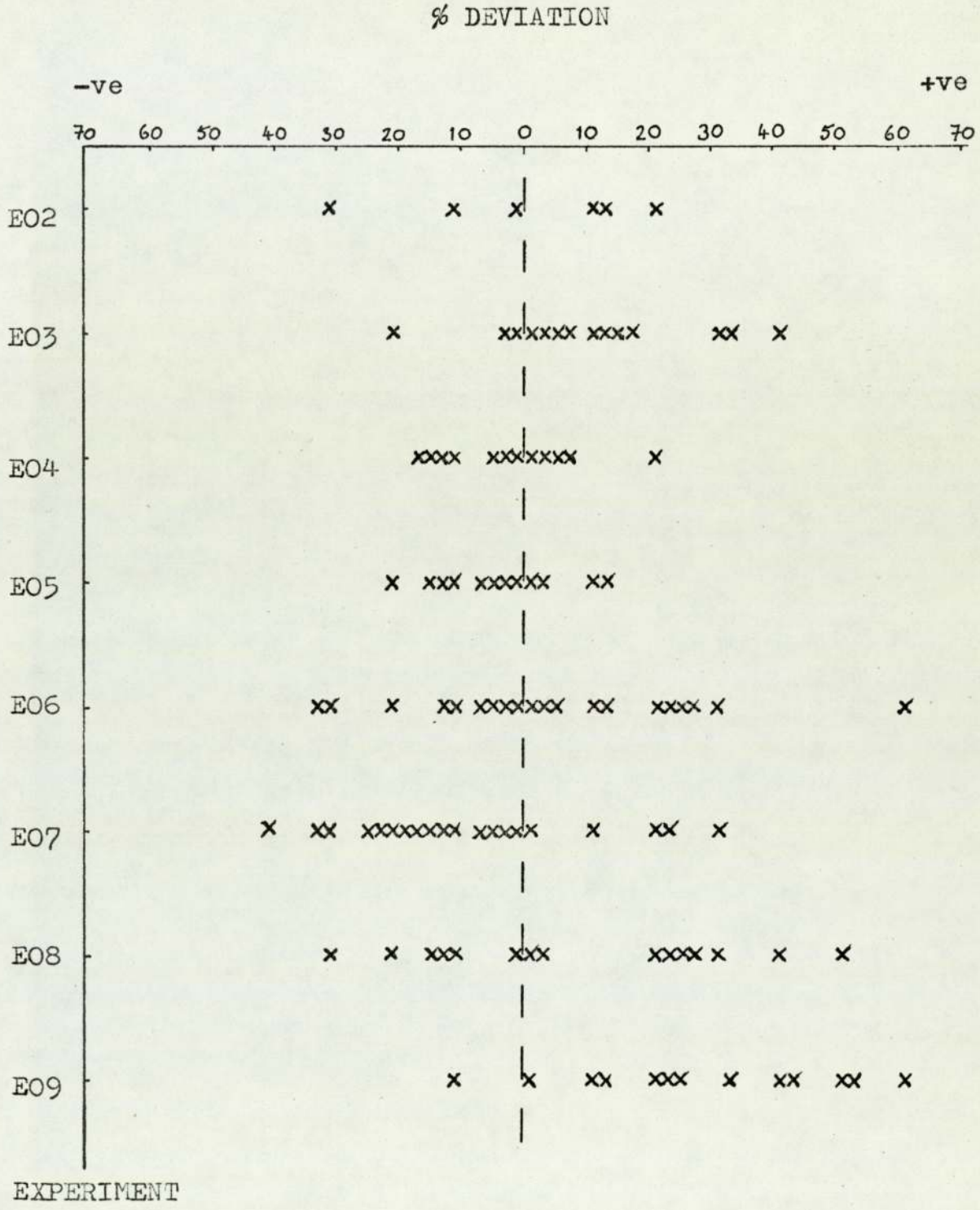
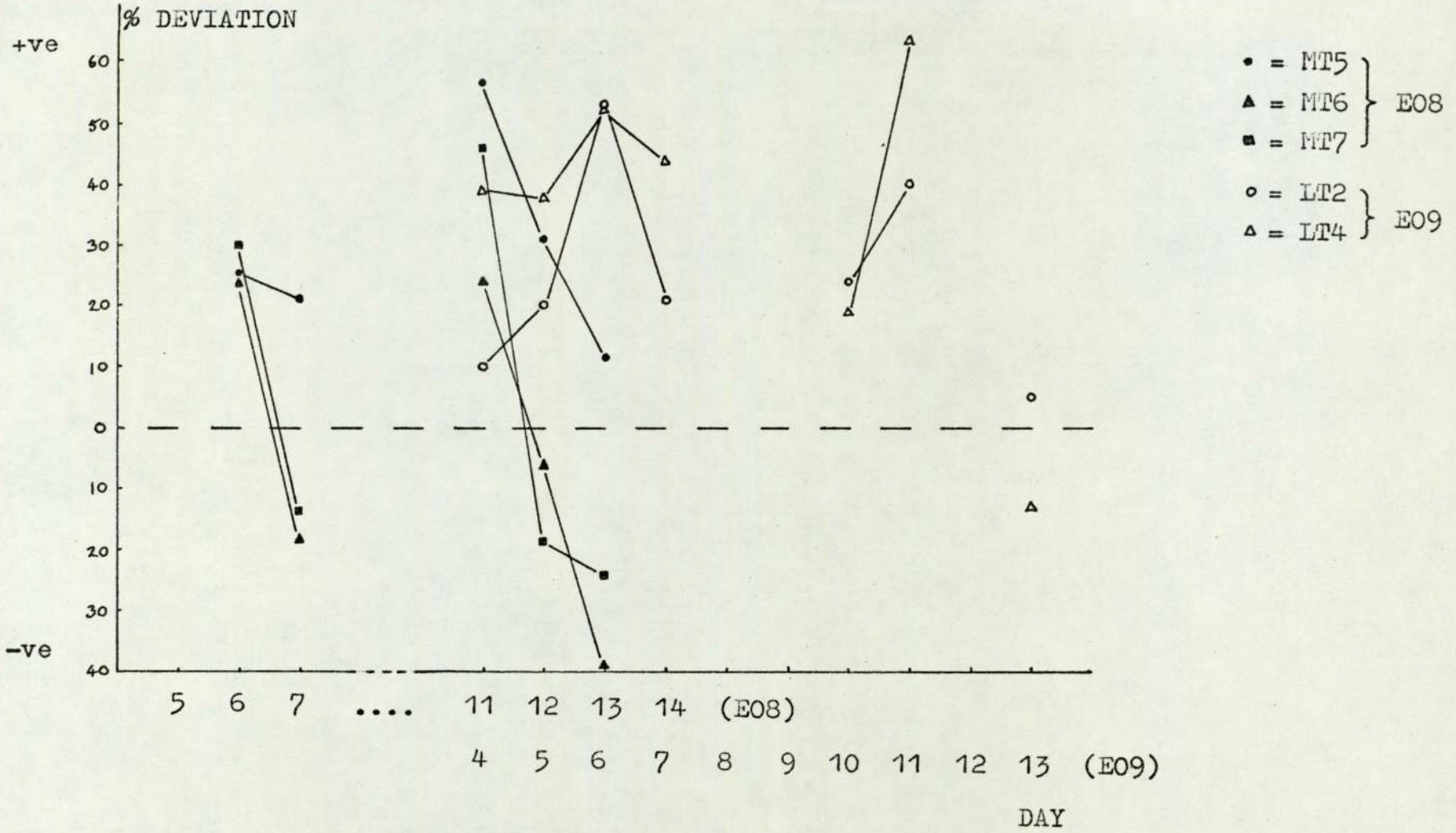


Figure C.1 % deviation of observed values from calculated values for model (4)

experiments took place within one month so that feed deterioration is unlikely to be involved). E08 and E09 overlapped by one week, and positive % errors also seem common in E08 (Fig.C.1) but to a lesser extent. This agrees with a hypothesis of mis-calibration for ammonia readings. Further analysis of Fig.C.1 shows that for E08, while MT5 results are generally high, MT6 and MT7 results are well spread. This would indicate a true tank difference rather than a calibration difference for E08. However, in E09 there is no apparent tank difference between LT2 and LT4, although there may be an overall difference between E08 and E09 due to tank size (this, however, is not shown in the similar comparison between E06 and E07).

C16 Graph C.10 shows the % error from Fig.C.1 plotted against time for E08 and E09. For each experiment taken separately it appears that there is no tank difference and that error variation follows a similar pattern in MT5, 6 and 7, and in LT2 and 4. This also agrees with a hypothesis of mis-calibration for ammonia. However, the crucial period is that covering E08 DAYs 11, 12 and 13 (4, 5 and 6 for E09). If faulty ammonia calibration were the true cause of the errors, all tanks would follow a similar pattern. In practice, the E08 tanks (MT) have a decreasing error over this period, while that of E09 tanks (LT) is rising. On each of these three DAYs, the same calibration was used for MT samples and LT samples. Thus, calibration can be ruled out, but the errors can be correlated with some difference between MTS and LTS.

C17 If observational overestimates cannot account for the high % errors in E08 and E09, then they must be due to model underestimates, i.e. the model is lacking in some feature which could explain them. This implies that the model requires to take into account some other, as yet unmeasured, variable (possibly one which is affected by tank size, see Graph C.10, although this is open to doubt as expressed above).



Graph C.10 % deviation in model (4) for E08 and E09

C18 Overall, however, the model chosen represents a good predictor in terms of the data used; it has a high multiple correlation co-efficient, and a reasonable magnitude of error (see Graph 7.5). The residual lack of accuracy of the model, as pointed out in Graphs C.8 and C.9, and Fig.C.1, is further discussed in its theoretical implications in Chapter 7 (DISCUSSION section).

D. STRESS, TOLERANCE AND TOXICITY

D1 Lloyd (1972) included in his discussion of water quality criteria assessment, a figure due to Hatch (1962) which is particularly useful in pointing out the fact that toxic effects are most often part of a continuum (Fig.D.1): there will be some level at which the fish will be exposed to the chemical in "normal" life, sufficiently small that it never takes the fish out of the region of "health". One important implication of this is that, in toxicity tests, controls should not be completely free of the chemical under observation, but rather should contain a low ("normal") level, if they are to be compared with field observations. More important to the current discussion is the implication of a transition region between "health" and "disability", where normal function becomes disturbed, and is either compensated for or allowed to become permanently disturbed. Two questions arise:-

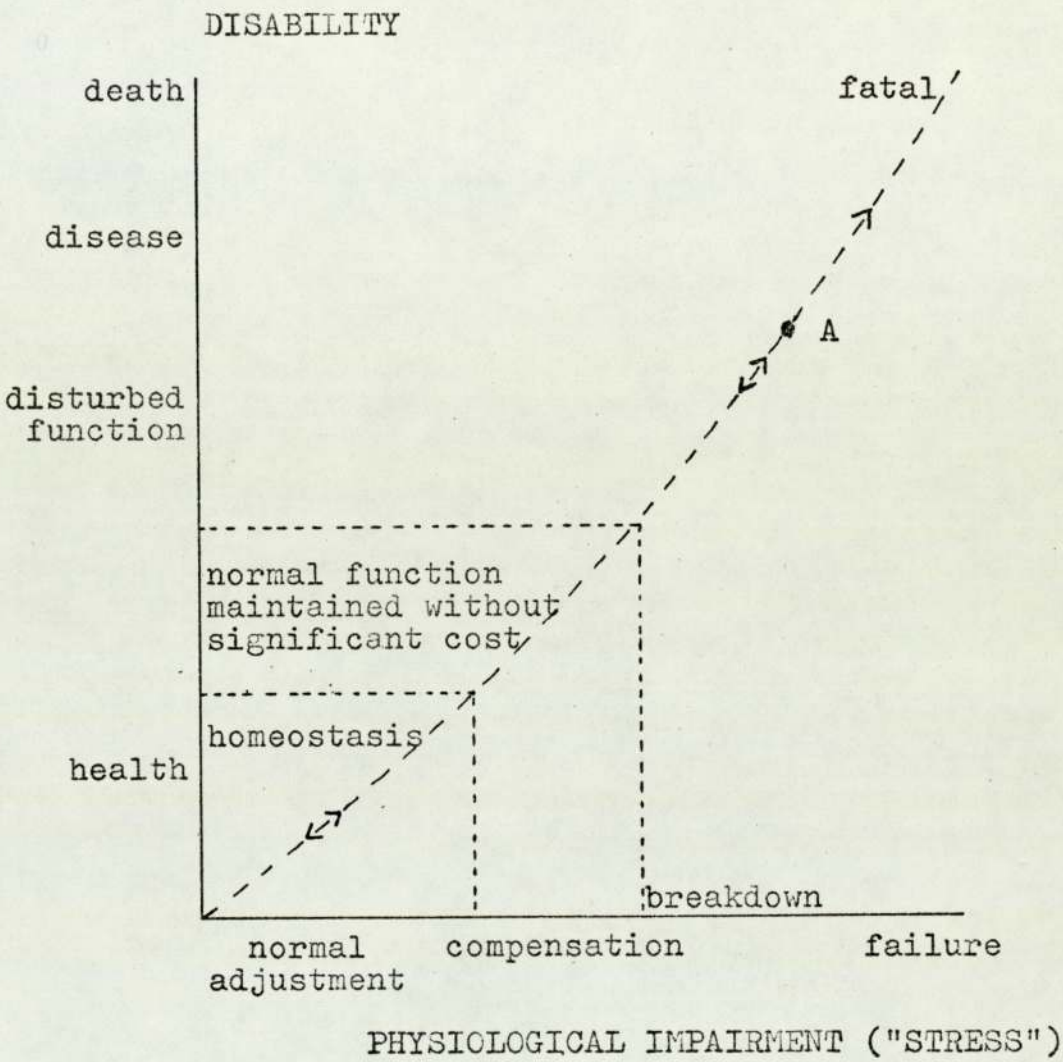
a) How can the boundaries between these different effects be delimited?

b) If they can, what are their values for this particular chemical?

The main problem, then, is identifying the meanings of the various criteria of sublethal toxicity which are available, in order to delimit effect boundaries.

D2 This kind of consideration for one chemical, as expressed above, can be put into the context of the normal environmental variation by reference to the work of Brett (1958).

D3 Brett (1958), in reviewing the problem of stress, commented that the standard medical definitions due to Selye were not only couched in terms most appropriate to human medicine, but gave little directive for biological investigation, as opposed to observation. Brett, in attempting his own definition of stress, resolved stress primarily into two types, discriminate (which affects individuals singly, and not the whole population), and indiscriminate (which applies to the whole population).



(The process is considered reversible up to point A, and irreversible after this.)

Figure D.1 Physiological impairment and disability

(from Lloyd (1972), after Hatch (1962))

Predation would count as a discriminate stress, whilst low oxygen would be indiscriminate in affecting all individuals. Physical and chemical stresses are almost invariably indiscriminate, and Brett subdivides this distinction with a scheme of stress categories similar to Fry's classification of environmental factors (1947).

D4 Brett describes four categories of indiscriminate stress:
LETHAL - describing the most extreme effects, measurable in such terms as LC-50, resistance times, etc.
LIMITING - as with Fry's limiting factor, a quantity affecting the supply of essential metabolites or interfering with a chain of energy release; this could be described as reducing the supply of metabolic "fuel" available.
INHIBITING - quantities which reduce the ability of organisms to operate or prevent the "usage of fuel" (e.g. narcotics or low temperature).
LOADING - quantities which impose a burden and require excess release of energy; equivalent to requiring more "fuel" to achieve the same result.
Any given quantity may act in several of these categories at different times, and Brett has provided a good example with temperature (Fig.D.2) as an inhibiting, loading or lethal stressor.

D5 In the figure, Brett shows how sockeye salmon (Oncorhynchus nerka), acclimated to a given level of temperature, react by some criterion at a particular test temperature. In his paper, values were only given for the criterion of LC-50 (lethal stress).

D6 The inner boxes show similar suggested boundaries for other criteria, LC-5 (5% mortality) being another measure of lethal stress. With temperature acting as a loading stress, Brett suggests a much smaller delimitation for the zone of tolerance, and suggests that measures of effect applicable here would be activity or growth. (Outside the box for tolerance, growth would be affected.) Similarly, inhibiting stress, measured by loss of spawning ability, would be envisaged as occupying a

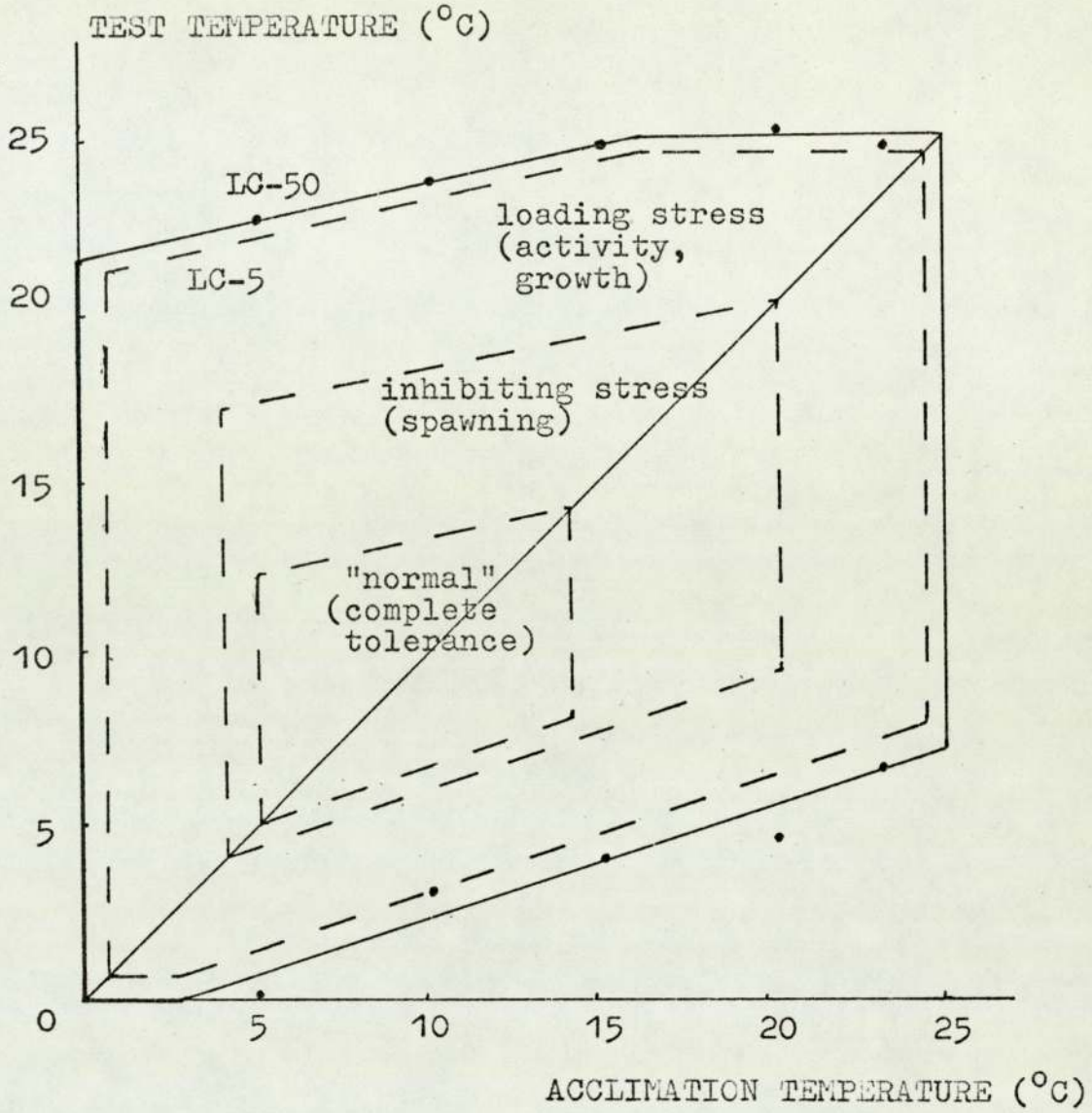


Figure D.2 Temperature stress/tolerance effects on sockeye salmon (modified from Brett(1958))

further area of the graph, leaving an even smaller tolerance box within.

D7 This illuminates the difference between toxicity and tolerance; to fully assess toxicity, as Sprague (1971) points out, requires knowledge of modes of action of toxicants, with the necessary histological, anatomical and biochemical studies that that entails. If a chemical toxicant, such as UIA, is substituted for temperature in Brett's figure, the fact that lethal and loading expressions of the stress may act in different physiological ways is not considered: what is derived from the figure is the range over which tolerance exists. Within the box delimitation, the fish tolerates the test-value and remains capable of responding according to the criterion chosen. Whether this tolerance is achieved by adjustment of the internal state (in step with the environment), or by homeostatic regulation, is a separate physiological question.

D8 Given that this basis allows the selection of a suitable criterion, the tolerance diagram gives rise to an experimental design capacity: fish acclimated to one level of a variable (e.g. ammonia concentration) can be tested at others, and those which cause loss of the criterion response will fix the boundaries of tolerance for that acclimation value. Brett's stressing of the importance of acclimation underlines such findings as those of Lloyd & Orr (1969) for UIA, where acclimation to experimental levels was observed: fish were shifting their position on the X-axis to come back within the box.

D9 Problems exist for this theoretical outline, however. How much effect is required to justify setting results inside or outside the criterion box? It is here that the toxicity terminology comes to the rescue, and there has arisen the concept of an EC-50 (median effective concentration) to set alongside that of the LC-50 (Sprague 1971). The EC-50 is far more widely applicable than the LC-50: LC-50 is just one particular kind of EC-50. Nevertheless, the same constraints apply:

the EC-50 is governed by the conditions of the experiment and in principle these include acclimation. By extrapolation, the EC-5 can be defined, and in theory the EC-0; although Sprague warns that the concept of a "no-effect" concentration is fundamentally different from the experimental estimation of response in relation to concentration.

D10 The final part of Brett's argument is that, ideally, stress should be measured in some fundamental metabolic terms which can encompass any of the categories (loading, limiting, etc.), and he nominates measures of metabolic rate (by means of oxygen consumption) as suitable, whether in terms of active or standard metabolism, or (for preference) scope for activity (Fry 1947). Thus determination of boundary levels (as in Fig. D.2) for particular physiological criteria, should then be supplemented by metabolic measurements over the whole range, which should show most "healthy" results when test values are near acclimation ones, and most "unhealthy" results when test values differ markedly from acclimation ones. Brett has shown (for example) that swimming performance (directly related to scope for activity) can be affected by temperature stress in the ways shown in Fig.D.3, for acclimated young sockeye salmon. Below $0^{\circ}C$ or above about $25^{\circ}C$ the stress is lethal (no performance). At low temperatures the stress is inhibiting (metabolic "fuel" inefficiently used) and performance is low. In medium temperature ranges stress does not occur, and at high temperatures loading stress occurs (extra "fuel" used to achieve the same result: or, in practice, the same "fuel" used and performance diminished).

D11 This shows that the concept of boxes in Fig.D.2 is in reality a continuum of ever-decreasing boxes, for which a particular chosen criterion represents only one: if Fig.D.2 was expanded into a third dimension (metabolic scope measurement, or power to perform), there would arise a 3-dimensional "hill", with a peak at the con-centre point of all boxes, and downward slopes in all directions. (This is a loose description:

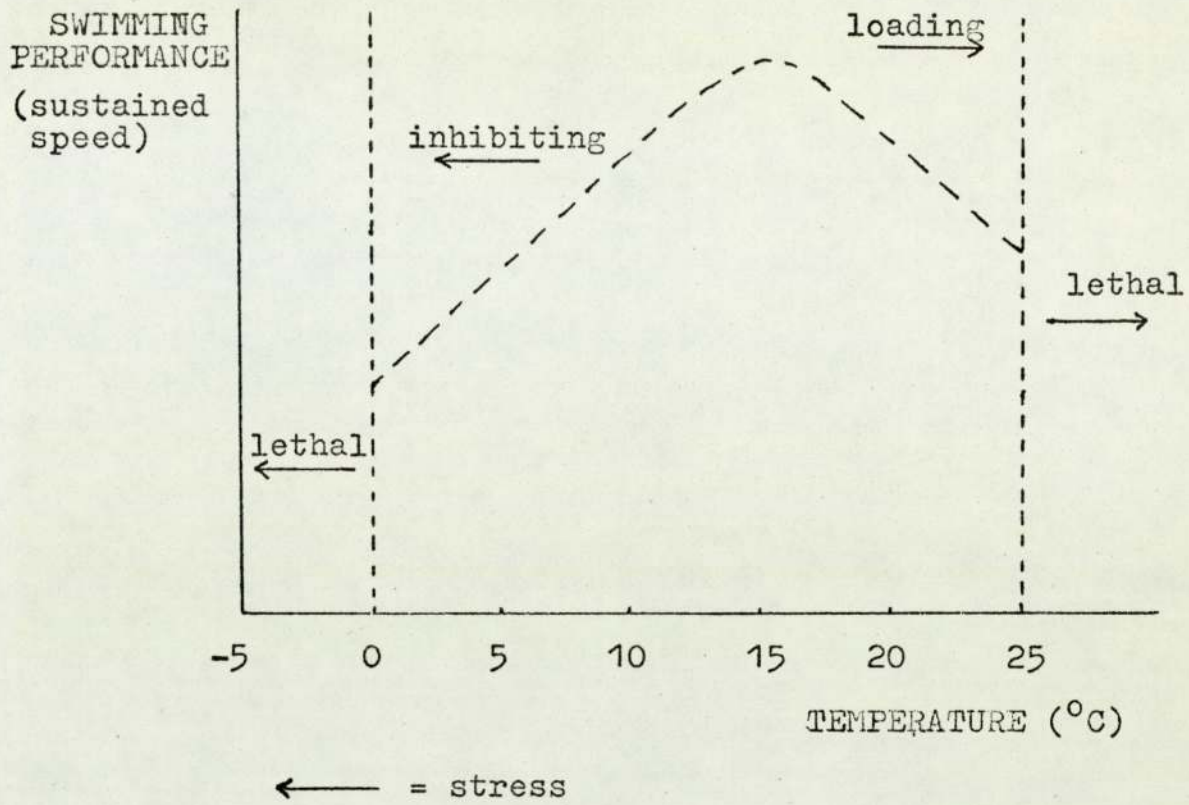


Figure D.3 Swimming performance and temperature in acclimated sockeye salmon (modified from Brett(1958))

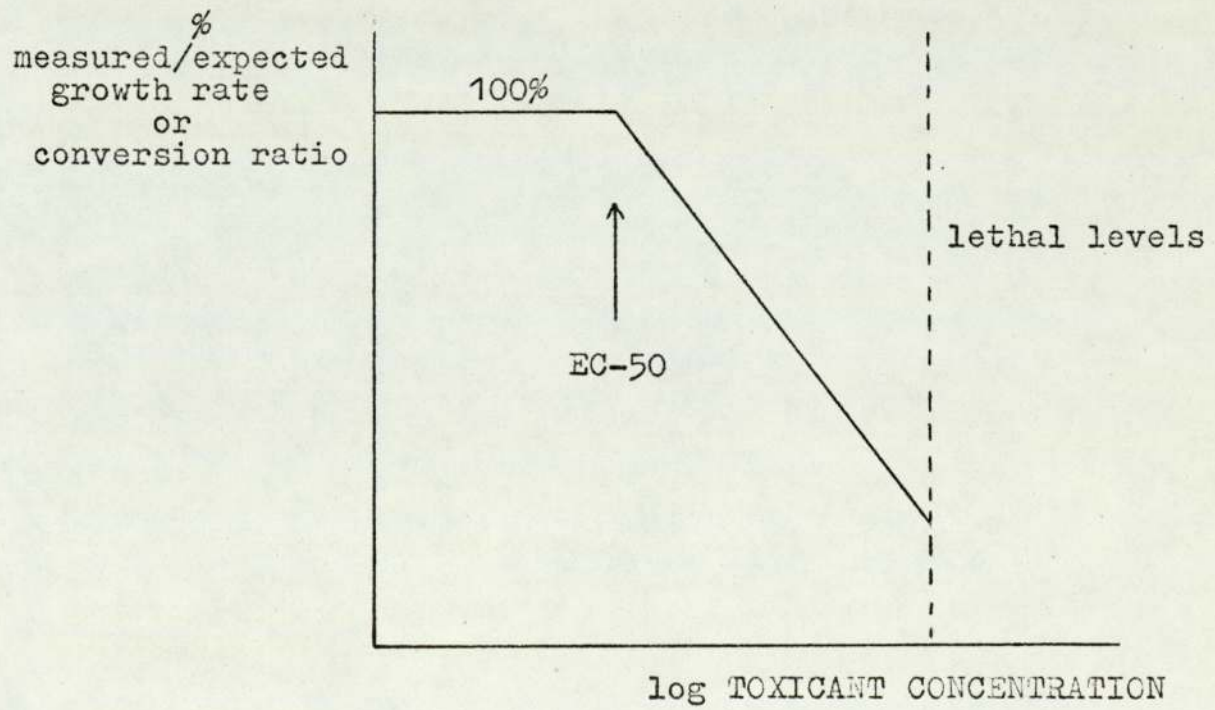


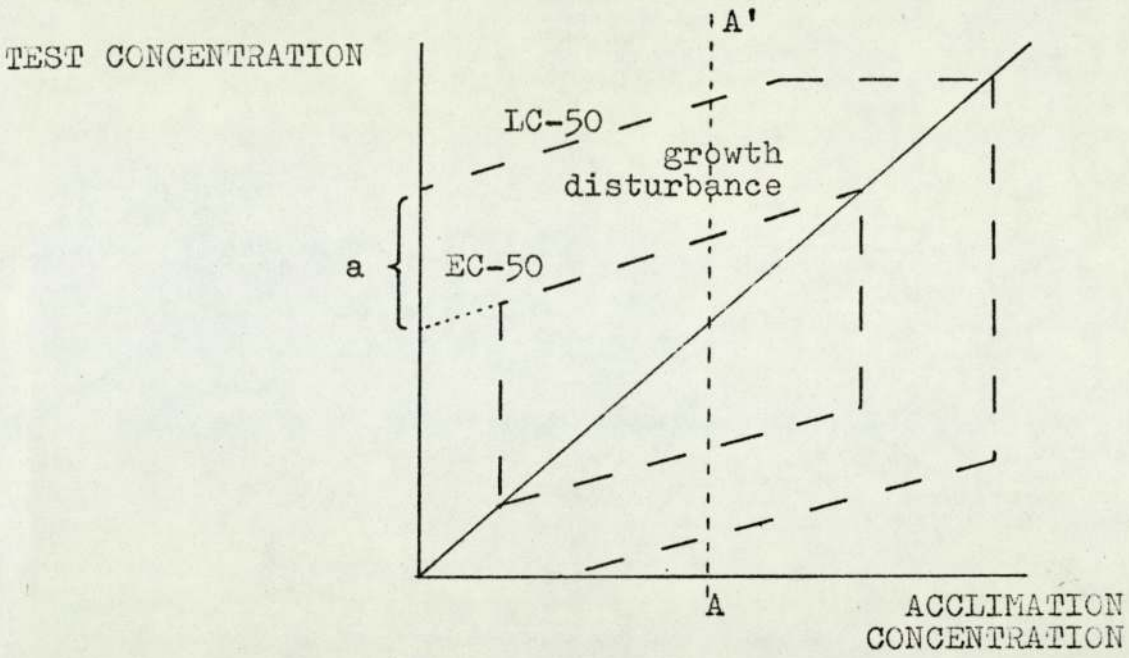
Figure D.4 The location of the EC-50 value
(modified from Webb & Brett(1973))

see Fry (1971) for a more rigorous treatment.) Thus the boxes are equivalent to contours on a map of terrain: they are artificial lines linking points of similar metabolic value. Further, the exact boundary between a stress and non-stress situation is similarly arbitrary and artificial: where should the "inhibiting" and "loading" arrows if Fig.D.3 really begin?

D12 The boundary is in fact the EC-50, which will be equivalent to the contour on the terrain map. Webb and Brett (1972, 1973) have demonstrated this approach in studies of pollutants on growth and food conversion efficiency of sockeye salmon. Plotting the mean % growth rate difference between experimentals and controls against log concentration of the pollutant sodium penta-chloro-phenate (Fig.D.4), they determined the EC-50 for change in growth rate by nominating the point at which mean growth rate was reduced. A similar procedure for change in conversion ratio gave rise to approximately the same value as EC-50. The effects were attributed to the elevation of maintenance energy demands of the fish, i.e. a loading stress, when the pollutant's mode of action was considered.

D13 Clearly, this last rigorous assessment of sublethal effects is not fully necessary for the fish farmer, but the determination of EC-50 for loss of growth or for loss of conversion ratio, may be ultimately of interest to him in setting his tolerance boundaries (Fig.D.2) and hence his water quality criteria.

D14 To summarise this consideration of stress, the situation of Fig.D.2 can be reconsidered. Re-drawing the figure for a chemical such as UIA, a box delimitation can be suggested for the growth rate or conversion ratio EC-50's described above (Fig.D.5a). Within the box "no stress" exists: outside it is the stress region leading to the extreme of death. Taking just one, acclimation level (A - A'), a relationship between test values and scope for activity can be suggested as in Fig. D.5b. At appropriate points the EC-50 is indicated.



If a is costant and known, EC-50 can be derived from LC-50

Figure D.5a Suggested tolerance diagram for a toxic chemical

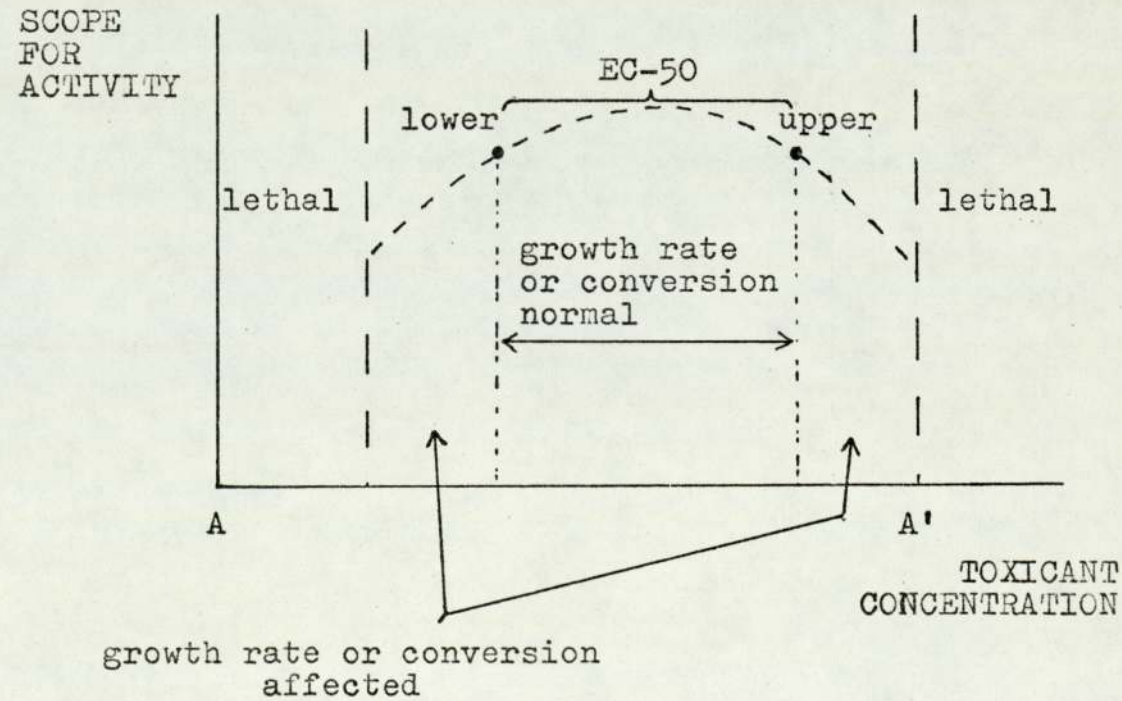


Figure D.5b Suggested relation between scope for activity and concentration of toxicant

D15 Clearly the second diagram, when experimentally evaluated, gives extra information, but for an appreciation of tolerance, only Fig.D.5a is required. The EC-50 "contour" limits the region of tolerance, within which no substantial loss of growth rate (or conversion) need be feared. Thus to describe tolerance, experimental evidence is required for the EC-50 at different acclimation levels. If the boxes for LC-50 and EC-50 are truly concentric and geometrically similar, then they will have a constant relationship, particularly along their top edges, which is likely to be the zone of particular interest. If this is so then only one acclimation value need be used for growth tests; the other points along the EC-50 line can be estimated by performing LC-50 experiments (much quicker and simpler) and using the constant relationship to derive EC-50 values. In this way, a few growth experiments coupled with a series of LC-50 determinations should enable the discovery of the upper tolerance limit for growth rate or conversion effects.

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EXCRETORY PROBLEMS

IN FRESH-WATER FISH CULTURE

IN CIRCULATING SYSTEMS

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639.93WAT
203640 21 MAR 1977

A thesis
submitted to the University of Aston in Birmingham
for the Degree of Doctor of Philosophy

September 1976

SUMMARY

Increasing interest in intensive fish culture in tanks has led to a need for reliable estimates of excretory productivity by fish, especially with regard to ammonia, to facilitate the design of efficient water treatment equipment for recycling or effluent disposal. Two main aspects of this are examined: (a) realistic assessment of excretory productivity in the limited but multivariable tank situation; (b) criteria for allowable persistence of dissolved excretory products in closed systems, consistent with maintenance of fish growth.

Assessment of ammonia productivity was investigated using rainbow trout. Laboratory facilities were designed and built, and an approach to a fuller understanding of the problem worked out using multivariate analysis, resulting in a multiple regression model which satisfactorily described the experimental situation.

Under experimental conditions, the specific excretory rate of young rainbow trout could be related to environmental conditions by the following equation (parentheses denote 95% confidence limits for one observation):-

$$y = 5.7522 + 0.0427x_1 + 0.0002x_1^2 + 0.0414x_2^2 + 0.4165x_2x_3 - 0.0014x_1x_4 (\pm 4.9783)$$

- where y = specific excretory rate, x_1 = fish number, x_2 = temperature, x_3 = mean free path, x_4 = stocking (mass/volume)

The implications of the technique are discussed, and it is proposed that the method used to derive the relationship is worthy of further application and refinement.

Under similar experimental conditions, a preliminary study was conducted, of the growth tolerance of trout to simulated recycled fish effluent, and a basis provided for

future investigation thereof. Small differences in growth patterns were found between treated and control fish for two strengths of simulated effluent, lending limited support to the concept of growth stimulation at low concentrations of otherwise apparently toxic substances. The details and implications of this are discussed.

The overall context of the work is discussed, and related to the concept of stress in fish culture. Suggestions are made for future experiments.

This work is dedicated to the love and memory of my mother.

ACKNOWLEDGMENTS

Many people have helped me, both directly and indirectly, in the completing of this work. At Aston, my thanks are due to Professor Matty for originating the project; and to Messrs. R. Burns, A. Lawrence and J. Berwick, who gave much technical assistance. Acknowledgments are also due to the Science Research Council, for financial support; to Shearwater Fish Farming Ltd. (B.O.C.) for their generous financial support in the latter stages; and to Dr. John Forster, of M.A.F.F. and Shearwater, for advice and encouragement.

Special thanks must go to my supervisor, Dr. Chris Thornburn of the Biology Department, for his cheerful encouragement and assistance for so long, especially in the latter stages of production of this thesis; and also to Dr. John Aston, of the Metallurgy Department, for his invaluable aid with statistical and computing techniques, and unstinting sacrifice of the hours involved.

No acknowledgment here can possibly repay what I owe to my main colleagues in the Fish Culture Unit: Philip Smith, whose drive, dedication and comradeship from the beginning was directly responsible for most of what we achieved in establishing the Unit; and John Roberts, whose wit and companionship brightened the weary path of recurrent problems and frustrations. I am privileged to have worked with them both.

I must warmly thank Mrs. B. Smith, of Bramley, for typing the thesis with such despatch; and extend grateful thanks to all others who have helped in any way at all.

Lastly, my especial gratitude goes to my wife Christine, for her continual support and encouragement, and for suffering many more tribulations than I during the course of this work.

C O N T E N T S

Contents (continued)

ABBREVIATIONS AND CONVENTIONS USED

SI units or units derived from them are used throughout*; time of day is given in the 24-hour convention.

Standard biochemical abbreviations are used for well-known compounds (e.g. ATP), oxidation states, and stereo-isomers.

In statistical discussion:-

P	=	probability of result occurring due to chance: 1.0 represents 100% chance.
NS	=	not significant
*	=	significant at 5% level
**	=	significant at 1% level
***	=	significant at 0.1% level

(according to standard tables given in Bishop 1966)

r	=	simple correlation coefficient
R	=	multiple correlation coefficient

Simple abbreviations sometimes occur in Tables (e.g. TEMP for temperature, EXPT for experiment).

Most symbols used are explained in the text; the following list emphasises the most important ones:-

PO1, PO2, PO3	pilot experiments 1, 2 and 3
EO1... EO9	E-series experiments 1 - 9
TO1, TO2	T-series experiments 1 & 2
Lot	fish batch (as purchased)
FCAT	fish category (see Table 1)
HC	holding conditions

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* except for dimensions in Figures depicting developmental work in Chapters 2 and 3; materials were ordered and built using feet and inches, hence these units are retained.

Abbreviations continued

ST(S)	-	small)
MT(S)	-	medium) tank (system)
LT(S)	-	large)
SER	-	specific excretory rate
SREF	-	simulated recycled effluent

General abbreviations :-

(e.g.)	1.1	-	chapter and paragraph number
	Fig.	-	Figure
	para.	-	paragraph
	TMAO	-	trimethylamine oxide
	Q_{10}	-	rate change coefficient for 10 deg C rise
	LC-50	-	lethal concentration (50% mortality)
	EC-50	-	effective concentration (50% affected)
	P_i	-	inorganic phosphate radical
	K_a	-	equilibrium constant
			($K_a = \frac{[H^+].[NH_3^o]}{[NH_4^+]}$ where
			a = dissociation constant)
	SEM	-	standard error of the mean
	ℓ	-	litre(s) [to avoid confusion with 1]

PART 1

INTRODUCTION

1. GENERAL INTRODUCTION

BACKGROUND

1.1 The culture of fish for food is a practice undertaken by man in many ways and places, and one can accept that its earliest occurrence was probably a consequence of man's transition from a hunting style of life to the settled conditions of agriculture. China provides an example of a very ancient settled situation, and correspondingly furnishes the oldest records of fish culture (475 BC), concerned with the common carp (Hickling 1971).

1.2 Yet in another sense fish culture, especially in the form of fish farming, is a very modern asset in the struggle to supply man's food requirements; its occurrence and importance is rapidly growing, and in the United Kingdom alone interest, research and commercial commitment have sharply increased over the last decade, principally utilising high market-value species which will bear the required research and development costs.

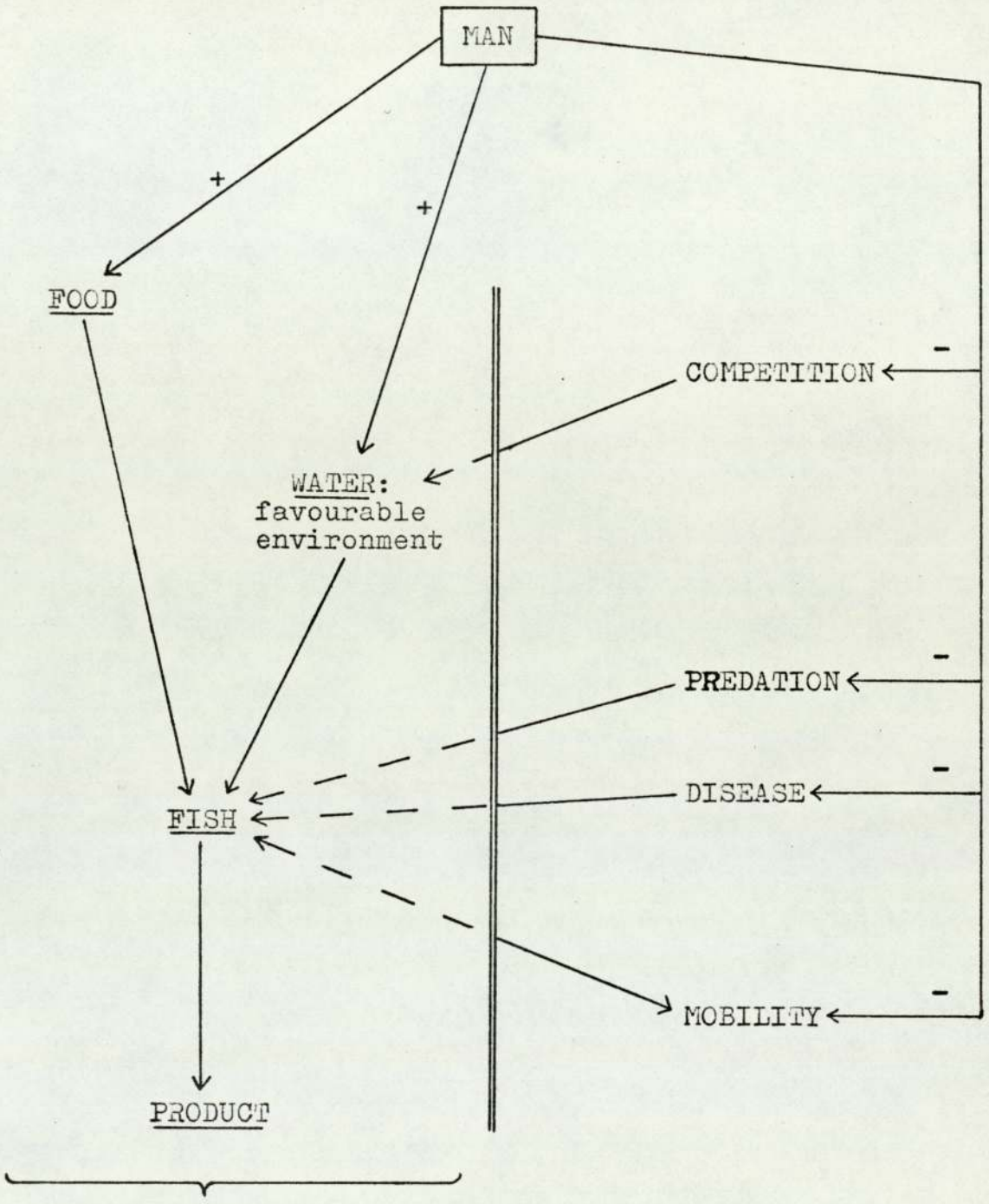
1.3 Much of the drive behind the recent surge in interest has come from the application of improved methodology and technology, some imported from the United States and some due to indigenous ideas and new equipment, together with a spirit of trial and investigation.

1.4 While fish culture may cover a wide-ranging group of activities (Hickling 1971, Fish Farming International 1973-1975), the concern of these studies is with one particular type of culture; the intensive rearing, in tanks, of salmonid fish. The exact definition of "intensive" is difficult, but for the present I shall take it to imply the production of large numbers of fish in an enclosure whose land area is considerably smaller than that which a "natural" distribution might require (where general behaviour would be unaffected by man). Salmonids have probably the fullest development history of all intensively cultured fish, largely due to the United States Bureau of Sport

Fisheries and Wildlife, whose research has generated salmonid rearing information for most of this century. All experiments documented here utilise the rainbow trout (Salmo gairdneri RICHARDSON), to which much American information applies, and which is currently the subject of the largest-scale British intensive fish-farming developments. One such development, that of Shearwater Fish Farming Ltd., is based on the employment of circular tanks at all stages of the rearing process, and it is with circular tanks that the work described has been carried out.

1.5 Foremost among the problems that the fish-farmer faces are the supplies of two vital ingredients, water and food. Like all animal culture, fish farming is essentially a function where a simplified path is chosen through a network of ecological interactions, resulting in man attempting to prevent certain interactions, while enhancing others (Fig.1.1). This simplistic model disguises a host of lesser problems, but emphasises man's particular role; he may enhance natural supplies or remove the natural supply and substitute it from another source. Food is frequently seen as the fish-farmer's first priority,and in many cases where the natural supply is substituted or greatly supplemented, it is his greatest commercial cost, although all of his activities may bear incidental costs.

1.6 Thus it is not surprising that nutrition has occupied a major place in fish culture research. Transition from early wet fresh-food diets for trout to modern dry pelleted feeds with easier storage, transport, handling and feeding requirements, has only been possible because of extensive research on fish nutritional requirements and correct diet formulations, spurred by the background of rising prices. Growth rates have been maintained or enhanced, but the principal cost involved remains the high protein requirement of salmonids, supplied largely from a world stock of fish meal which has recently become erratic and increasingly expensive (Smith 1976). Consequently a search has begun, in animal feed production generally,



+ = supply or enhancement effect

- = prevention effect

Figure 1.1 Some major considerations in the fish-farming situation

for substitute proteins which will yield equally good results at lower cost. Thus in trout nutrition at least, the emphasis has shifted from the basic ability to supply a convenient food, to a refining of technique, and while this is less true for the many other fish species at present under consideration for intensive farming, it is possible to feel that the major groundwork in this field has been performed.

1.7 However, when attention is turned to a favourable fish environment expressed in the form of water, it soon becomes clear that there are several topics of interest involved, and that the pattern of knowledge is frequently lacking or indeterminate. The position is further complicated because different systems of intensive rearing may require different husbandry rules. Thus information generated for use in the American raceway-based industry (Piper 1972, Liao 1970), may be much less useful in a high-flow circular tank system, particularly if oxygen enrichment is used to boost fish-loading levels.

1.8 Oxygen is usually the first limiting factor in trout production environments. Fish which are actively growing require abundant oxygen supplies, and the fish-carrying capacity will be limited at the point where fish oxygen consumption cannot be met by the oxygen present in the water supply.

1.9 Given that an environment suitably rich in oxygen can be provided, it has been increasingly felt that the next limiting factor involved is an inhibitory or "toxic" component (see Chapter 8) due to the fish themselves, identified with their excretory products. A variety of information has led to the conclusion that ammonia (the major excretory compound) is the offender. (Brockway 1950, Kawamoto 1961, Burrows 1964).

1.10 If excretory material is important in limiting fish-carrying capacity, it is clear that a second function of an abundant water supply for trout (besides oxygen supply) is the

removal of excreta as swiftly as possible so that each fish's local environment is continually renewed, and inhibition prevented. In this respect, in order to match water supply to requirement, the fish farmer needs to be able to estimate excretory production in his tanks, and manipulate either water supply or fish stocks to achieve the correct balance.

1.11 Since intensive rearing may entail great numbers of fish densely packed into relatively small volumes, it is clear that in many cases trout farming requires a large supply of water; the quantitative aspect of this will be discussed later, but the essential point at this stage is the setting of this requirement into a national background of a limited number of potential farm sites in the UK. Within the geographical limitation, the administrative limitations involved in operating a fish farm have been clearly stated by Cracknell (1974).

1.12 The recent response to these problems both in the USA and the UK has been to look forward to farms where water is recycled and the supplementary water requirement is hence minimised. This process carries many important implications for fish farms, but the aspect of greatest concern to this study is the waste product accumulation which is implied in such a system. At this point, a clarification of terminology may be useful; in this study, all systems where water is re-used (conventionally called recycled, recirculated or closed-circuit) are referred to as <u>closed systems</u>. The alternative, which may be referred to elsewhere as a single-pass, flow-through, open-circuit or discharge system, is here termed an <u>open system</u>.

1.13 While solid waste is the more visually-obvious problem of closed systems, the unseen problems of dissolved waste may be essentially more important. With the possible harmful effects of excretory products born in mind, it is clear that a fish farmer using recycled water must know the excretory productivity of his tanks. Only then can he efficiently design a water-treatment unit for incorporation into his system, knowing the

excretory loading it must deal with, and by reference to the water standard required for re-use, knowing the degree of efficiency required.

1.14 Unfortunately, the fish in a farm tank do not constitute a stable system; at the very least the farmer is promoting growth, and a range of other variables may be involved, possibly the most important ones having to do with the level of stocks carried. Thus a meaningful assessment of exretory productivity will only be one which takes into account these variables, and hence is able to express the prediction of excretory levels in operational terms. For many years such an approach was not forthcoming, but recently work at the Salmon Cultural Laboratory in Washington (Burrows 1964) and the Bozeman Fish Cultural Development Center in Montana (Piper 1972) has resulted in simple numerical guides for fish farmers. It is my submission that as trout farming begins to take a more scientific turn, and problems of the micro-environment of a fish-rearing tank begin to merit detailed study, it is of fundamental importance to attempt a fully scientific and comprehensive assessment of excretory productivity in the multivariable situation involved. The major part of this study is directed towards this goal.

1.15 With water re-use borne in mind, a secondary excretory problem is implied; as previously suggested, the filter efficiency required must be matched to some standard for the water delivered back to the fish. How clean must the filtered water be; or, what kind of water quality can the fish tolerate, consistent with the desired productivity? (Fig.1.2). Knowledge of the acute (lethal) toxicity of ammonia is well developed, but this information is too extreme: the interest is rather in the kind of excretory concentration where growth is affected. The terms become those of tolerance rather than toxicity, and the concept of median effective concentration (EC-50) affecting growth is more relevant (Webb & Brett 1972). Thus an attempt has been made during this study to provide basic guidelines, by

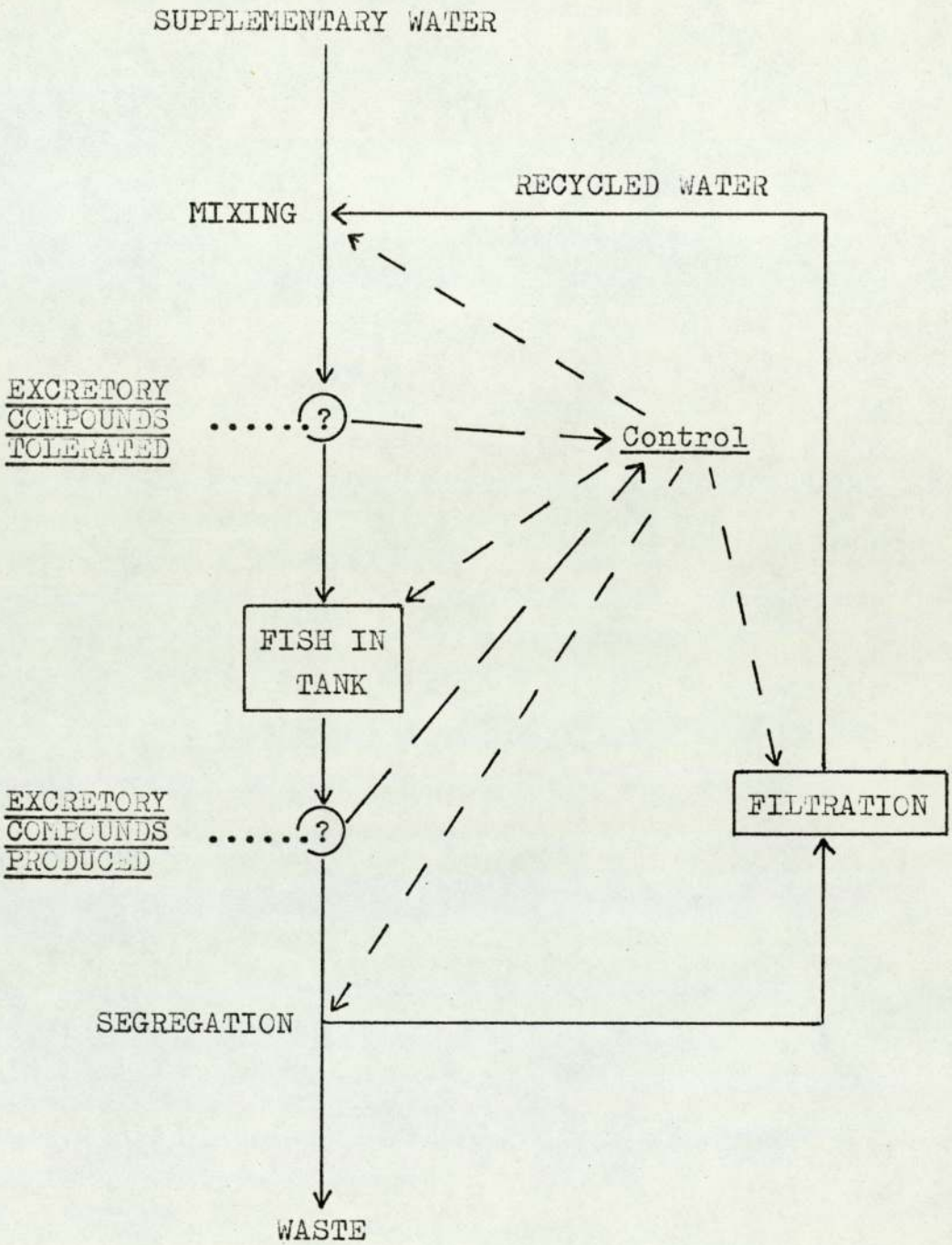


Figure 1.2 Fundamental control required in closed system operation

means of preliminary experiments, for an experimental approach to this problem.

1.16 Finally, this study discusses these problems in the general contexts of circulating systems (those where water is directed round a tank peripherally), closed systems, and the general fish culture environment. It is important to point out that small-scale work in the laboratory can be integrally different from the farm situation; the magnitude differential is expressed in many ways and the potentials of the two situations for measurements are also different. For this reason the aim of this work is to produce ammunition for theoretical discussion (supported by illustrative data), rather than to put forward the generated data in the form of authoritative statement. It is to be hoped that consideration of the ideas involved, in partnership with future experiments on full-scale research systems, will be of industrial or commercial benefit, while scientific interest may lie in the study of the production fish-tank environment as worthy of academic attention in its own right.

FISH AND HOLDING CONDITIONS

The rainbow trout

1.17 The experimental animal used throughout was the rainbow trout, Salmo gairdneri RICHARDSON. This spelling is used in preference to "S.gairdnerii" for simplicity, following McPhail & Lindsey (1970). This is a non-migratory fish, in contrast to the anadromous steelhead trout of the same species. Under experimental conditions the rainbow can be converted to living in sea-water, under which conditions it is said to grow faster. All work in this study was carried out in fresh-water.

1.18 This animal was used for a variety of reasons:-

a) its ease of availability,

b) its importance in commercial fish-farming, especially in current investigations into water-recycling,

c) its prominence in physiological, biochemical and fish-culture literature, providing a high level of general knowledge of the animal,

d) the importance of the salmonids as the fishes most sensitive to water pollution, and the extensive literature arising from toxicity studies.

1.19 The salmonid family belongs to the Order Salmoniformes, a sub-group of the Superorder Protacanthopterygii according to the classification of Greenwood et al (1966) (quoted in Alexander 1967). This Superorder represents a fairly primitive teleost group which may have given rise to most of the others. The primitive nature of the body shape is emphasised by the possession of an adipose fin without fin rays behind the main dorsal fin: this is typical of the Superorder. In contrast to more specialised fish which probably evolved later in other groupings, the paired fins of the salmonids are in the primitive positions; pectorals low down on the sides posterior to the gills; and pelvic fins just anterior to the vent. The salmonids in general are a group of relatively non-specialised, carnivorous, fresh-water and anadromous fish; the anadromous capability might also be considered fairly primitive.

1.20 Salmo gairdneri is readily identifiable from the other common British Salmo species, S.trutta (brown trout) and S. salar (Atlantic salmon), by its possession as adult of an irridescent streak along the flanks, a black-spotted green-brown mottled dorsal surface, and a red lateral band when in spawning condition (often clearest on the operculum). This contrasts with the silver appearance of the smolted S.salar, and the conspicuous red or orange dorsal spots on the brown trout. The parr of these species are less easy to distinguish (see McPhail & Lindsey 1970).

1.21 The rainbow trout is a native of the rivers of the North-West American seaboard, first described from the Columbia River; but it has in recent times been spread by man to many of the

temperate waters of the world, also into high altitude regions in lower latitudes (MacCrimmon 1971). The fish is said to have several intra-species varieties, the most commonly quoted being "Idaho" and "Shasta". The latter is characterised by autumn spawning (as opposed to spring), and Bernhart (1969) claimed to find haematological differences between these strains. Nevertheless, for most purposes the common strains are indistinguishable (save in spawning), and since no guarantee of strain could be given by the suppliers for the fish used in this study, no account will be taken of strain as a source of variability.

Holding conditions

1.22 Fish used were bought in as alevins, usually of between 3 and 7 cm length for growing up prior to experiments. Transportation was in large tied-off plastic bags placed inside plastic dustbins, half-filled with water from suppliers, and aerated continuously. On arrival, fish were placed in holding tanks in their own water, supplied with extra aeration via diffusers, and over a period of time, Birmingham tap-water was gradually allowed to flow in and displace the water in which the fish had travelled. In this way, minimum stress was associated with the transfer from hard, alkaline hatchery water to soft Birmingham water, and temperature shock was avoided. This routine kept initial losses of fish to a very few, and was usually followed by good feeding behaviour on the next day. Fish were retained in holding conditions for several weeks before being used for experiments.

1.23 Two major types of holding circumstances were used, designated HC1 and HC2 respectively. The main points of these conditions are summarised in Table 1.1.

1.24 During holding, populations in different tanks varied according to size, state of grading, cleaning activity, etc. but loading was usually maintained below 1.0 kg min l^{-1}, and reduced when high temperatures were encountered (following Burrows 1972).

Table 1.1 Details of holding conditions

DETAIL	CONDITIONS	
	HC1	HC2
Shelter	Outside (2nd-floor roof) but some protection from clear plastic "greenhouse" mounted over tanks	Inside (unheated wet laboratory)
Water supply	From main University building reservoirs, via reservoir tank on roof	From tap-water main, via constant head reservoir tank
Lighting Photoperiod	Natural	Artificial (controlled)
Tanks	3 x circular (250 ℓ), or 1 x rectangular (1000 ℓ)	6 x circular (250 ℓ)
Feeding	By hand, usually twice per day	Automatically, to schedule, five times per day
Disturbance	Occasional; by wind effects, drop in water flow, people	Rare (tanks screened); only by experimenter
Siting	Large distance from holding tanks to experimental system	Experimental system in same room
Grading	Occasional	Regular

1.25 Birmingham tap-water is chemically soft (see below), and originates mostly from the Elan Valley catchment area in Wales. It is brought directly to Birmingham with a small proportion (7%) of River Severn water added. Thus water treatment before addition to the mains supply is minimal; in particular the water is low in chlorine content. At all times fish were found to live and grow well in tap-water whose only form of treatment was a boost in aeration by spray discharge into a header tank before being conducted to the fish. In this respect the timing of the work was fortunate, since in the future greater proportions of Severn water will be required, necessitating more treatment and higher chlorine levels. The chemical nature of the water was described by the local Water Authority as in Table 1.2; periodic tests at Aston are in good agreement, but regular measurement of pH normally gave values between the minimum and mean values quoted.

1.26 Water supplies were at the approximate rates of 8 $\ell\ min^{-1}$ (HC1) and 9 $\ell\ min^{-1}$ (HC2), but this was liable to fluctuation in HC1. A constant-head tank kept the flow steadier in HC2, but in both cases the flows to individual tanks were adjusted according to the populations held at any particular time.

1.27 In the case of the 1000 ℓ tank in HC1, flow was introduced at one end, and the water was lost by over-flow from an exit port at the other end, thus creating a linear flow. In the 250ℓ circular tanks water was peripherally introduced at an angle, circulated round the tank, and left by a central exit hole, sweeping out faeces and any uneaten food in a self-cleaning action; the water was brought up by a U-tube beneath the tank, to overflow from the U-tube beside the tank, at a height which governed water level (and hence volume) inside the tank. U-tubes were regularly cleaned out to prevent debris accumulation. Fish normally swam against the direction of flow; this is well shown in Fig.3.14 in Chapter 3.

Table 1.2 Chemical nature of Birmingham tap-water

(Results in mg ℓ^{-1} except for pH) (from CBWD 1973)

CHEMICAL	MEAN	MAXIMUM	MINIMUM
Ammonia -N	0.042	0.208	nil
NO_2^- -N	< 0.001	0.004	nil
NO_3^- -N	< 0.5	2.4	trace
pH	7.65	8.60	6.80
free CO_2	1.3	3.0	nil
Carbonate)	12	16	9
Total) Hardness	23	29	18
Calcium) (as $CaCO_3$)	16	20	10
Fe	0.17	0.46	< 0.04
Cu	0.005	-	-
Zn	0.02	-	-

1.28 Fish were restrained in the 1000ℓ tank by a hinged wooden lid, opened for feeding. (The translucent tank sides admitted light from outside). The circular tanks were fitted for HC1 with flat covers of 6 mm square mesh green Netlon (see Chapter 3) bolted to the flange round the tank lip. Food could be dispensed through the mesh, or a small hole cut in it would allow demand feeder operation. For HC2 restraint was as described in Chapter 3.

1.29 Photoperiod, or day-length, was natural for HC1. Since the tanks were outside, daylight effect would last for a time varying from about 8h minimum during December to about 16 maximum during June, with gradual daily shifts between these points. Combined with seasonal temperature changes, this fluctuation would allow any seasonally-controlled metabolic effects in the fish to vary fully, and would, further, cause disjunction on the removal of fish to the indoor experimental system with its controlled photoperiod. In the improved HC2, fish were held indoors under a controlled 12h photoperiod with artificial lights. Since all main experiments were performed under this regime, no disjunction occurred. However, the HC2 photoperiod was displaced from a natural photoperiod; it began at 0800 and finished at 2000, whereas the natural 12h photoperiod would be from about 0600 to 1800. Thus the displacement was by 2h in a late direction, with "mid-day" occuring at 1400.

1.30 The effect of the HC2 photoperiod on fish would be to suppress the daily "re-adjustment" which is postulated to occur in natural inherent biological clocks (Lofts 1970). However seasonal information would still be partly available by means of the water temperature. Under these conditions, photoperiod was removed as a possible source of variability, and seasonal effect was minimised.

1.31 Feeding in holding tanks was conducted according to Table 1.3. This is a chart for use with CNP feeds, adapted from Deuel et al (1952), and is based on increases in % of body weight fed

Table 1.3 Feeding chart for CNP Beta Salmon and Trout feeds

FCAT	1	2	3	4	5	6	7	8	9	10
WEIGHT (g)	<1.5	1.5-5.1	5.1-12.0	12.0-23.0	23.0-39.1	39.1-61.7	61.7-91.7	91.7-130.6	130.6-179.2	179.2+
LENGTH (cm)	2.54-5.08	5.08-7.62	7.62-10.16	10.16-12.70	12.70-15.24	15.24-17.78	17.78-20.32	20.32-22.86	22.86-25.40	25.40+
TEMP (°C)										
3	2.4	2.0	1.7	1.3	1.2	0.95	0.8	0.7	0.65	0.6
4	2.5	2.0	1.7	1.4	1.2	1.0	0.8	0.75	0.7	0.6
5	2.7	2.2	1.8	1.5	1.3	1.1	0.9	0.8	0.75	0.65
6	3.0	2.4	2.0	1.6	1.4	1.2	1.0	0.9	0.8	0.7
7	3.3	2.6	2.2	1.7	1.5	1.3	1.1	1.0	0.9	0.8
8	3.5	2.8	2.3	1.8	1.6	1.4	1.2	1.1	1.0	0.9
9	3.9	3.1	2.6	2.0	1.8	1.5	1.3	1.2	1.1	1.0
10	4.2	3.3	2.7	2.1	1.9	1.6	1.4	1.3	1.2	1.1
11	4.6	3.6	3.0	2.3	2.0	1.8	1.5	1.4	1.3	1.2
12	4.9	3.9	3.1	2.4	2.2	1.9	1.6	1.5	1.4	1.3
13	5.3	4.2	3.4	2.6	2.4	2.1	1.8	1.6	1.5	1.4
14	5.5	4.5	3.6	2.8	2.5	2.2	1.9	1.7	1.6	1.5
15	6.0	5.0	3.9	3.1	2.7	2.4	2.1	1.9	1.8	1.7
16	6.5	5.3	4.2	3.4	2.9	2.5	2.2	2.0	1.9	1.8
17	5.5	4.5	3.6	2.9	2.5	2.2	1.9	1.7	1.6	1.5
18	4.5	3.8	3.1	2.3	2.1	1.8	1.5	1.4	1.3	1.2

Notes:-

a) All figures in FCAT columns in the lower part of the table are % BWD (% of body weight fed per day).

b) During measurement, length readings were taken to the nearest o.5 cm, thus FCAT 1 embraced 3.0, 3.5, 4.0, 4.5, 5.0 cm, FCAT 2: 5.5, 6.0, 6.5, 7.0, 7.5 cm, etc.

per day (%BWD) with temperature to an optimum (16°C) and thereafter a fall, for any single size-category of fish (FCAT). With increase in size, %BWD at any given temperature falls. Thus the highest %BWD is fed to the smallest fish at the optimum temperature. It should be noted that the commercial chart prepared by CNP recognises a smaller size-category (not encountered in this study) and a separate brood fish category (also not encountered).

1.32 CNP also recommends which sizes of foods (Beta Salmon and Trout diets) should be fed to different sizes of fish. In practice the best test was acceptability to the fish, and the No.4 (floating) Trout diet was generally acceptable to fish in all categories from FCAT 3 upwards. All main experiments utilised this diet. Under normal circumstances, trout will feed both at the surface and off the bottom, so the sinking diets used for small fry (Beta Salmon No.2 and No.3) were not a drawback. Major characteristics of the diets (as published by CNP) are listed below:-

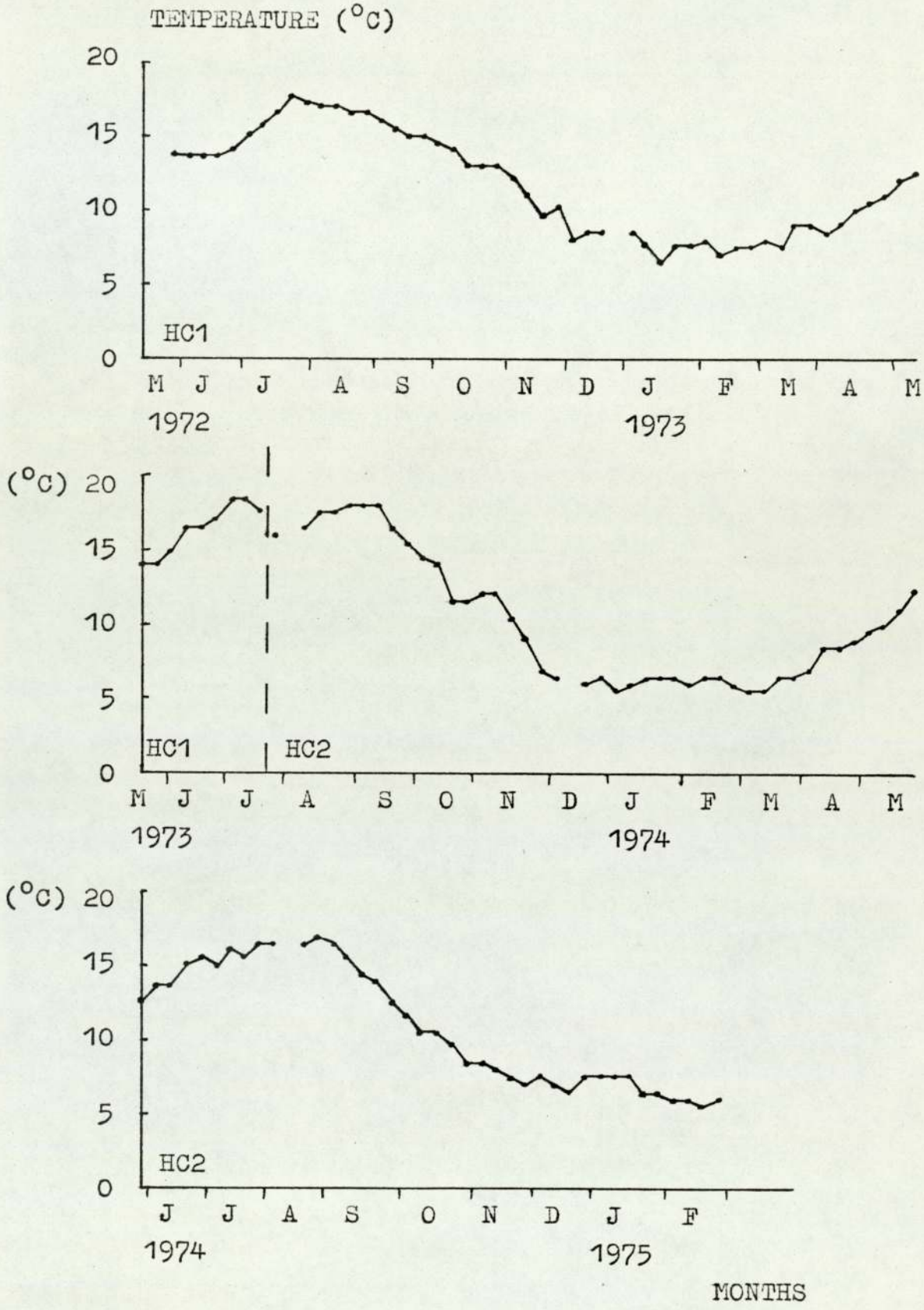
a) Salmon No.2 and No.3: sinking granules, 7.5% oil, 58% protein, 1.5% fibre, 2000 iu kg^{-1} vitamin A, 2000 iu kg^{-1} vitamin D, 36 iu kg^{-1} vitamin E.

b) Trout No.4: floating pellets, 4.5% oil, 40% protein, 4.5% fibre, 1000 iu kg^{-1} vitamin A, 2000 iu kg^{-1} vitamin D, 30 iu kg^{-1} vitamin E.

Trout No.4 pellets are cylindrical, approximately 3 mm long by about 2 mm diameter.

1.33 Since %BWD is controlled by temperature and size, records of temperature (and periodic re-grading of fish) were essential in determining feed rates. Average weekly water temperature was recorded throughout all work, and is shown in Graph 1.1.

1.34 Four batches of fish were used during the study, each designated by a Lot number (01,02,03,04). Table 1.4 gives details of utilisation of each Lot; all fish were supplied by Vortex (Donnington) Ltd. trout farm.



Graph 1.1 Temperature records

Table 1.4 Details of fish Lots and utilisation

DATE (wb)	LOT	No.of FISH	FCAT	HC		EXPERIMENTS USED FOR
22/5/72	01	480	1	1	until wb 16/7/73	P01,P02,P03
2/4/73	02	126	5	1		
16/7/73				2	Lots 01 & 02 pooled	BMP
3/12/73					Lots 01 & 02 written off	
10/12/73	03	1350	1	2		E00,E01,E02 E03,E04,P04, E05
26/8/74	04	1500	2	2		
11/11/74				2	Lots 03 & 04 pooled	E06,E07,E08, E09,T01,T02
24/2/75					Lots 03 & 04 written off	

Notes:-

a) wb indicates "week beginning".

b) Codes quoted in the final column (P01,etc.) refer to individual experiments, and are explained in Chapters 6,7 and 9.

c) "written off" indicated that fish were becoming too large for laboratory holding, or were otherwise finished with, and were passed on to colleagues for use in other work.

1.35 Fish handling was by different-sized hand nets of fine nylon mesh which were kept clean and wetted before contact with fish. Similarly, for manual handling of fish, wetted rubber gloves were worn: these precautions avoided damage to fish epidermis.

PART 2

DEVELOPMENT OF

EXPERIMENTAL SYSTEMS

2. DEVELOPMENT OF PRELIMINARY SYSTEMS

INTRODUCTION

2.1 An integral part of the work described in this account was the planning and construction of experimental facilities. At the commencement of the studentship (October 1971), facilities available comprised one 1000 ℓ rectangular fibreglass holding tank in an outdoor location supplied by a (shared) water line with maximum delivery about 16.5 ℓ min^{-1}; and half-share in an indoor area measuring 3.3 m x 2 m. Thus available building space was about 3.3 m x 1 m x 3 m height.

2.2 Before acquisition of a suitable laboratory in June 1973, an exploratory pilot system was built to cater for three small-scale pilot experiments, in order to provide background experience for the subsequent larger-scale facility.

2.3 In October 1971, a colleague, Philip Smith, also commenced work on applied fish culture research, and throughout the planning and building periods there was much common discussion, simultaneous usage of fish batches and mutual assistance, especially in designing the laboratory and common facilities required by both. However, all experimental work and decisions of experimental policy regarding this study, were carried out by this author alone, after due consultation with the research supervisor.

2.4 Planning, acquisition of equipment and building accounted for at least one-third of the time spent on the project work, with frequent uncontrollable delays in delivery of equipment and also the problems consequent upon the industrial 3-day week of early 1974.

PILOT SYSTEM

Basic plan

2.5 The pilot system took a proportionally longer period of time to construct than the later larger-scale system, due to

its necessarily evolutionary nature.

2.6 Requirements were for:-

a) controlled water supply to each of 8 small tanks,
b) controlled overflow-levelled drainage from each tank,
c) imposition of a controlled photoperiod,
d) isolation from as many uncontrolled external stimuli as possible,
e) ease of access to tank water for sampling,
f) containment of fish within tanks,
g) some ability to control, or at least modify, temperature variation.

2.7 The tanks were positioned in a row inside a rectangular trough which formed a water bath, and a water line from the outside supplied each tank from the mains. The central tank exits were connected through the trough bottom to U-tubes whose longer sides emerged outside the trough, where the U-tube overflows governed the tank water levels. (Fig.2.1) The whole system, except for U-tube overflows, was inside a light-proof enclosure whose roof housed the lighting system. Overflow drainage was to a pipe and drain-cup. A separate water circuit maintained the water bath at a controlled temperature: temperature exchange was by means of a Pyrex heat exchanger.

Tanks

2.8 Two small tank (ST) types were used in pilot experiments. ST Type 1 was a modified polythene bucket (Plysu brand) 25.5 cm high and of diameter tapering downwards from 28 cm to 20 cm. The tank was 10ℓ in capacity, coloured blue, with handle removed and bottom modified; the trough accommodated eight tanks, but only four were used at any one time. ST Type 2 was a modified polythene circular frozen-food contained (Ekco-ware brand), of 12ℓ total capacity, 15 cm high and 33 cm in diameter. It was translucent-white, and the tank rim was well below the rim of the trough when in position, making careful control of water-

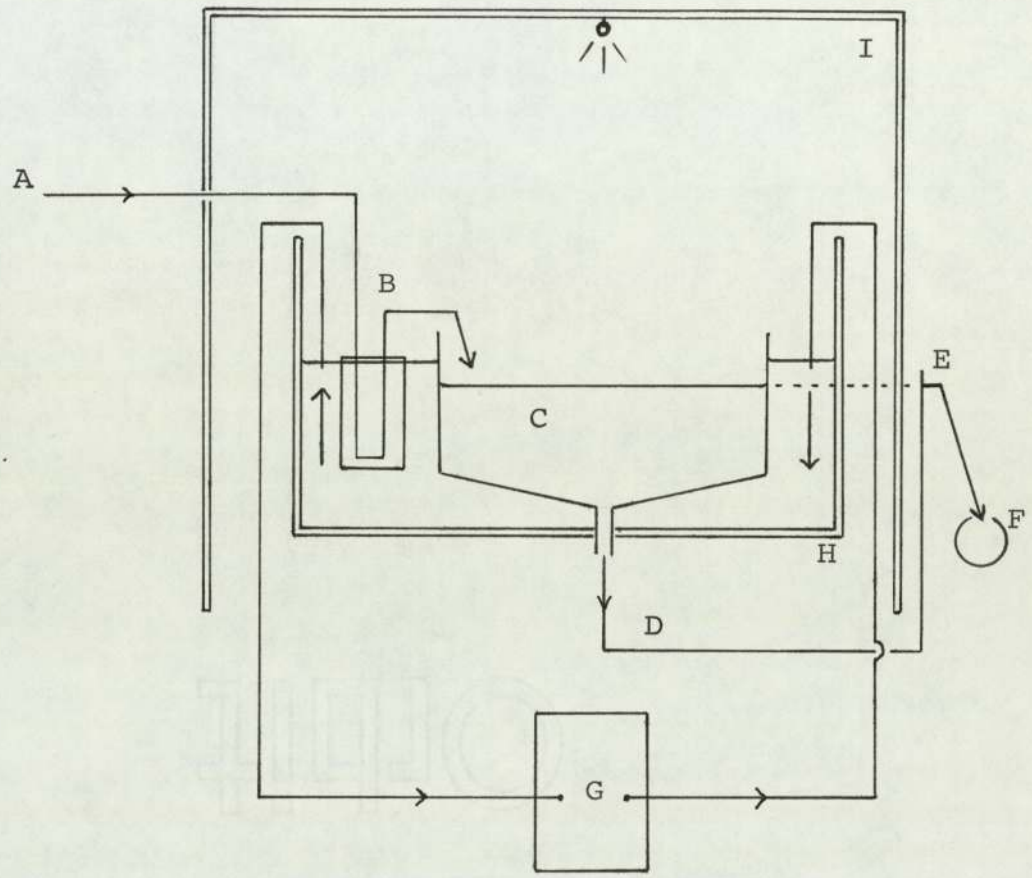


Fig. 2.1 Pilot system basic plan : cross-section

A = mains supply
B = heat exchanger
C = tank
D = U-tube
E = overflow controlling tank level
F = drainpipe
G = Temperature control unit
H = trough water bath
I = light-proof enclosure

bath level obligatory. Four suitably modified ST Type 2 could be accommodated. ST Type 1 were used for the first pilot experiment, but their lack of stability, restricted swimming room for fish, and poor hydraulic pattern (incomplete circular flow due to greater height than diameter), suggested that ST Type 2 were more suitable.

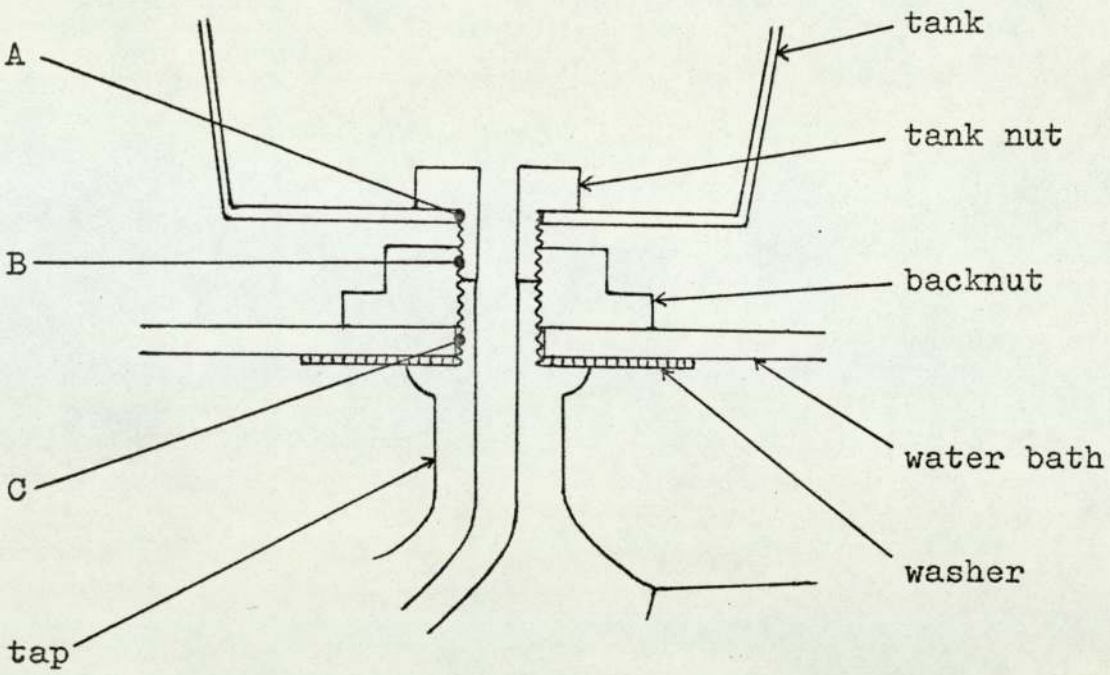
2.9 Each tank was fixed in position by a central tank sealing joint with a hole in the water-bath floor. Water left the tank through this joint, which also connected with the effluent U-tube. Since tanks were intended to be removable and interchangeable, the joint was necessarily complex, and Fig.2.2 shows both the first type (for use with ST Type 1) and the later, strengthened type used for ST Type 2. The early type allowed the tank to be unscrewed directly from a mounting, but potential leakage at points A, B and C suggested that a joint in which tank and trough-floor were clamped together was superior.

2.10 Fish were confined in the tanks by means of transparent hoods (large plastic bags) placed over the tank tops and secured (by waterproof adhesive tape) around the rims. For feeding and general inspection, tank access was via a hole in the top of the hood, otherwise the hood was tied off below the hole. Periodic checks were made of the surrounding water bath to ensure that no fish escaped. Figure 2.3 shows an ST Type 2 in position with hood furled back.

Arrangement of system

2.11 Fig.2.4 shows the system layout for pilot experiments; in this case P01, using ST Type 1.

2.12 Tanks were positioned, suitably spaced, inside the water bath so that their central drain points were in the mid-line of the trough's long axis. The fibreglass trough was internally 2.44 m long x 18 cm wide x 9 cm high, and was internally lined



a) EARLY TYPE (for ST Type 1) ↑

(not to scale)

b) LATER TYPE (for ST Type 2) ↓

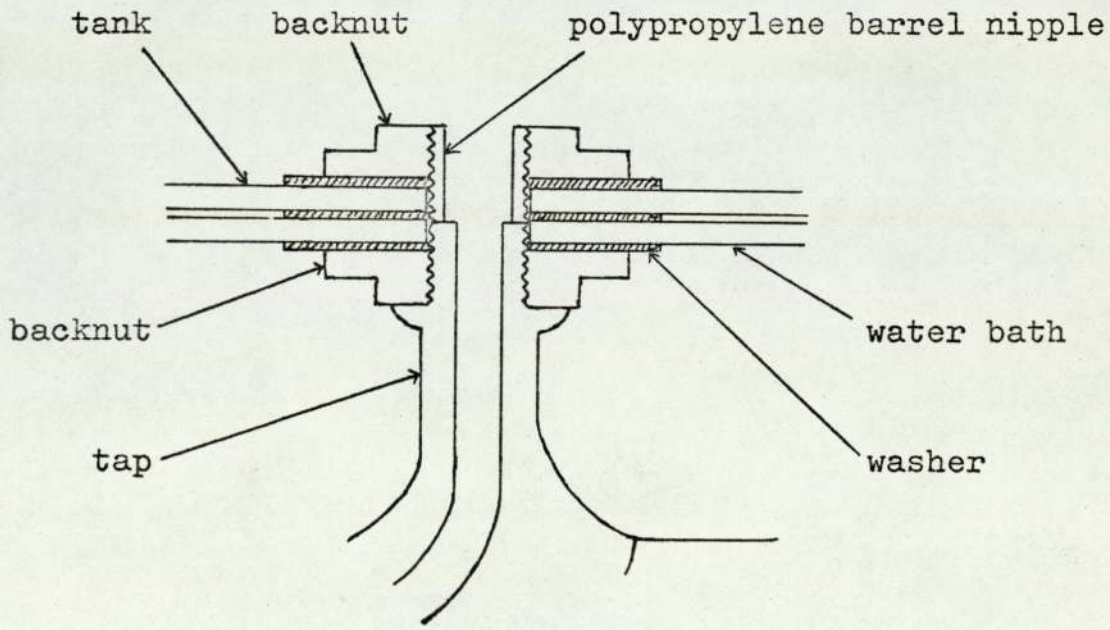


Figure 2.2 Pilot system tank sealing joints

Figure 2.3 (p.15)

ST Type 2 during pilot experiments

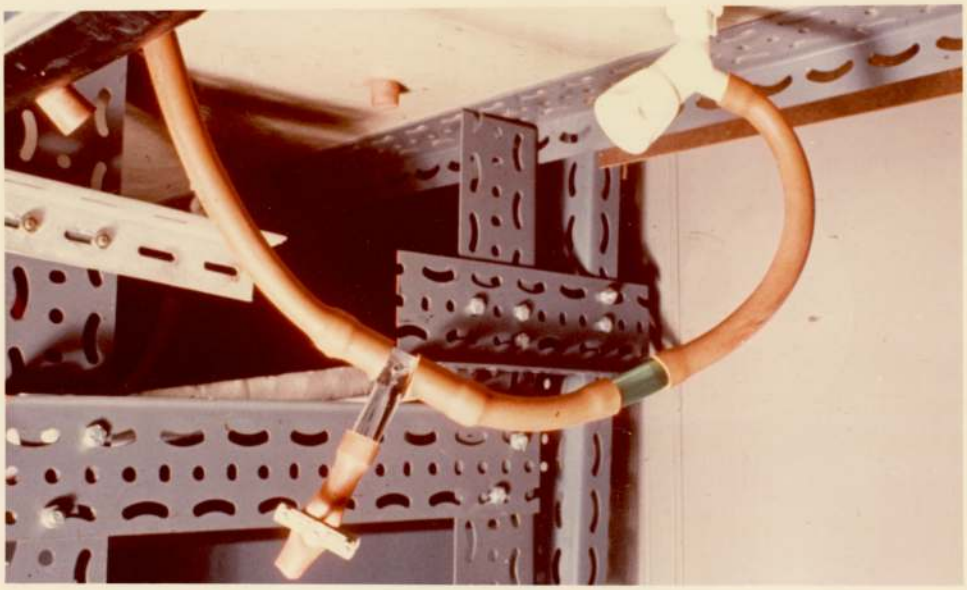
Figure 2.5 (p.16)

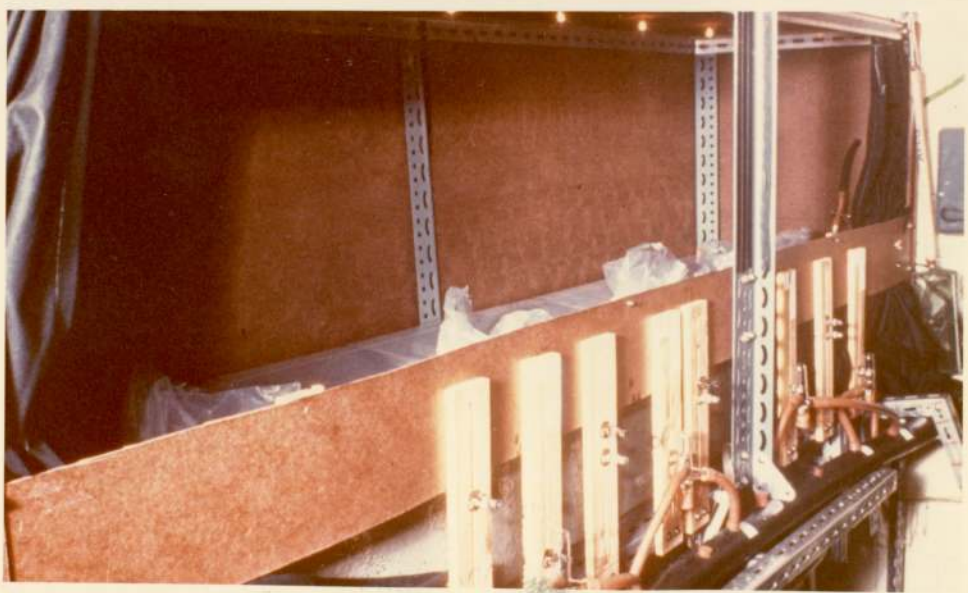
STS lower plumbing (pilot experiments)

Figure 2.7 (p.16)

Pilot system, front view

(overflow levelling controls at front)





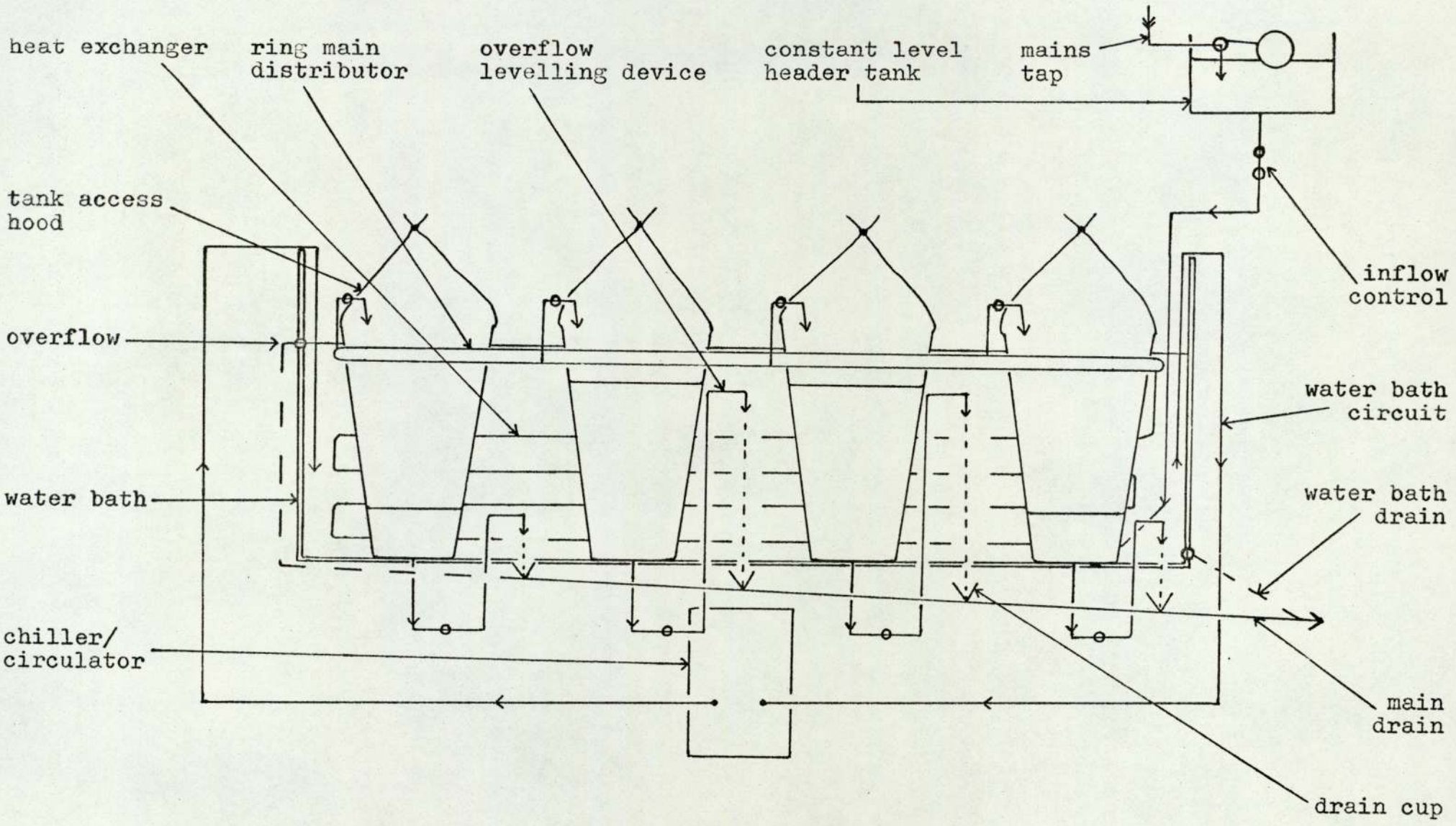


Figure 2.4 P01 apparatus layout

with PVC; it had two apertures at opposite ends, at suitable heights for overflow and drainage respectively (Fig.2.4).

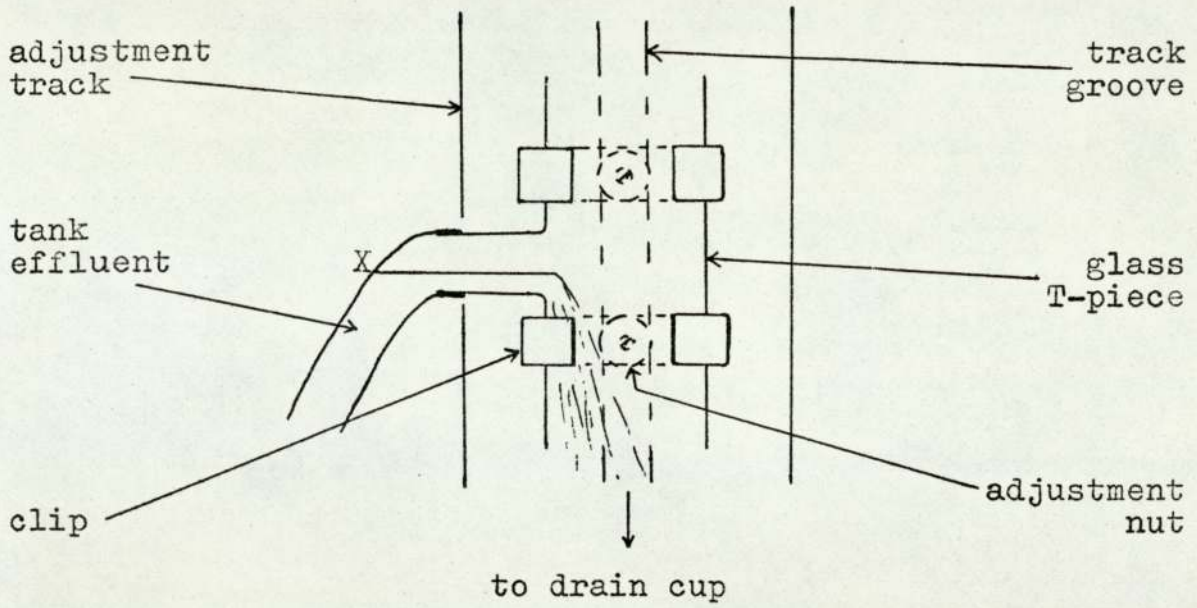
Plumbing

2.13 Mains supply from a 9ℓ ballcock-controlled header tank, placed on an angle-iron scaffolding, had a water-head of about 1.6 m above the tank inlets. Water passed via the heat exchanger (submerged in the trough) and ringmain distributor into the experimental tanks, with a branch tube for inlet water sampling just before the heat exchanger. The heat exchanger was constructed of 8 x 1.5 m lengths of Pyrex tubing, in two manifolds of 4, placed along each side of the trough, interconnected by green plastic tubing, and held in place by short lengths of PVC piping drilled through and stood on end (Fig.2.3).

2.14 Individual tank inlets left the ring main by glass T-pieces, and plastic tubing directed the influent water over each tank side to provide angled drive for the tank circular flow (Fig.2.3). The tubing was attached to the tank side by means of rigid plastic tubing.

2.15 Each tank effluent passed outside the water bath, round the U-tube upwards to a levelling outlet for volume control and then passed into a large main drain and so to waste. Fig.2.5 shows the outlet from below, with nylon control tap (open during experiment) and U-tube bleed-off (for removing any accumulated debris). Fig.2.6 shows the overflow levelling control in its two design stages, and Fig.2.7 shows the levelling controls in front of the full system. Tank water-level control was accomplished by sliding the glass tree up or down the groove in a fixed wooden track, and locking its position by means of two tubing-clips with wing nuts. The tree top aperture allowed a thermometer or pH electrode to be inserted to measure water parameters.

2.16 Pipe dimensions were as follows:-



a) EARLY DESIGN

(not to scale)

Adjustment nuts allow T-piece or tree to move in vertical plane along groove in track.

Height of side-arm X controls water level in tank.

b) LATER DESIGN

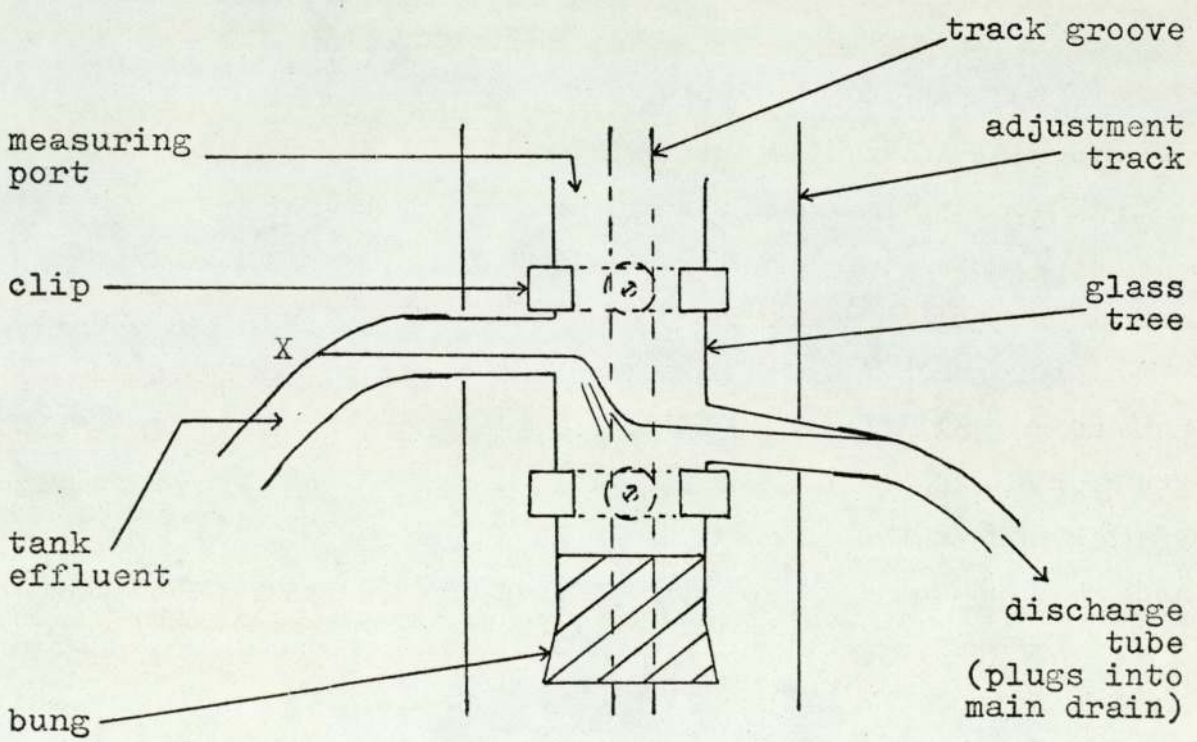


Figure 2.6 Overflow levelling device

a) 13 mm: main inflow,
heat exchanger

ring main distributor

individual tank outlets to overflow levelling controls

b) 6.5 mm: individual tank inlets

c) 9 cm: main drain pipe

2.17 Flow control along flexible tubing was by means of Hoffman clips, except as in Fig.2.5 below the tank.

Illumination control

2.18 As Fig.2.7 shows, the tanks and trough were enclosed inside a light-proof hardboard box (with angle-iron framework) and rested on an angle-iron trestle. The front portion of the box was closed off by means of black cloth curtains during experiment; these allowed easy access when required.

2.19 Set in the roof of the box was an array of 8 light bulbs (Fig.2.7), each of 6W @ 12V AC, positioned one above each tank in MES screw fittings. Control of the lights to the desired photoperiod was automatic, by means of a simpler version of the system described in Chapter 3.

Temperature control

2.20 In the 80 cm high space below the system, which provided for access to U-tubes and tank sealing joints, a Churchill chiller/heater circulator was installed. This received water from the water bath by means of 13 mm tubing, imposed temperature control, and pumped water back to the water bath via a further length of tubing. A thermometer built into the chiller allowed temperature monitoring. In practice the heat exchanger was found to be limited in value. Tank water temperature was governed largely by the inflow temperature, since this was an open system, and the water bath was only sufficient to buffer minor fluctuations.

Aeration

2.21 Dissolved oxygen was kept non-critical, and any chlorine in the inflow was removed, by vigorous aeration in the header tank using 2 laboratory air-pumps with air-stones, (one during experimental dark periods).

Pilot equipment sources

2.22 The following list details the manufacturers of important apparatus.

ST type 1 (buckets)	- Plysu Products Ltd., Bletchley
ST type 2 (containers)	- Ekco Plastics Ltd. Southend-on-Sea
Fibreglass/PVC trough (to specification)	- Cago Ltd., Birmingham
Chiller thermo-circulator	- Churchill Instrument Co., Perivale
Pyrex tubing	- James A.Jobling Ltd. Laboratory Division, Stone, Staffs.
6W light bulbs	- Vitality Ltd., Bury St. Edmunds
Glass overflow-levelling control trees (to specification)	- J.A.R. & M.K.Hill, Walsall

INTERIM PLANS

2.23 During construction and operation of the pilot system, plans were laid for a larger-scale laboratory. Six circular tanks of diameter 91 cm and depth 46 cm were designed, and built to order by a local firm. They were of translucent white polypropylene, each with a lip-flange round the top and mounted on three tubular PVC legs with the central drain-hole 30 cm off the ground. Each tank floor was slightly sloped for self-cleaning drainage, and the drain-hole was of 2 cm diameter with a short length of PVC piping sealed into the hole. The tank held about 250ℓ and was designated LT (large tank).

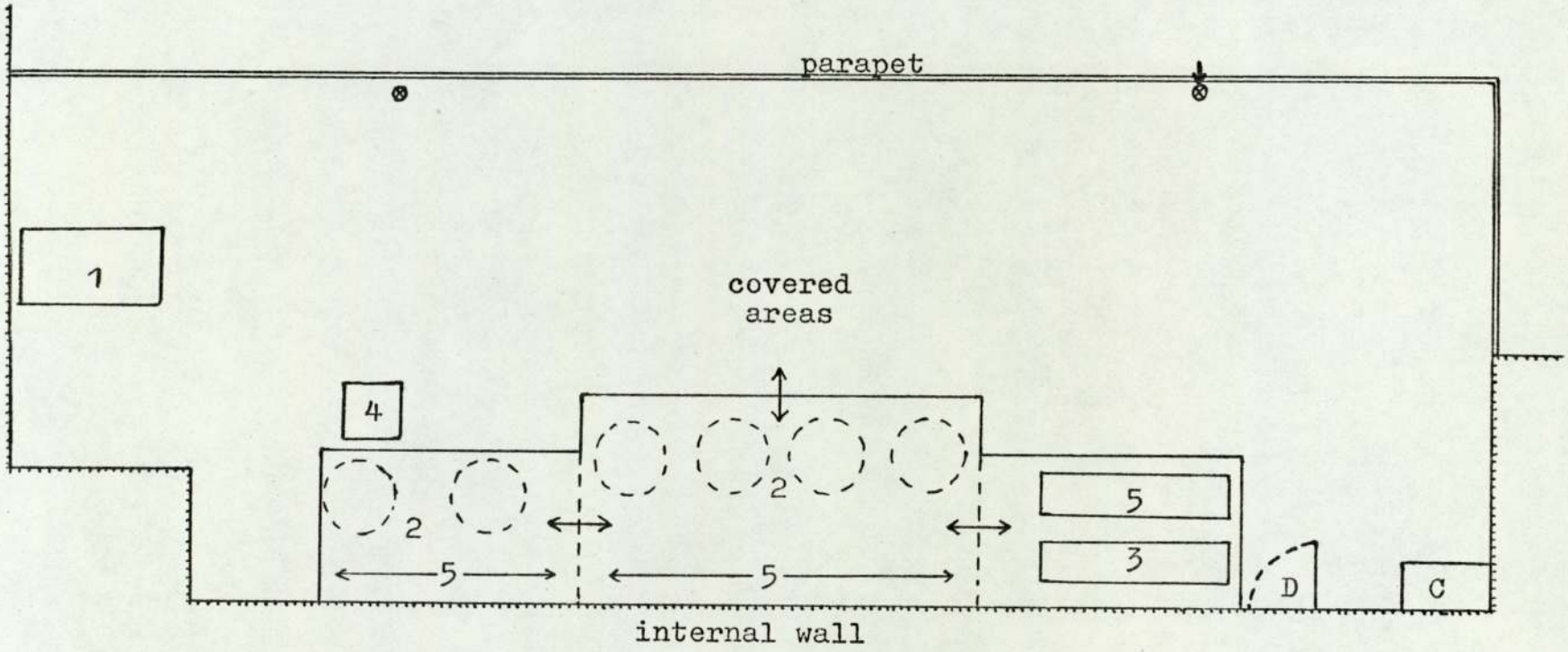
2.24 The first plan for a larger laboratory was based on conversion of a roof-area (Figure 2.8). The plan was disallowed by University authorities on structural grounds.

2.25 Following this planning session, the merits of a fully indoor working area and an independent water supply were becoming apparent, and attention for the next plan was focussed on a similar area in the basement of the main University building, following the example of the MAFF (Ministry of Agriculture, Fisheries and Food) Salmon and Freshwater Fish Laboratory in London. After consideration by the University, this plan also had to be abandoned, and it became clear that a site outside the main University building would stand most chance of success.

2.26 Eventually a promising site was found at the rear of a fully walled-in outdoor area. Plans envisaged either a prefabricated construction or the setting up of ready-made huts on the site (Fig.2.9). A firm commitment by those responsible had not been made when another nearby site was discovered which was superior to all those so far examined.

2.27 The last site, which eventually was turned into an experimental fish facility, was a two-storey shed-type building behind a laboratory complex, with a concreted base floor, two brick walls and two corrugated-sheeting walls, and a wooden-beam and floor-boarded first storey with a sloping roof. Windows were present in both storeys. When first encountered the building was in some disrepair (Fig.2.10).

2.28 A new series of plans was formulated, and after approval and confirmation, the initial technical drawing for basic conversion work was prepared by the University Estates division in December 1972.



1 = 1000 l rectangular tank 2 = 250 l circular tank

3 = STS (trough) 4 = 350 l square tub

5 = other research tanks (colleague)

C = compressor housing (air supply)

D = access door to interior

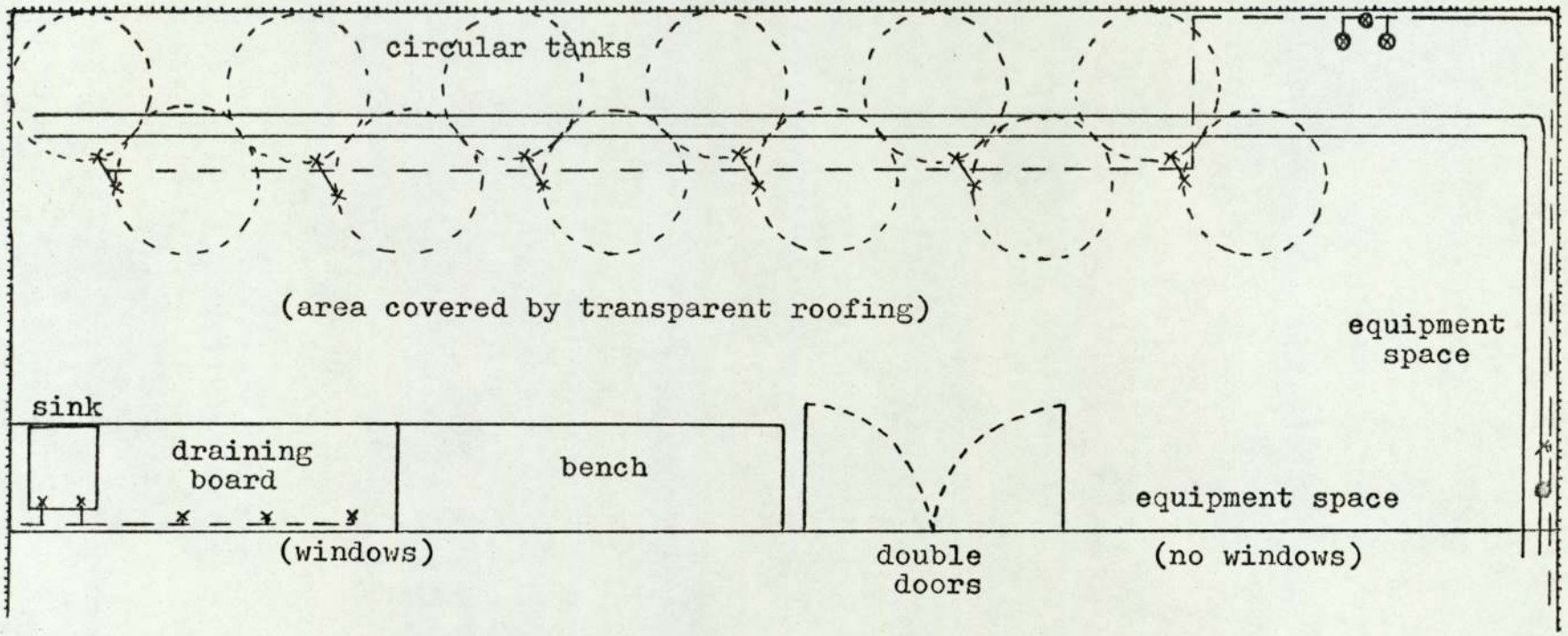
⊗ = drain

↕ = access to covered areas

↓ = water supply tap (supplying other fish holding as well)

Scale: ¼ inch rep. 2 feet

Figure 2.8 Plan for roof-area conversion



All facilities shared with colleague.

x = tap ⊗ = stopcock

= gutter — — — = water line

Scale: $\frac{1}{4}$ inch rep. 1 foot

Figure 2.9 Plan for outdoor area conversion

2.29 The necessary conversion work, including removal of old fittings, repair of walls and ceiling, installation of systems and benches as in Appendix B, was carried out by contractors during early 1973. A steel walkway was included, allowing access to the upper storey from an adjacent fire-escape; the trapdoor connecting the two storeys was supplemented by a vertical steel ladder; and the lower storey windows were boarded over to make it light-proof.

2.30 Water was supplied by a branch off the University main supply, and was made to empty via a large ballcock into a 1000 ℓ fibreglass rectangular tank mounted on wooden trestles in the upper storey of the building, at a point where the wooden floor of the prefabricated part of the building gave way to a concrete floor (part of a rather larger building immediately adjacent). The stone-floored section was that detailed on the plan (Appendix B) as "header tank room". Contractors installed the header tank and supply, and finished its plumbing with four PVC 13 mm outlet pipes descending to the lower storey, terminating above head height with angle-seat valves. Water entrance to the header tank was by cascade from the valve, and the mains pressure during operation caused an effective jet-spray ensuring excellent aeration.

2.31 Conversion work was finished in June,1973 and by July it was possible to instal the 6 LT and to institute fish holding under HC2. The laboratory facility was shared with P.Smith, and for experimental purposes one half of the lower storey was allotted to each project. This was designated as a wet lab., with facilities for water drainage, while the upper storey accommodated bench work and desk space.

3. DEVELOPMENT OF FULL EXPERIMENTAL SYSTEMS

PLANNING

3.1 After laboratory conversion the following facilities were available:-

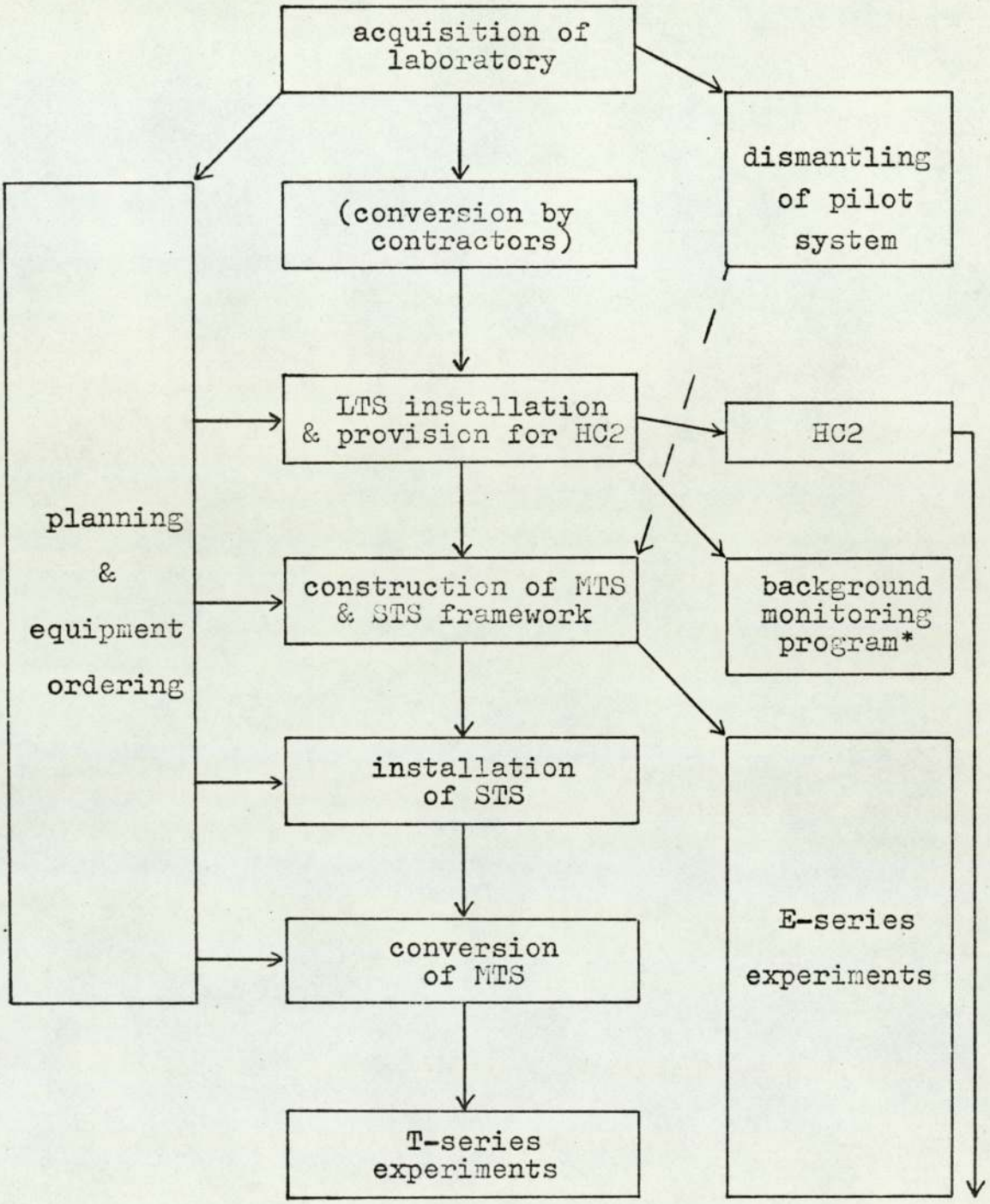
a) 2.5 cm diameter main water supply issuing by ballcock to a 1000 ℓ fibreglass constant-head reservoir tank with four separate 13 mm outlet pipes (with control valves), and suitable overflow, drain-tap and hinged lid attachments. Two of the outlets were available for this project work.

b) Building-space of 6.4 m long x 2.5 m height x 1.2 m depth. At one end the trapdoor ladder formed a boundary; at the other, a corner space was available. The floor had a drainage gulley, and there were twin electric points at 1.2 m above the floor at each end of the long wall; also, the end wall nearest the trap door had supplementary electric points. Illumination was by a single standard fluorescent light. Supplementary water supplies (hand taps) were provided, one at each end of the wet lab.

c) Half-share in the upper storey (dry) laboratory space including bench, cupboard, sink, water and electricity facilities.

3.2 Figure 3.1 show the activities scheme pursued during the project after laboratory acquisition. Development, construction and experimental work had to be organised for simultaneous operation.

3.3 Fig.3.2 shows the elevation view of the wet lab building space, and in this space 3 separate systems were accommodated, the small (STS), medium (MTS) and large tank systems (LTS). The STS and LTS used the tanks previously described, but for the MTS a number of fibreglass tanks were purchased. These were of 15 ℓ capacity, coloured light green, with diameter 49 cm and depth 10 cm; they were modified from the circular hatchery tray design used by Vortex Ltd., and in place of the standard central fittings had a well of 12.5 cm diameter and



*see Chapter 7

Figure 3.1 Chart of major developmental activities

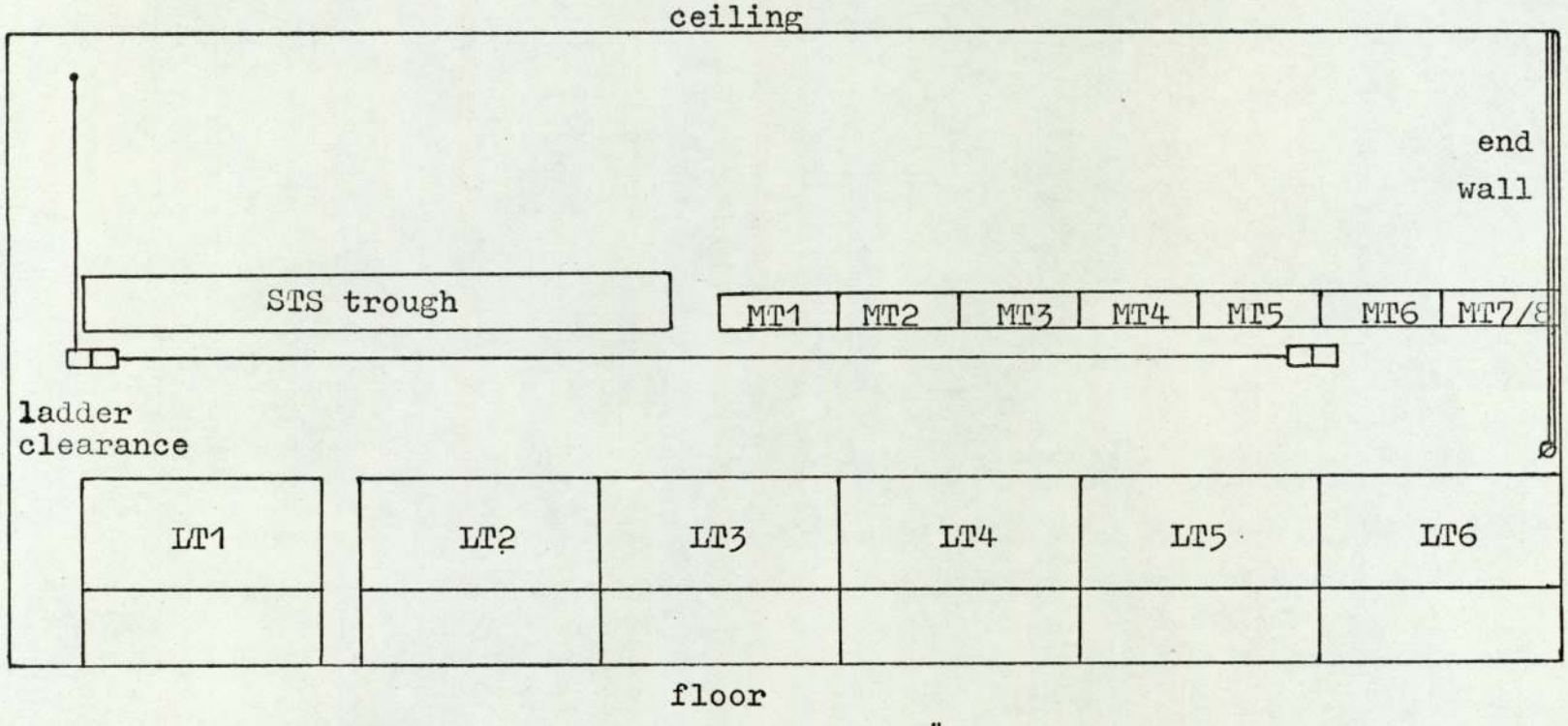


Figure 3.2 Elevation view of wet lab building space

2.5 cm depth, leading to a central exit hole of 2.5 cm diameter. A rim-ledge allowed a mesh to be placed across the well, and the upper rim of the tank was rolled to allow stacking and cover attachment.

3.4 Assembly work was performed in the following order:-

a) Main plumbing to all systems.

b) Angle-iron framework to support MTS and STS, and enclose LTS.

c) Installation of LTS (6 tanks) and LTS drainage.

d) MTS angle-iron superstructure installation and drainage; STS trough installation, superstructure and drainage.

e) Lighting systems and controls.

f) Feeding systems and controls.

g) LTS and MTS tank tops.

h) Blackout.

3.5 Items (a) to (c) were required before fish could be held in the LTS, and items (e) and (f) were required before HC2 could be instituted.

3.6 Systems were designed, profiting from pilot experiment, with several major objectives in mind:-

a) to allow simultaneous fish holding and experimentation with a dual-purpose LTS;

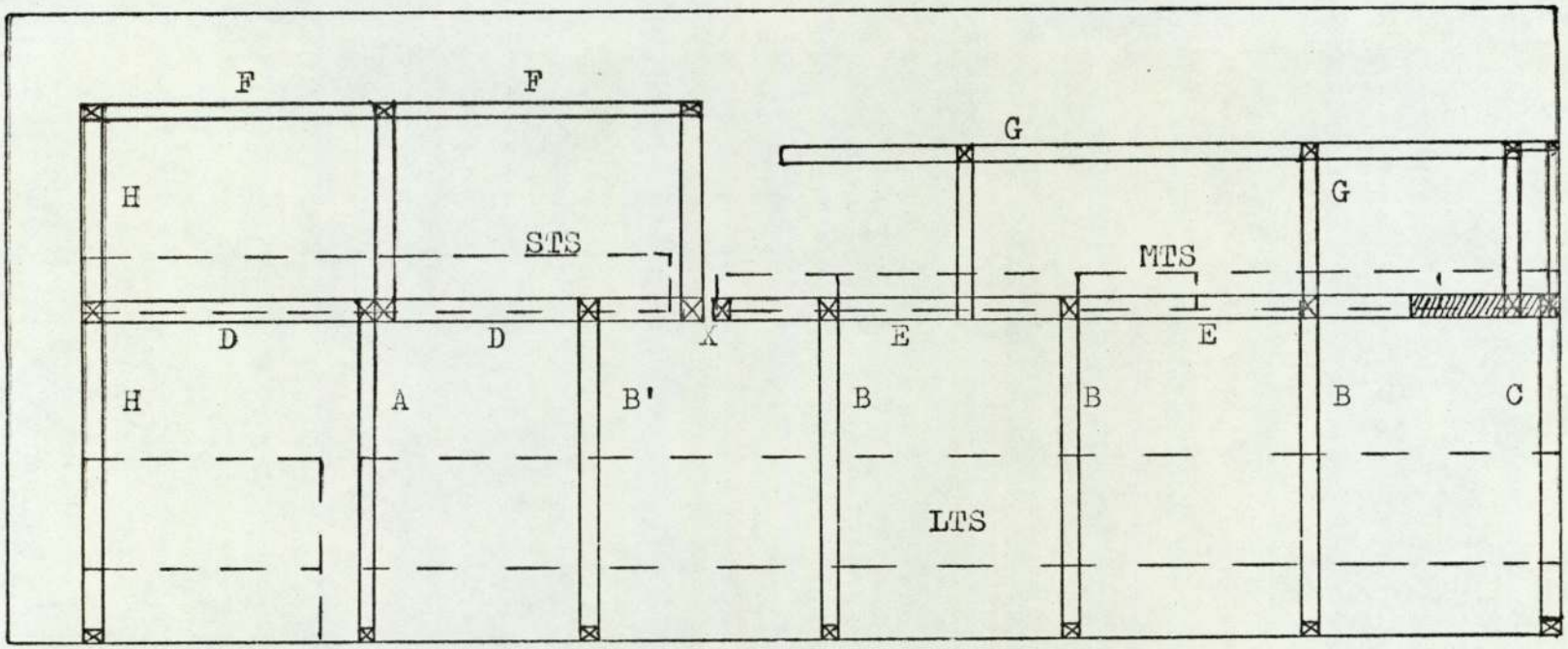
b) to provide an MTS for the bulk of the excretion/environment work (E-series experiments), comparable in arrangement with the LTS when used for the same work, but also readily adaptable for tolerance work;

c) to allow easy access to all systems, so far as was compatible with space limitations;

d) to provide an STS specifically geared to tolerance work (T-series experiments);

e) to provide ease of cleaning and removal of components;

f) to provide automated controlled lighting and feeding systems for each tank;



= angle iron frame = angled cross strut to support MTS

⊠ = cross strut A = arch of depth 2'6"

B = B' = arch of depth 2'3" C = arch of depth 3'6"

D = STS trestle E = MTS trestle

F = STS superstructure G = MTS superstructure

H = end frame; arch depth 2'9" X = break between sections

Figure 3.3 Elevation view of angle-iron framework

(Scale: 1 cm rep. 1 foot)

g) to exclude unwanted external stimuli, particularly visual. In view of considerations (c) and (e), open gutters were preferred to drain pipes; and in order to fulfil condition (g), blackout curtaining was preferred to box enclosures. Blackout was necessary since it would allow other work in the laboratory and use of external lights without fish disturbance.

3.7 A complete system for air supply to all tanks was originally planned. This would have required a large compressor, and despite preliminary negotiations, such a supply was not in practice authorised and purchased. All systems received excellent initial aeration from the header tank, and small laboratory air pumps were used in emergency, at the introduction of new fish Lots, during experimental anaesthesia and sorting, and for air supply in T-series experiments.

3.8 After the major assembly work and progress on the E-series of experiments, a later assembly stage was involved in preparation for T-series experiments, (see Chapter 9). In their final configuration the three systems were in use for fish holding, E-series and T-series experiments simultaneously.

EQUIPMENT AND CONSTRUCTION

Framework

3.9 Surrounding the LTS, and supporting the STS and MTS, an angle-iron framework was built in two sections; Fig.3.3 shows all basic framework.

3.10 The STS section consisted of 3 arches (H,A,B') on which rested longitudinal trestle runners (D). Arch (H) was continued to form an end frame, and superstructure (F) was erected on top of the trestle to enclose the STS in box-like fashion. This was similar to the pilot system construction, and allowed similar arrangements to be made for curtains, overflow levelling and internal fixtures.

3.11 The MTS section abutted closely to the STS section (X) and also employed an arch-supported trestle (E; arches B,C). However, the MTS superstructure was formed of two arches surmounted by runners (G) forming girders for fixture attachment. At the wall end, the girders were continued at 90° to the general direction, and a larger arch (C), and supporting cross-strut and stanchions, allowed space for an extra MT. Thus seven MT were accommodated along the long wall and one against the short wall. A girder-type superstructure was used in preference to a box for ease of access to, and removal of, MT and tank services.

3.12 Both superstructures were curtained-off, as was the LTS beneath the trestles, by use of blackout attachments at the walls, ceiling, and suitable points on the framework.

3.13 Angle-iron was of the stove-enamelled Bartangle variety, and according to strength required and position, use was made of the three width-sizes, 38 mm, 64 mm and 89 mm. The framework was levelled and bolted together with suitable strengthening corner struts where necessary, to give rigid scaffolding.

3.14 Arches (H,A,B,C) stood out further from the long wall than the superstructures above. This allowed access and removal space for the LTS, support for drainage systems, and protection of superficial equipment.

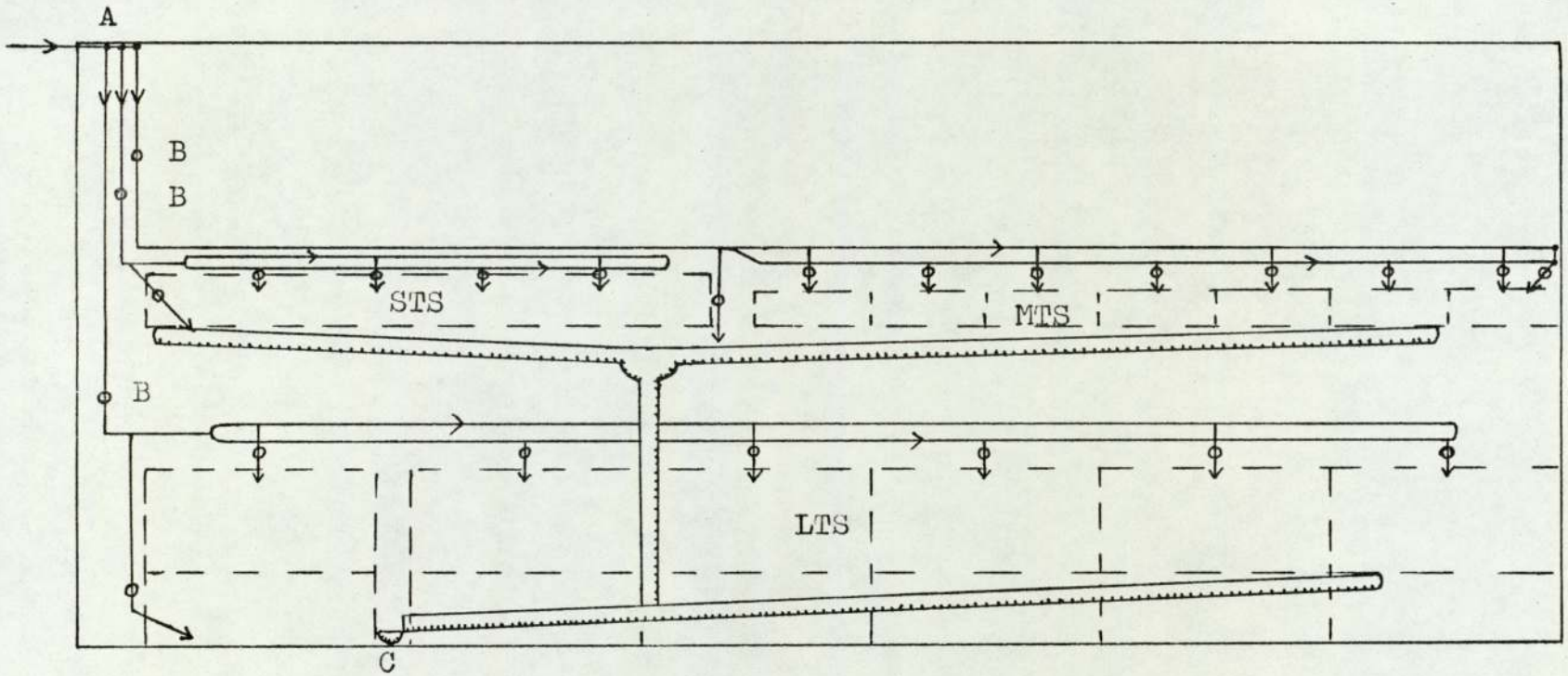
Plumbing and drainage

3.15 Water supplies for the MTS and LTS were taken from the two available outlet pipes from the header tank. Green transparent plastic tubing of 13 mm diameter was used to convey water supplies to arrive at the experimental system area at ceiling height. Water for the STS was brought from the header tank drainage tap (by similar tubing) through the ceiling next to the MTS and LTS supplies. Water-head above the STS/MTS was 180 cm.

3.16 Inlet plumbing and drainage are shown in Fig.3.4. Water supplies from point A were distributed by the green tubing with occasional short sections of rubber tubing (e.g. at the main shut-off controls). Each supply line had a main shut-off (large Hoffman clip), and subsequently split into a ring main to supply the tanks, allowing greater facility in individual inlet control. Just before each line split, it was provided with a clip-controlled side branch for inlet water samples. Supply lines were held in place by spring clips attached to wooden battens fixed on the wall. The STS supply rested on the front trough flange to allow ease of access to control clips.

3.17 15 cm above each MT or LT, the ring main branched by a glass T-piece to give a 6 mm clip-controlled inlet supply. This passed through a 90^{o} glass bend to enter the tank at an angle, thus providing circular tank drive. MTS inlets were suspended in position by cord from above; each LTS inlet was held in a groove through a small wooden block fixed onto the end of an angle-iron support arm (see Fig.3.14 later). Thus the inlet was held at a constant position. The connection between T-piece and inlet bend was made with flexible 6 mm tubing, which supported the control clip; inlet sample supplies were similarly furnished, with greater lengths of tubing to allow easy manipulation and stowage. The U and Y-tubes delimiting the ring main were 13 mm glass tubing. STS inlet supplies were similar to those of the pilot system.

3.18 Outflow from STS and MTS, and trough overflow and drainage, passed from the overflow level controls or trough outlet holes via short lengths of rubber tubing to the appropriate drainage gutters; these were mounted in the angles of the framework arches, and discharged into a central saddle fitment and down a 94 cm length of 6 cm diameter vertical PVC pipe. At the lower end, waste water entered the main LTS gutter (Fig.3.4)



ϕ = Hoffman clip ↓ = tank inlet or inlet sample tube ⎓ = drainage

A = arrival of water supplies from header tank C = cross-gutter to gulley

B = supply main shut-off control

(Scale: 1 cm rep. 1 foot)

Figure 3.4 Elevation diagram of inlet plumbing and drainage

and flowed into a cross-gutter discharging into a drainage gulley. These arrangements allowed the lab floor to be kept fairly dry when required. Guttering was of 10 cm diameter PVC.

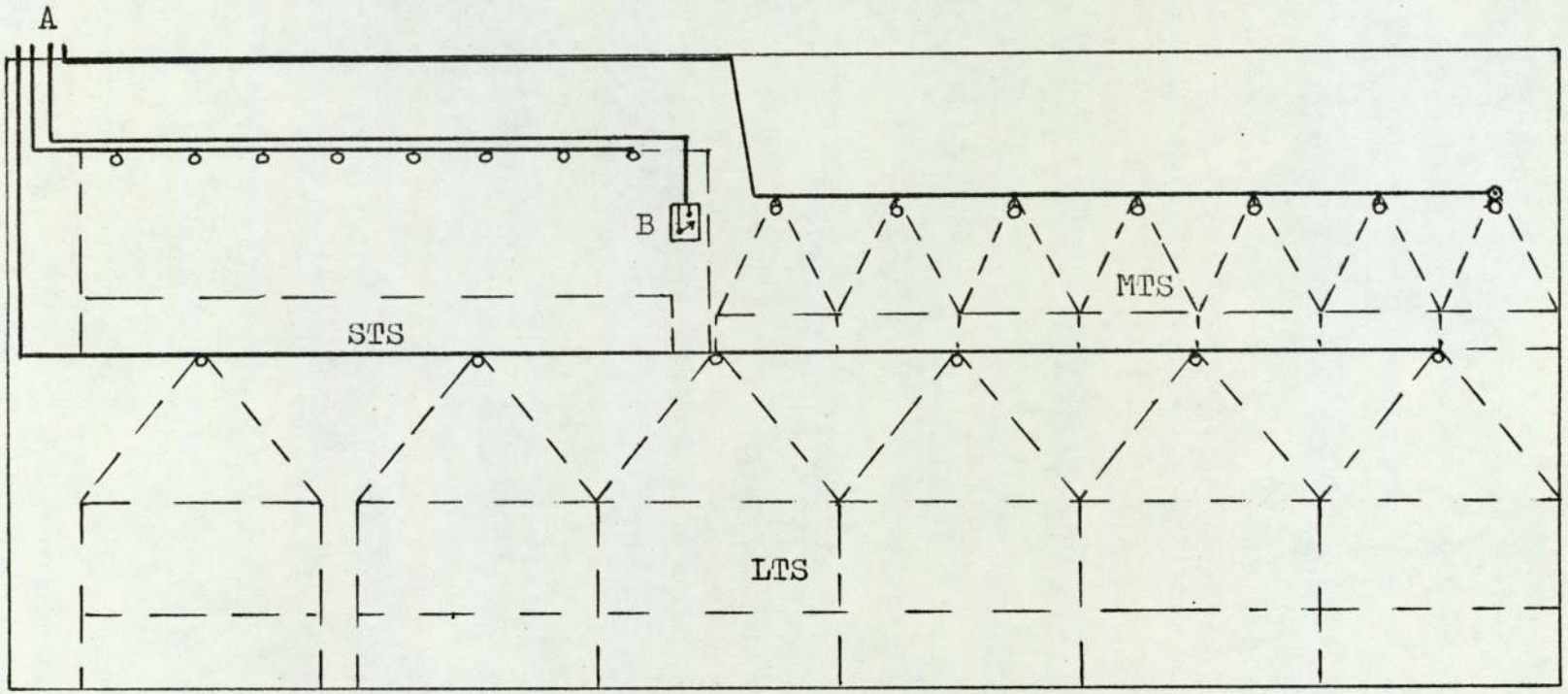
Electric fitments

3.19 All systems had electric lighting; one bulb per tank for MTS and LTS, and 8 bulbs in the STS system (Fig.3.5). There was also a remote override switch for the feeder system (see below) mounted on the side of the STS superstructure, and sealed into a wooden block.

3.20 Electricity control was performed by means of a master control box mounted for safety on the upper (dry) laboratory wall; cables passed through a special aperture in the wet lab. ceiling.

3.21 Since LTS lighting was below the MTS and STS water level, it was necessary to protect LTS lights from flooding. This was done by mounting, sealing and covering light fitments on hardboard plates positioned on angle-iron girders just below the STS and MTS trestles. An inverted length of guttering served as roof for each fitment (Fig.3.6). MTS lights were similarly mounted on the superstructure girders, and STS lights were set in a hardboard roof over the box superstructure.

3.22 Lighting control was accomplished as in Fig.3.7. The mains supply directly powered a 24h Venner timer, imposing a 12h-on, 12h-off photoperiod routine by means of control pointers. The regulated mains (240V) supply was then transformed to 24V for safety, and fed three supply circuits (independently switched) serving the tank systems. The bulbs were 12W pearl bus interior lamps, with SBC fittings and holders, mounted in parallel to prevent failure of one from extinguishing the rest. A master switch allowed the lighting system to be switched off without affecting the feeders (see below), if required.



= double cable

= 12W bulb

A = cables to control box

B = remote manual override (for feeders)

Figure 3.5 Elevation diagram of wet lab electric cables

(Scale: 1 cm rep. 1 foot)

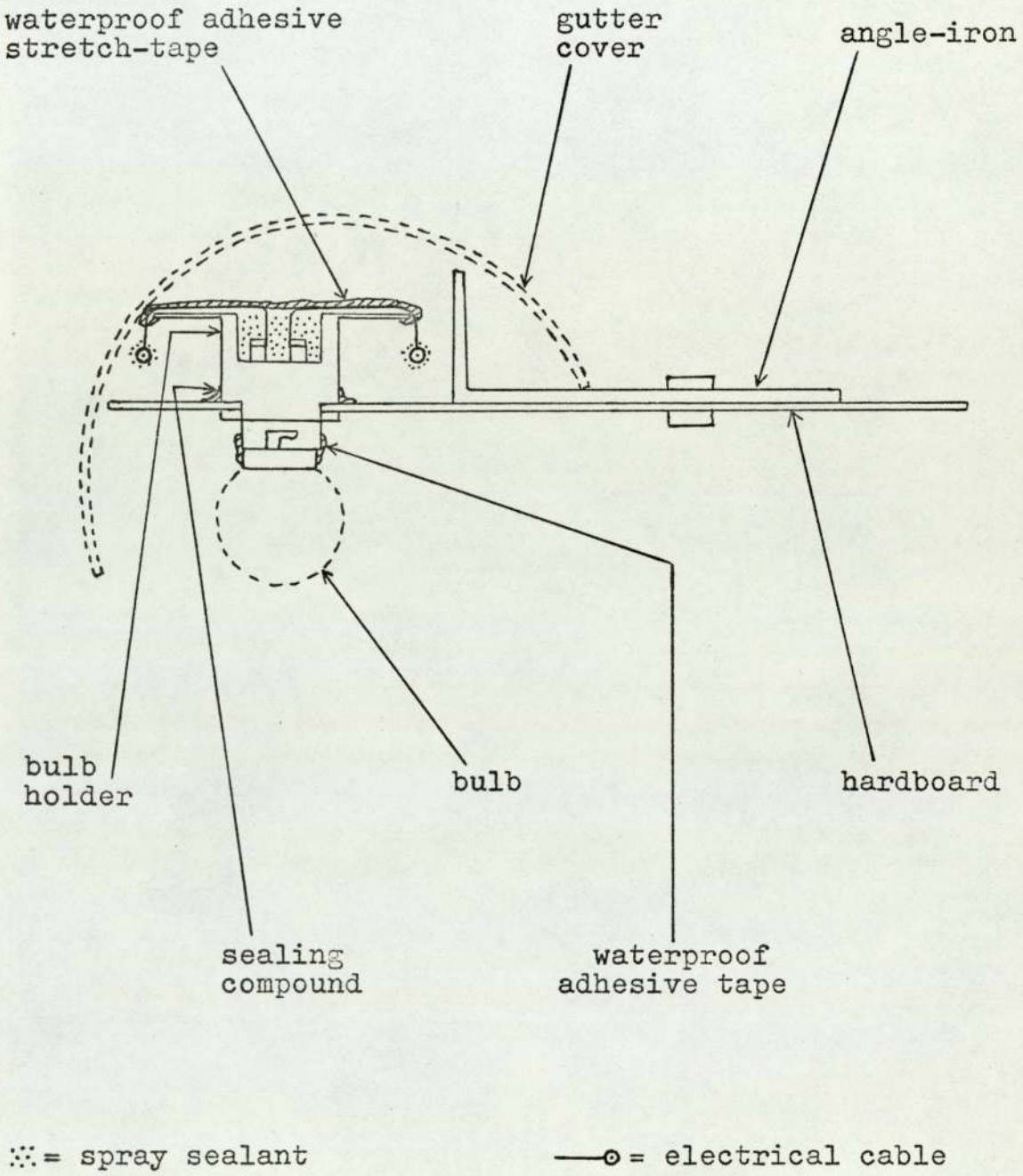
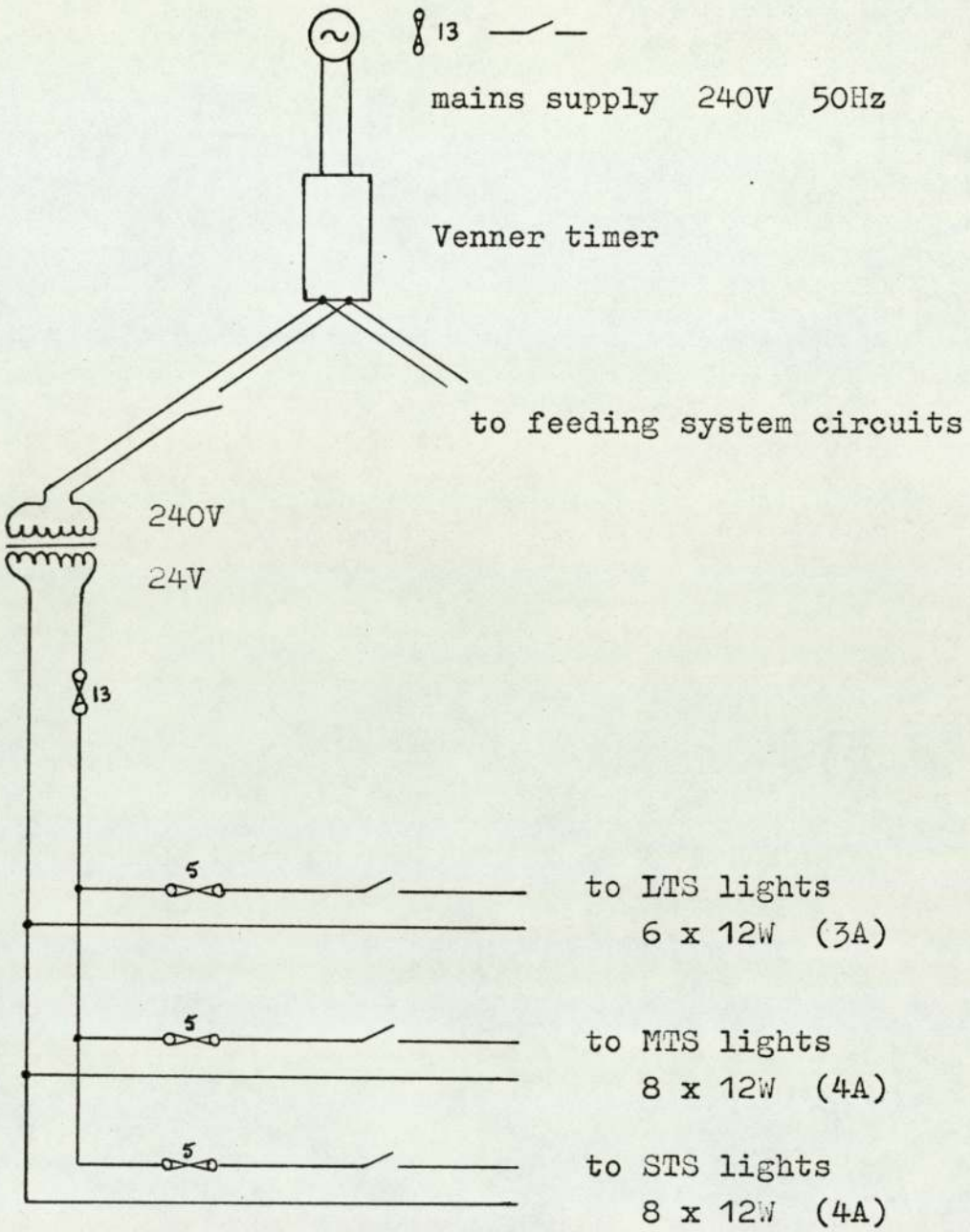


Figure 3.6 MTS & LTS: light-mounting and sealing



(number by fuse indicates current rating)

Figure 3.7 Diagram of lighting control circuit

3.23 Electrical components for the feeders were contained in the master control box. The feeder general supply also derived from the Venner timer controlling photoperiod but was independently switched (Fig.3.8). The 12h supply was then further regulated by No.1 process timer, delivering a 1.5 min pulse at the end of each 2h cycle. This pulse was the power for a feeding session. The photoperiod control switched on at 0800 and pulses occured at 1000, 1200, 1400, 1600 and 1800. The 2000 pulse was just prevented by careful setting of the Venner. The 1.5 min pulse powered No.2 process timer, delivering a 6s pulse at the end of each 1 min cycle. Thus for each 2-hourly feeding session a 6s pulse of power reached the feeder discharge mechanisms. Since No.2 timer only received a 1.5 min power supply, it was cut off during its second cycle. As the process timers were automatically reset to zero when switched off, this ensured a routine of 6s of firing every 2h. (Fig.3.9)

3.24 The 6s pulse fired a solenoid, which operated a release valve built into the feeder system (see below), and caused food to be dispensed. During feeding, a red neon light, mounted on the control box and wired across the feeder solenoid, was lit to allow remote visual check of feeder operation.

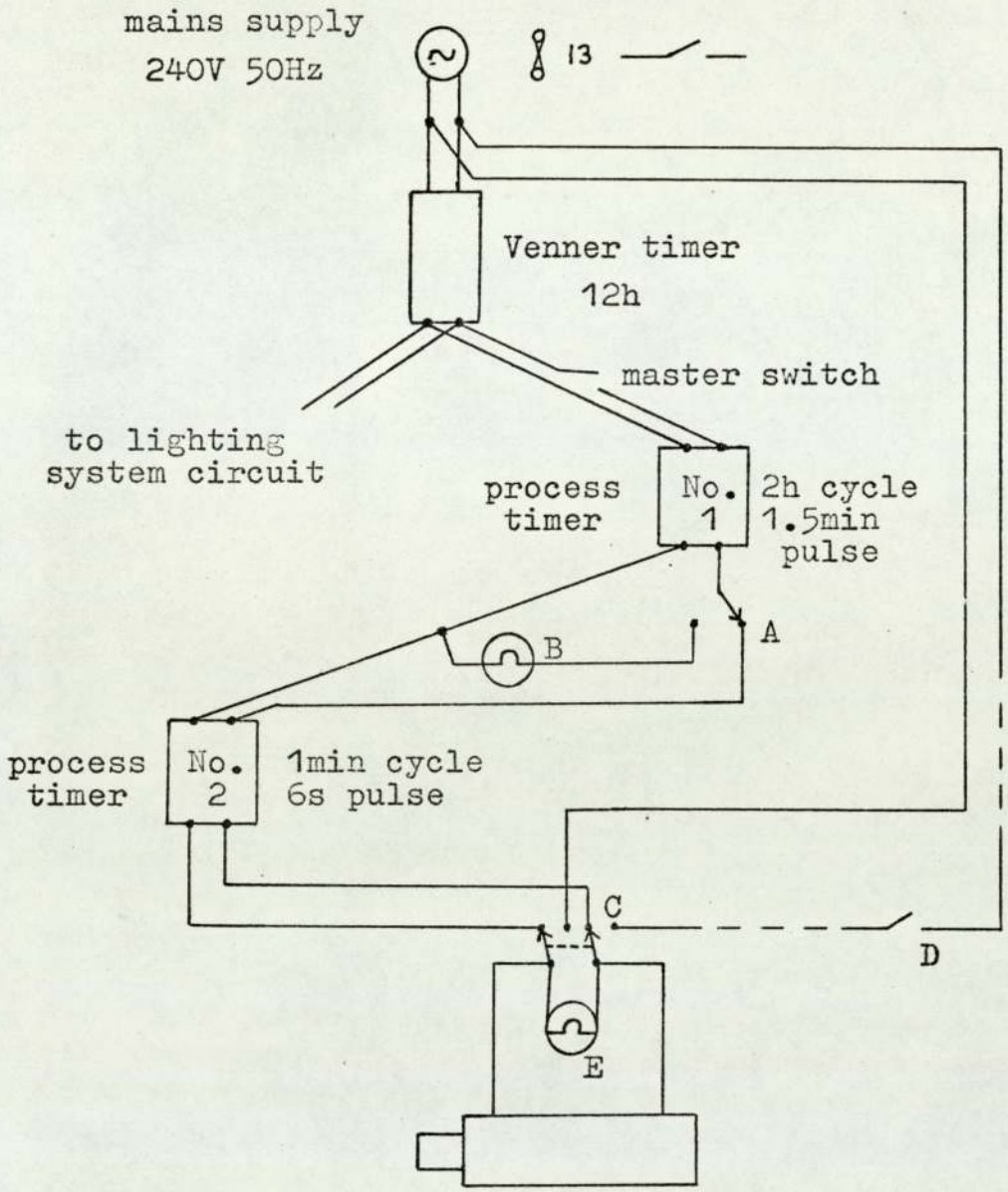
3.25 For convenience, especially when calibrating feeders at the beginning of an experiment, and when sampling overran its time, it was desirable:

a) to be able to prevent a feed manually by delaying it until the control pulse had passed;

b) to be able to feed whenever required by manual override without affecting the timing circuits; and

c) to be able to feed by manual override either at the control box, or by a remote control mounted in the wet lab.

These objects were accomplished by the circuitry of Fig.3.8, which also incorporated a warning light (orange) as a visual reminder of delay switch action. Thus complete flexibility



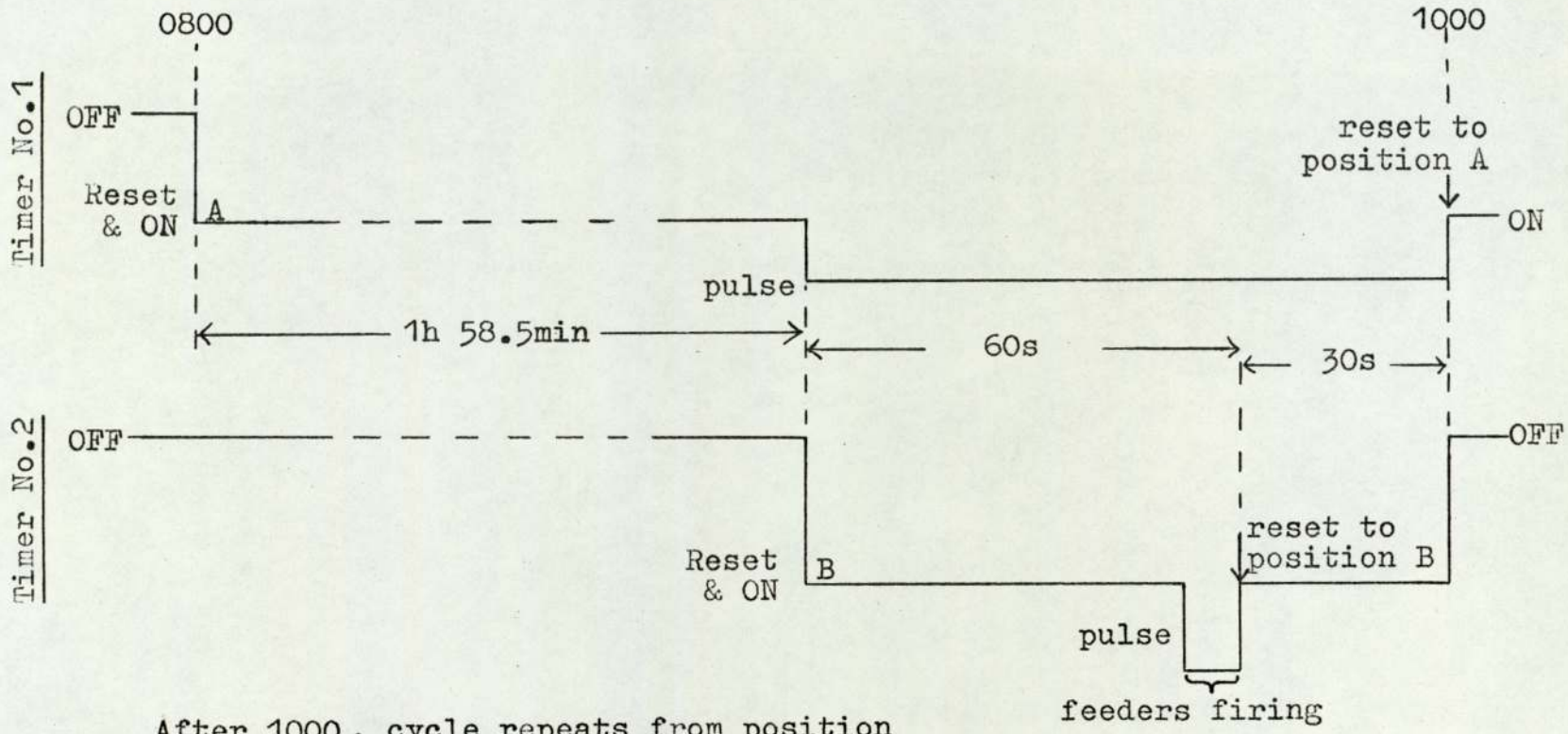
A = manual delay switch

B = delay warning light

C = manual override switch)
) - used in conjunction
D = remote override switch)
(in wet lab)

E = feeding monitor light

Figure 3.8 Diagram of feeding control circuits



After 1000, cycle repeats from position A four times (to 1800); but in the next cycle, the 12h control turns all timers off just before position B at about 1957.

Figure 3.9 Automatic timing cycle for feeders

was obtained over the lighting and feeding systems, allowing automatic or manual control as required.

3.26 The control box was constructed of hardboard panels with a wooden backboard (2 cm thickness) to which the components were fixed. All components were suitably connected by soldered wiring, earthed, fused and insulated, and a hinged door allowed access. The faces of the process timers, and the control switches were mounted on the front face of the box for operation, with neon lights alongside, but for safety the Venner timer could only be operated by opening the door. The box was served from a mains electric point with switch kept on all the time except when components were being checked.

Individual tank fittings

3.27 Each of the 8 MT had an array of fitments as detailed below (see Fig.3.10).

3.28 A short length of 2.5 cm diameter PVC pipe was inserted tightly into the tank exit hole and sealed with a non-toxic sealing compound. 19 mm diameter rubber tubing formed a U-tube below the trestle in the inter-LT spaces, with one end fitted over the PVC pipe and the other end rising to the front of the trestle, inboard of the MTS gutter. At the bottom of the U bend a glass T-piece and bleed-off tube allowed debris removal as in the pilot system.

3.29 The U-tube front end was inserted into a PVC 2.5 cm diameter T-piece which formed an overflow level control, held in 2 spring clips on a track formed of two short vertical pieces of Bartangle strip; it was adjustable over the height of the track by means of wing nuts on the clips. The T-piece side-arm controlled level, and rubber tubing conveyed the effluent water to the gutter.

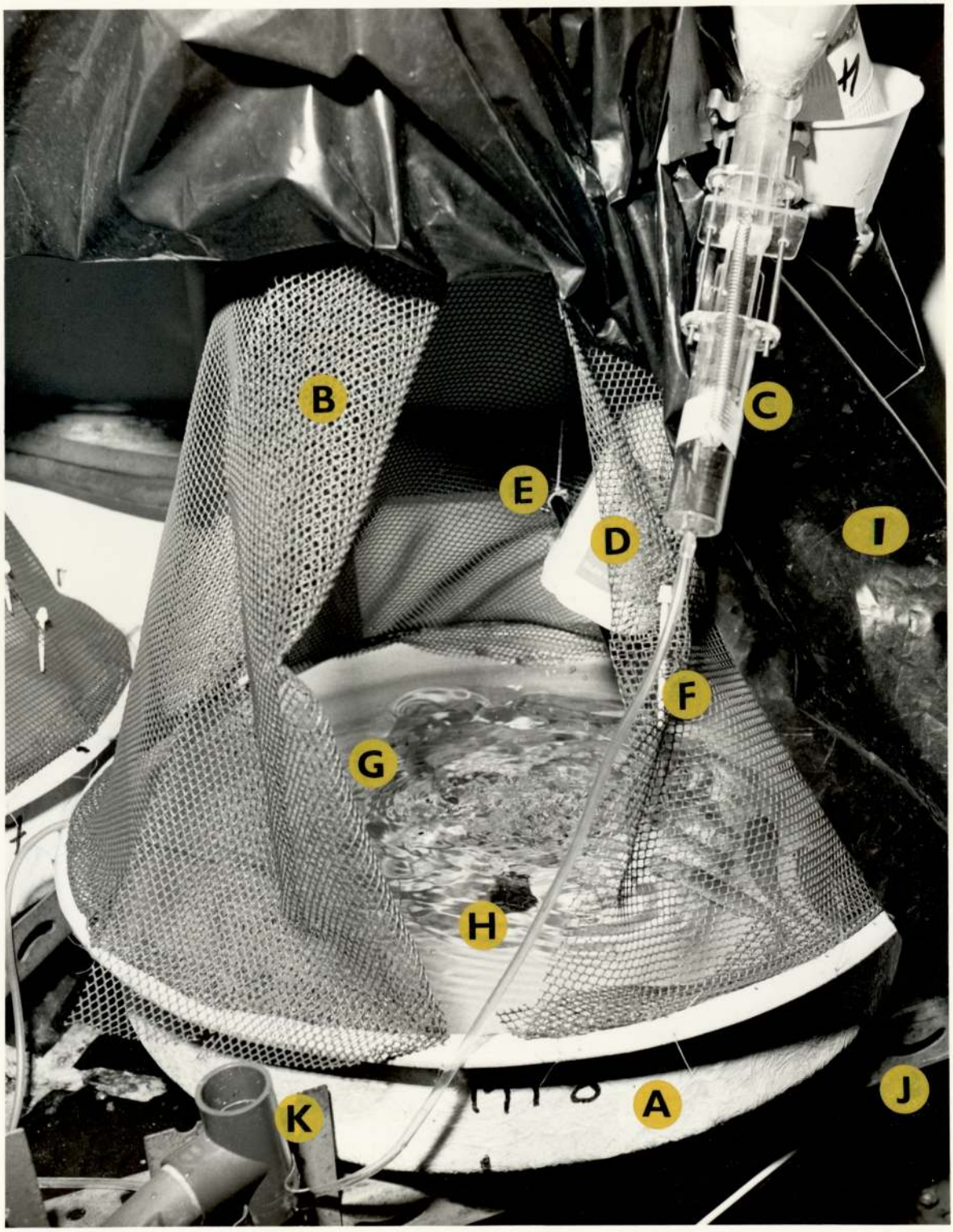
Figure 2.10 Laboratory before conversion

A) Upper laboratory/office, with header tank room beyond partition and trapdoor in floor at right.

B) Lower (wet) laboratory, with future experimental space along right-hand wall.

Figure 3.10 MT in E-series configuration

A MT8 in position
B Tank shroud pinned back
C Feeder in operation (spring extended)
D Delivery tube protruding through blackout and shroud
E Tank inlet suspended in place
F Shroud pin
G Swirling denotes feeding activity
H Mesh plug in tank exit hole
I Blackout panel
J MTS trestle cross-strut supporting MT8
K Overflow level control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3.30 The well of each MT could be screened either by a circular disc of 6 mm hard square green plastic mesh fitted tightly into the well ledge, or by a short roll of the same mesh forming a plug in the hole (as in Fig.3.10). In either case the purpose was to allow removal of faeces or uneaten food but prevent loss of small fish; the latter method proved better since the former caused a "dead space" in the well and inhibited self-cleaning of the MT.

3.31 A length of flexible 6 mm plastic tubing was led over the tank rim to attach to a 30 cm length of 4 mm bore straight glass tubing mounted on a diagonally placed graduated scale (card and board assembly) across the front of the tank as in Fig.9.4 (Chapter 9). This formed the volume indicator. Water was sucked through the tube by the operator (to form a siphon over the rim) and left to find a level in the external indicator. By prior calibration of the indicator with known water volumes, tank volume readings could be obtained. The diagonal scale allowed more accuracy than a vertical scale would have done due to its greater length. The volume indicator was fixed in place to the trestle, and to the overflow level control Bartangle supports.

3.32 Fish were retained in the MT by means of a conical tank shroud of soft green plastic mesh (3 mm diamond), rolled round so that the larger end of the cone fitted over the tank, and the smaller end fitted over the light bulb hardboard plate (see Fig.3.6). Holes were drilled around the MT perimeter, and the cone sewed in place with nylon fishing line of 4 kg breaking strain. The cone overlapped at the front of the tank and one side was left free to allow for handling of fish into and out of the tank. During experiment, the overlap was closed by pinning through the mesh. Apertures through the mesh allowed water inlet and feeder discharge tubes to protrude inwards.

3.33 The LTS was equipped with similar tank shrouds to the MTS, attached to the tank sides by nylon bolts and nuts. When necessary (with small fish) mesh plugs were inserted into LT exit holes. The LTS was not equipped with volume indicators: instead the water level was observed through the translucent tank side and its height measured from the tank bottom. Prior calibration thus allowed experimental volumes to be measured. LTS overflow level controls were similar to those of the MTS but were mounted at convenient points on the framework. No U-tube bleed-offs were present because of the lack of room for operation below an LT; satisfactory debris removal was accomplished by "pumping" by stamping the U-tube against the floor several times.

3.34 The STS was equipped similarly to the pilot system, employing ST type 2. The U-tubes below the tanks hung in the inter-LT spaces and the only major modications were deletion of the plastic tap below the tank, and the use of horizontal fitted plastic lids for the tanks. All later modifications of the STS and MTS for T-series experiments, are discussed in Chapter 9.

Feeders

3.35 The feeding system which supplied all tanks was made to a design* by P.Smith, and was powered by compressed air cylinder. The basic plan, as in Fig.3.11, was a ring main compressed air circuit supplying a feeder at each tank. Firing of the control solenoid pulled open the master supply valve for 6 seconds. This allowed compressed air at about 1520 millibars to enter the ring supply, and at each feeder a piston discharged food from a barrel, acting against a calibrated stop nut. When the solenoid ceased pulling, the valve closed again, the pistons withdrew, and the compressed air was exhausted at the valve.

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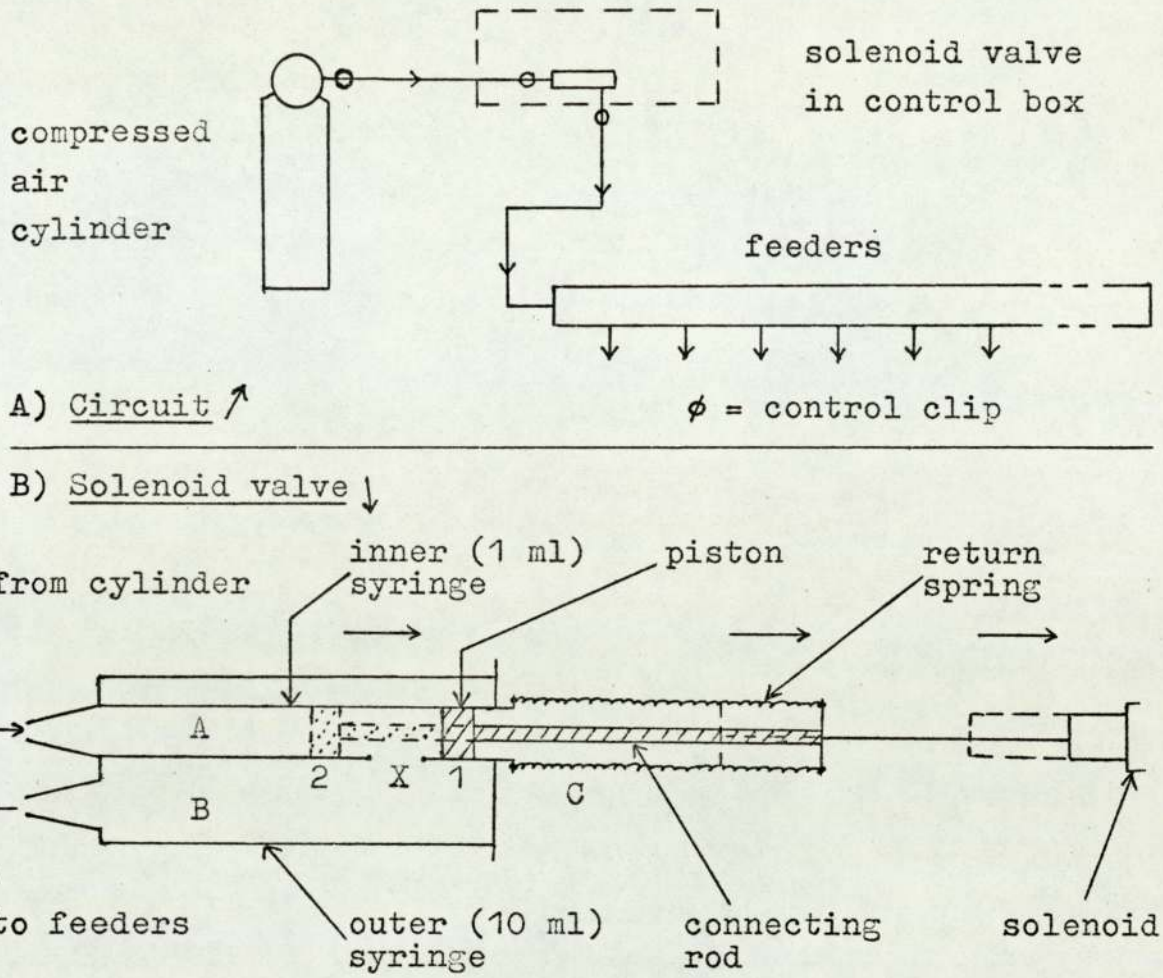
* currently under consideration for patent



Valve open: Solenoid pulls piston to position 1 and compressed air passes from cylinder via inner syringe (A) through valve aperture (X) into outer syringe (B) and so to feeders.

Valve closed: Return springs pull piston to position 2, shutting off air supply (A) and allowing air from feeders to exhaust from B through X past the connecting rod to outside of the valve (C); return springs maintain position 2 against the compressed air supply.

Figure 3.11 Compressed air feeding system

As each feeder was on a branch supply off the ring, individual feeders could be taken out of use as required by means of clips. The compressed air lines were of 3 mm flexible plastic tubing.

3.36 Each feeder unit was built of inexpensive materials, based on the use of plastic syringes, with plastic cups for supply hoppers. The basic design was as in Fig.3.12, and this was modified for the LTS by substituting 50 ml syringes for the 20 ml ones, and using plastic bottles for the hoppers. The hoppers were covered by plastic (petri-dish) lids to prevent water from entering.

3.37 Fig.3.13 shows an LTS feeder; the top of the barrel was protected from spray by an inverted plastic cup which deflected discharged food downward into the receiver cup, whence food fell via an angled delivery tube protruding through the tank shroud as in Fig.3.14 (delivery tube at top centre). This 2-stage operation facilitated calibration of the feeders, as a blind cup could be placed in the receiver cup to allow observation of discharge but prevent food delivery.

3.38 LTS feeder units were mounted on the framework between the tanks, for easy access. MTS feeders were mounted on hardboard plates bolted to the superstructure girders. STS feeders were mounted in pairs on angle-iron support arms, just above the tank lids.

3.39 Feeder calibration was required at the beginning of each experiment. First the amount to be fed daily was calculated (see Chapter 7), and the feeder hoppers filled at some convenient time after the day's last automatic feed at 1800 (supplying the fish on HC2). Blind cups were inserted into the receiver cups to prevent food delivery. The calibration nut was then set on each feeder to a likely position and the feeders

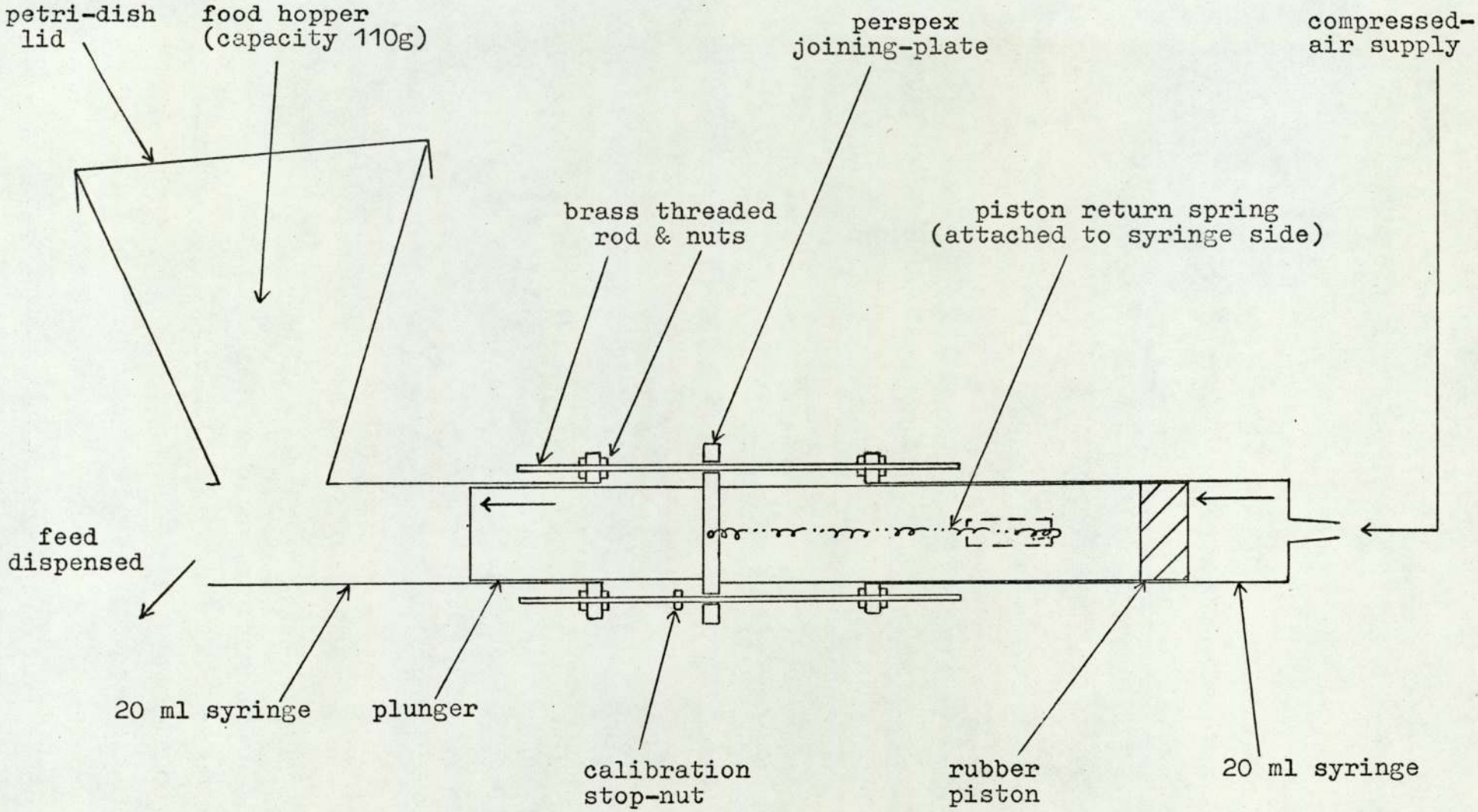


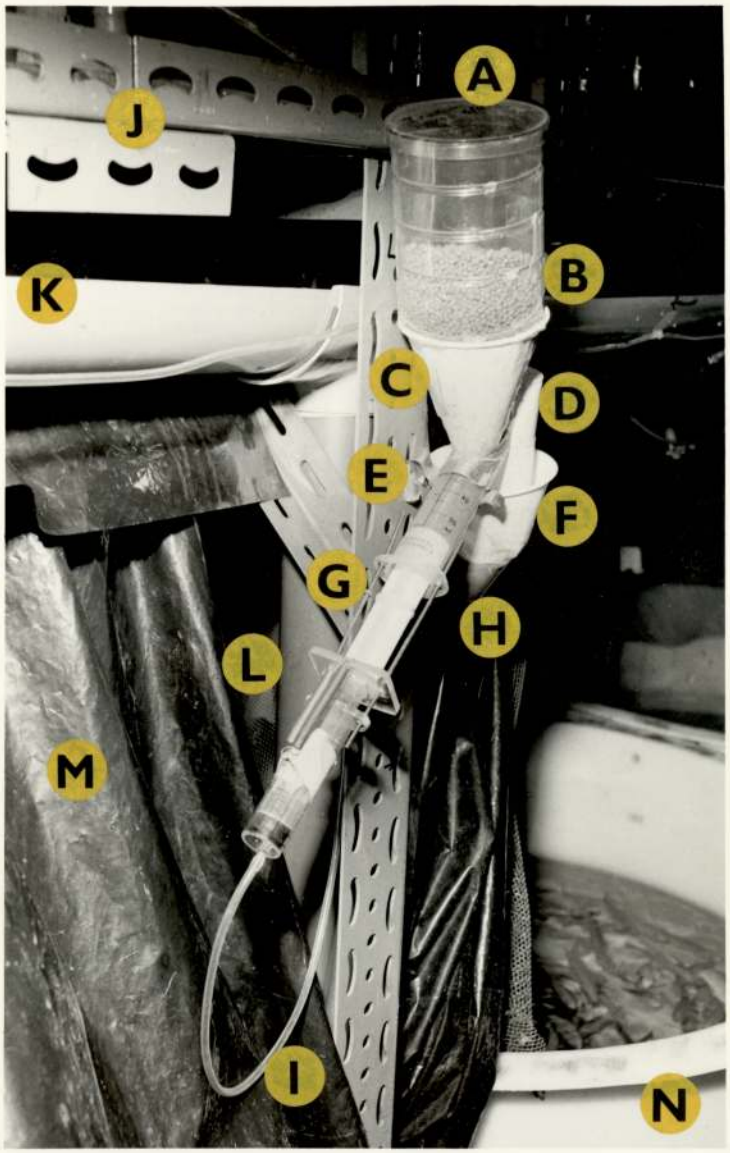
Figure 3.12 Plan of MTS feeder (approx. to scale)

Figure 3.13 LTS feeder

A Plastic hopper lid
B No.4 pellets in upper part of hopper
C Lower part of hopper
D Protective deflector
E Spring clip attachment to framework arch
F Receiver cup
G Calibration nut
H Delivery tube extending through blackout and shroud
I Compressed air supply tube
J STS trestle framework
K STS drainage gutter
L Drainage down-pipe
M Blackout curtain
N LT

Figure 3.14 Food delivery to an LT

A Delivery tube
B Food pellets
C Shroud attachment bolt
D Angled water inlet
E Inlet support arm
F Fish swimming against direction of flow
G Exit hole with simple mesh screen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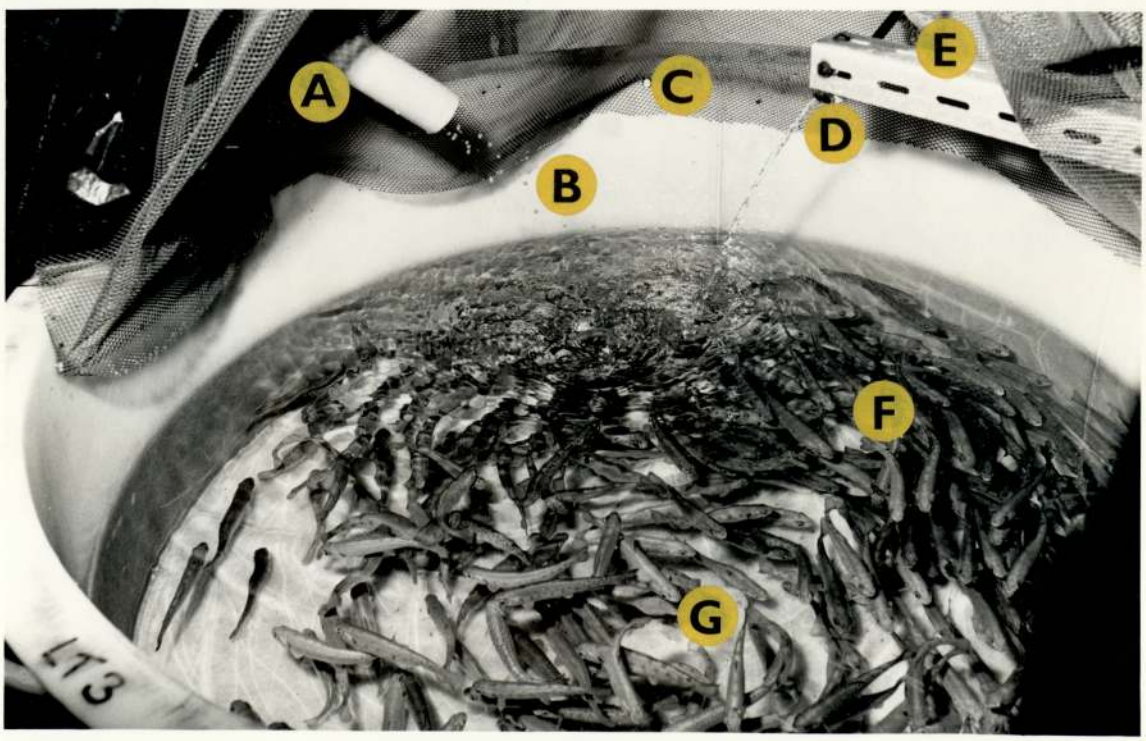
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operated five times by switching the remote override for about 6 to 8s each time, allowing for full piston return in between. The blind cups were then collected and their food content weighed. Any required calibration adjustment was made, and the process repeated several times until feeders were dispensing the correct amounts. Then the blind cups were removed, the hoppers filled with known weights of food, and the control system left to cause the first feed of the subsequent photoperiod automatically.

3.40 Feeders operated successfully with minimal maintenance over a period of 18 months, and were fundamental to the success of experiments (see Chapter 7). Receiver cups and delivery tubes required periodic cleaning as they collected dust from the food.

Blackout

3.41 An efficient blackout system was required and for simplicity and ease of access panels of heavy-duty black polythene sheet were chosen. These had the advantage over cloth curtains of being unaffected by water spray, but were rather less robust. They could be modified with ease, and were conveniently fixed in place to the walls, ceiling and framework, as required, by strips of double-sided adhesive tape. LTS panels were formed into curtains which could be drawn aside on curtain runners for tank access, but overlapped when closed. In addition, horizontal panels were placed below the MTS (and STS where necessary) to shut off the LTS from disturbance, and extra panels were positioned to supplement the STS cloth curtains, so that all three systems were light-isolated from each other and from the rest of the wet lab. The following facilities were outside the blackout and could be accessed without disturbance of the fish: feeders (except STS), volume indicators, overflow level controls, LTS U-tubes, inlet sample supplies. Access behind the blackout was required for tank inlet controls, STS

and MTS U-tubes and bleed-off tubes.

Measuring equipment

3.42 During the development of experimental systems, the following pieces of apparatus fundamental to measurement were procured:-

a) Sauter 10 kg automatic pan-loading balance (this replaced a Sartorius Model 707/10 manual pointer balance and a Gallenkamp manual balance)

b) Sartorius 1100 top-loading 200 g balance

c) Corning-EEL Model 12 Research pH meter (used with Corning or Activion rugged Combination electrode)(this replaced a Pye Dynacap pH meter).

3.43 At an early stage of development it was envisaged that an extra long electrode cable (about 6 m) would allow a reading to be obtained from each tank overflow level control. This proved impracticable due to problems of electrical conductivity. Instead, the pH meter was kept in the dry lab and samples brought to it.

Equipment sources

3.44 Manufacturers of important equipment are listed below.

Angle iron	-	Bartangle Ltd., Bilston
Glassware (to specification)	-	(J.A.R.&.M.K.Hill, Walsall (Glassblowers, Department of (Physics, University of Aston
Medium-size tanks (MT) (to specification)	-	Vortex (Fishery Equipment) Ltd., Meriden
PVC guttering	-	Hunter Plastics Industries Ltd., Woolwich
Process timers	-	Crouzet Ltd., Manchester
Light bulbs	-	Osram (GEC) Ltd., Wembley
24-hour timer	-	Venner Ltd., New Malden

Small electrical components	-	R.S. Components Ltd., London EC2.
Plastic mesh	-	Netlon Ltd., London WC2.
Large-size tank (LT) (to specification)	-	Cago Ltd., Birmingham

4. MEASUREMENT OF AMMONIA

INTRODUCTION AND LITERATURE REVIEW

4.1 The analysis of fish tank water for ammonia played a fundamental part in the work here described. Thus the methods used for ammonia analysis, and their validity and accuracy, are of prime importance in helping to assess the study of excretion and excretory tolerance.

4.2 It is important to note that in all following discussion, ammonia is quoted as ammonia-nitrogen. The importance of the ionisation state is discussed in Chapter 5; all methods used estimated the concentration of nitrogen due to total ammonia ($NH_3^o + NH_4^+$). Estimation of nitrogen due to unionised ammonia alone (NH_3^o) involves measurement of pH and temperature.

4.3 The most common standard method for ammonia analysis has for long been the use of Nessler's reagent (IWE 1960, APHA 1971). Nessler's reagent is an alkaline mercuric iodide solution which turns from red to yellow or brown, the colour intensity (due to a complex ion) being a measure of the ammonia present. Recommended levels of ammonia in the sample for good results are from 400 $\mu g\ \ell^{-1}$ to 5.0 $mg\ \ell^{-1}$ with a sensitivity to 200 $\mu g\ \ell^{-1}$ (APHA 1971), and the process when used directly is subject to interference (causing turbidity or greenish colouration) from calcium, iron, magnesium, sulphide and a wide range of organic molecules.

4.4 Another standard method is the phenol-hypochlorite reaction, which produces an intense blue colour with ammonia due to indophenol formation. First described by Berthelot (1859), it was applied to ammonia by Van Slyke and Hiller (1933), and has since been modified many times. Russell (1944) increased the sensitivity of the method and Riley (1953) applied it to seawater. Lubochinsky & Zalta (1954) introduced the use of nitroprusside catalyst. Since then the method has been used

extensively (e.g. Emmet 1969, Solorzano 1969, Nimura 1973). Cocking (1967) applied it to ammonia excreted by goldfish, and found that it allowed estimation of 25 $\mu g\ l^{-1}$; urea, creatine and creatinine did not interfere even at high concentrations. The method is recommended (APHA 1971) for values up to 500 $\mu g\ l^{-1}$. Croston (1969) quotes the method as more sensitive, stable and reproducible than Nesslerisation. With these points in mind it was decided that the phenol-hypochlorite method would be used for spectrophotometric determination.

4.5 The particular version used was that of Harwood & Kuhn (1970), which has the advantage of simplicity with a relatively small amount of preamble before photometric measurement.

Reagents:-

Buffer: 5% (weight/volume) Na_3PO_4 solution

Phenol stock: 500 g phenol dissolved in methanol, diluted to 800 ml with methanol, stored at $2^{o}C$.

27% NaOH: 270 g NaOH pellets dissolved in water, cooled, diluted to 1 l.

Reagent A: 15 ml phenol stock plus 0.02 g sodium nitroprusside, diluted to 100 ml with water.

Reagent B: 15 ml diluted commercial bleach (hypo) solution ($\pm$ 3% free Cl) plus 15 ml 27% NaOH, mixed, diluted to 50 ml with water.

Stock standard solution: 38.2071 g NH_4Cl dissolved in water, diluted to 1 l (gives 10 g l^{-1} ammonia-N); 1 ml of this diluted to 1 litre (gives 10 mg l^{-1} ammonia-N).

(All chemicals were of analytical reagent grade.)

4.6 In all cases ammonia-free water was required as the diluent. Following Harwood & Kuhn (1970), use was made of distilled water which had been deionised by passing it through a Permutit Mk.17 portable cartridge ion-exchange equipment. Such water should contain $<10\ \mu g\ l^{-1}$ ammonia-N (Beckett & Wilson 1974). Reagents A and B were stored in a refrigerator but

allowed to reach room temperature before use; they were made up weekly from the stock reagents when required. Stock standard solutions were made up fortnightly and similarly stored.

4.7 Procedure:-

To each 25 ml volumetric flask (one per sample or standard) was added (by pipette);

5 ml sample or standard,

2 ml buffer,

3 ml deionised water,

5 ml reagent A while swirling,

2.5 ml reagent B while swirling,

and deionised water to make up to 25 ml.

The flask was stoppered and mixed well, and left for at least 25 min. Two flasks were prepared for each sample or standard solution used (standards were made by appropriate dilutions from the stock standard); a blank using deionised water was also prepared. Since the developed colour was stable for 4 h, photometric measurement could be delayed for a short while if necessary.

4.8 Measures of the solution from the flask were pipetted into 1 cm glass cells and measured at 630 nm against the blank in a spectrophotometer. For pilot experiment P01 a Beckman DB was used, but for later work a Unicam SP 500 was used. The recorded measurement for each sample/standard was the average of the duplicates.

4.9 During P01 good calibration graphs were obtained for values between 0.1 and 1.0 mg ℓ^{-1}, reading the absorbance scale to give a straight line calibration. Unfortunately, most of the experimental values found in P01 were at or below 0.1 mg ℓ^{-1} and hence on the most uncertain part of the line. Although Harwood & Kuhn (1970) recommend the use of 4 cm cells for the range 0.1 to 1.0 mg ℓ^{-1}, the adequacy of the calibration lines between these values suggests that 1 cm cells are

satisfactory, although naturally somewhat less precise. On average, the correlation found between absorbance values and ammonia-N concentration was expressed by r = 0.9972. (Harwood & Kuhn (1970) found r = 0.9993 for 4 cm cells, and r = 0.9989 for 1 cm cells in the range 2 to 10 mg ℓ^{-1}.) Harwood & Kuhn also tested their ammonia measurements against those produced by an Auto-analyser method, finding very high similarities. When samples were presented for Auto-analyser analysis at Aston, it was necessary to use a range-expander with some loss of sensitivity and precision. The calibration graphs of transmission against concentration (plotted on semi-log graph paper) were of variable curve-form. For this reason, and also due to problems of machine access, the Auto-analyser was eliminated from consideration as a routine ammonia-measurement method.

4.10 The spectrophotometric method was satisfactory down to about 0.1 mg ℓ^{-1}, but its major drawback was the amount of manipulation needed to deal with large numbers of samples. In later stages of experimental work it was necessary to handle up to 40 samples per session. This made error or spillage extremely likely, and the time required would have interfered with other necessary operations during the day. As it was considered better to analyse samples as soon as possible rather than to store them, the procedure would become increasingly unwieldy with large numbers of samples.

4.11 An alternative speedy method of ammonia analysis was therefore sought, capable of handling large numbers of samples with minimum effort, and reliable over the ranges of values anticipated i.e. down to 100 $\mu g\ \ell^{-1}$. For this purpose an EIL laboratory ammonia probe (Model No.8002) was purchased in September 1972 and used for all subsequent experiments (from P02 onwards - see Chapter 6).

4.12 Up to the time of purchase, such instruments had been little used by other workers for ammonia analysis, although Barica (1971) had used a univalent cation glass electrode for determination of NH_4^+ ion. The probe, in contrast, operates by detecting the amount of free NH_3^o. More recently literature on the use of the probe has appeared, besides the information sheets issued by the major American manufacturer of selective-ion electrodes (Orion 1970 a & b, 1972). Barica (1973) described use of an Orion probe for fish tank water, and Midgley & Torrance (1972, 1973), Beckett & Wilson (1974) and Evans & Partridge (1974) tested the EIL probe in various other applications.

4.13 Barica (1973) evaluated an Orion model 95-10 for determination of total ammonia. He found that for values below 0.1 mg ℓ^{-1} it was unsuitable, and in the range 0.2 - 0.5 mg ℓ^{-1} it yielded results differing from those found by automated spectrophotometry by $\pm$ 17%. In the range 0.1 to 14.0 mg ℓ^{-1} he found that calibration curves were necessary, but above this range and up to 14 g ℓ^{-1} response was Nernstian (i.e. a plot of mV response against log ammonia concentration gave a straight line); below 14 mg ℓ^{-1}, mV response was rather less per (log) concentration unit.

4.14 Beckett & Wilson (1974) tested the EIL probe from 0.1 to 4.0 mg ℓ^{-1}. They found Nernstian response over this range (which roughly agrees with Evans & Partridge (1974) and the manufacturers' advice (EIL 1971)); but found that the calibration line slope may vary from time to time or between individual instruments. They found variability of results from about 10% to 3% as concentration increased from 0.1 to 4.0 mg ℓ^{-1}. This confirms the findings of Midgley & Torrance (1972). Beckett & Wilson found that 5 mg ℓ^{-1} of urea present in the water caused a comparable sized error to that due to diluent water, which they considered negligible. They estimated calibration time as 20 min, with 5-6 min necessary for probe

stabilisation per reading (agrees with Barica (1973)). Good agreement was found between the probe and other methods, and Beckett & Wilson recommended the probe for various applications, mentioning its potential for on-line water analysis as in Midgley & Torrance (1973). Midgley & Torrance (1972) preferred the probe to a phenol-hypochlorite method for power-station high-purity water (0.1 to 1.0 mg ℓ^{-1} ammonia-N).

PROBE METHOD FOR AMMONIA

Introduction

4.15 The EIL ammonia probe functions in a similar way to a selective-ion electrode but operates on a different principle. It consists of a transparent hard-plastic tube with a thin hydrophobic polymer membrane across one end, across which free ammonia (NH_3^o) can diffuse but ions cannot. Within the tube is an ammonium chloride solution, bathing the interior of the membrane and surrounding a glass pH electrode and a silver/silver chloride reference electrode. The pH electrode is pressed against the membrane, trapping a thin film of solution. Unionised ammonia will diffuse through from a sample, and since the NH_4Cl solution provides excess NH_4^+ ions, the important effect will be to cause an increase of OH^- ions, according to the amount of unionised ammonia, until the partial pressure of ammonia is equal on both sides of the membrane:-

$$NH_3^o + H_2O \longrightarrow NH_4^+ + OH^-$$

This changes the pH of the internal solution: a change which is sensed by the pH electrode, and can be displayed as an electric potential change measured in mV, where the potential change (E) depends on concentration of NH_3^o (C) thus:-

$$E = B - \left(\frac{2.303\ RT}{F}\right) \log C$$

(B = constant, R = gas constant, T = absolute temperature, F = the Faraday (96500 coulombs per equivalent))

4.16 Due to the temperature dependence, the probe is used for measurement at a constant temperature; thus standards and samples must be brought to a common temperature before measuring.

4.17 Initially the probe was used with a milli-voltmeter/pH meter (Pye Dynacap), but for most of the experiments it was used with an EIL Model 7030 equipped to function as pH meter, millivoltmeter or specific ion electrode meter; the latter function depends on a built-in ability to vary slope correction as necessary (for the slope of the graph of log C against E) and supplying readings on a logarithmic scale corresponding to direct concentration readings (e.g. mg ℓ^{-1}). Calibration graphs were thus unnecessary provided two standards were used, separated in value by a factor of 10 (e.g. 0.1 and 1.0 mg ℓ^{-1}). The slope correction control allowed standardisation of the log concentration scale against the standards, and hence direct readout for unknowns. Calibration was carried out before each day's measurements.

Procedure

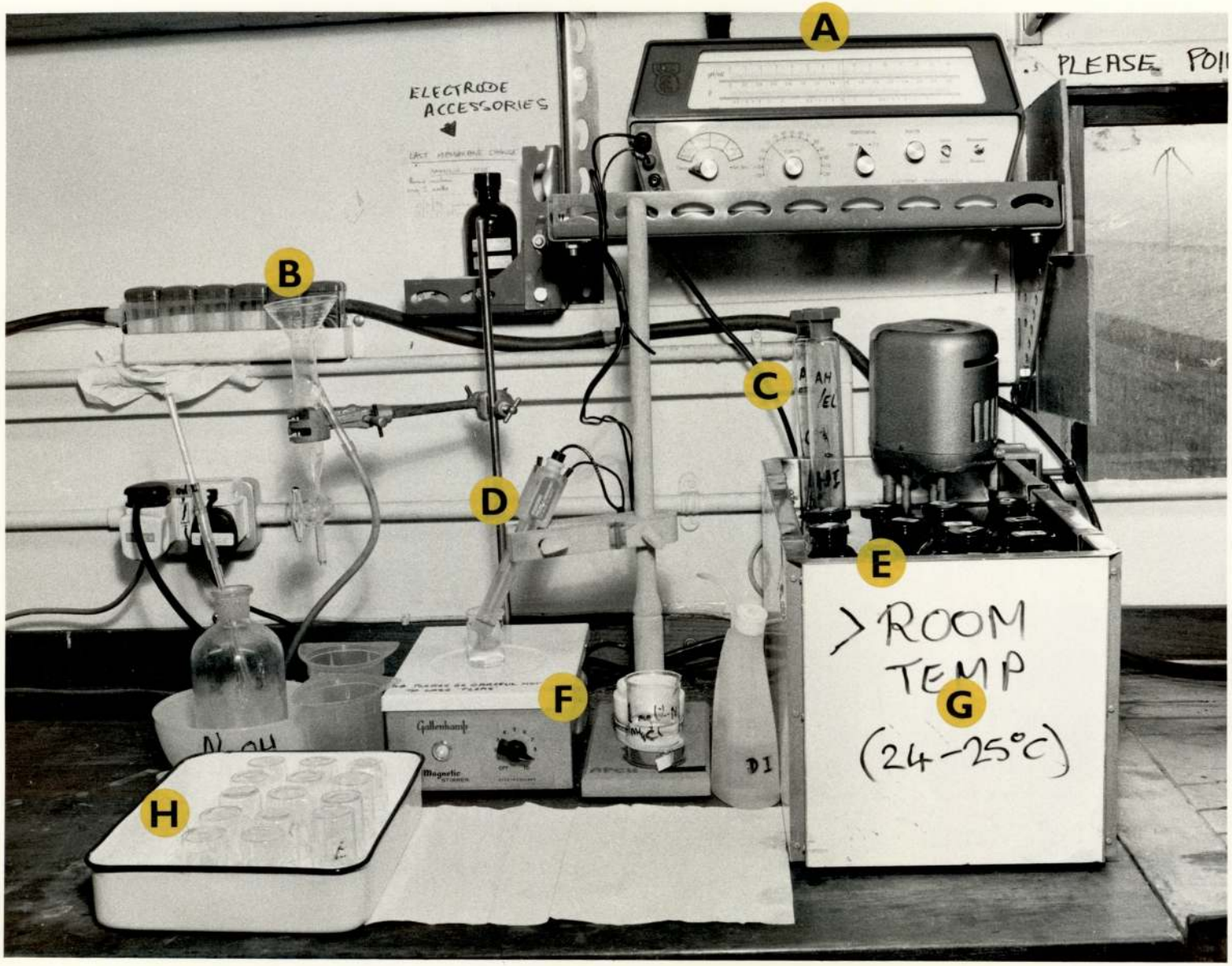
4.18 The measuring equipment is shown in Fig.4.1. Duplicate samples were taken from tank overflows (or inlet-sample lines) in 60 ml amber glass bottles with ground-glass stoppers. Each bottle was washed out twice in the water to be sampled before collection, then overfilled and stoppered without any air trapped inside. Sample bottles were transferred to a water bath at $\pm 25^{o}$C to reach measurement temperature.

4.19 Standards were prepared by diluting with deionised water the stock standard solution of NH_4Cl (10 mg ℓ^{-1}), and the standards (in 100 ml stoppered measuring cylinders) were also transferred to the water bath. A magnetic stirrer plate was set up, and a strong solution of NaOH (40 mg ℓ^{-1} or 1.0M) prepared.

4.20 Samples and standards were dispensed with the apparatus shown in Fig.4.1. This dispenser, made to specification by the University glass-blowers, was used because of its combination of speed and precision in delivering a fixed quantity ($\pm$ 22 ml), after being filled to the level controlled by the overflow arm with tap closed. The dispenser was first pre-

Figure 4.1 Ammonia measurement equipment

A EIL Model 7030 pH meter
B Sample dispenser
C Ammonia standard solutions
D EIL Model 8002 laboratory ammonia probe
E 60 ml amber sample bottles
F Magnetic stirrer plate
G Temperature-controlled water bath (25°C)
H 25 ml measurement beakers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Magnetic Stirrer
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washed with the solution to be measured, and then filled to overflow. The delivered aliquot was collected in a 25 ml beaker containing a magnetic stirring-rod. 0.25 ml of NaOH solution was added to raise pH to $\pm$ 12.5, at which all ammonia present would be converted to the unionised form. The beaker was then placed on the stirrer plate (set to a stirring speed which would not cause bubbles or excessive vortex).

4.21 The probe, connected to the meter, required rinsing with deionised water and dabbing dry with soft medical tissue paper, in between each solution measured, in order to prevent carry-over. For measuring, the probe was lowered at an angle into the solution to prevent bubble formation and the formation of a "dead space" under the membrane, and was then allowed to settle completely before the reading was taken.

4.22 Calibration was carried out as follows. A measure of "low" standard (e.g. 0.1 mg ℓ^{-1}) was dispensed and its pH adjusted. The probe was introduced into the beaker, and time for stabilisation of reading allowed (about 5-6 min at 0.1 mg ℓ^{-1}). The calibration "BUFFER" control was then adjusted to set the reading to the lower calibration point on the middle (p) scale (Fig.4.1). A measure of "high" standard (e.g. 1.0 mg ℓ^{-1}) was then substituted for the "low", the probe introduced and allowed to stabilise (2-3 min at 1.0 mg ℓ^{-1}), and then the meter was set to the higher calibration point on the p scale, using the slope calibration control (second from left in Fig.4.1). This compensated for slope change between measurement sessions.

4.23 The process was then repeated, using both standards, a sufficient number of times (usually only once) to check the accuracy of the first attempt, and then the BUFFER control adjusted so that the standard used last registered its true value on the CONC scale (lowest of the three, Fig.4.1).

4.24 Each sample was then treated in an exactly similar way to the standards, and the reading, after sufficient stabilisation time was noted from the CONC scale.

4.25 All glassware used was rinsed in distilled and then deionised water after washing, and prewashed with deionised water before use to avoid ammonia contamination.

4.26 With each batch of readings taken, an inlet-sample (of water as it entered the fish tanks), and a "zero" sample (from the deionised water supply) were measured as checks. The values recorded were always similar, never exceeded 50 $\mu g\ l^{-1}$, and rarely exceeded 30 $\mu g\ l^{-1}$. Given the unreliability of the probe's measurement at such low concentrations, it is felt that neither of these sources would give rise to an error above 10 $\mu g\ l^{-1}$ for concentrations greater than 100 $\mu g\ l^{-1}$ (see Beckett & Wilson (1974), Evans & Partridge (1974)).

4.27 It was found, in agreement with Evans & Partridge (1974), that stabilisation time varied according to the concentration difference between the current and the previous sample under the probe. For a number of samples in ascending order of concentration, stabilisation time could be reduced below expectations, while widely differing concentrations required longer internal probe readjustments. Duplicate measurements were taken in all cases, and the mean of the two was accepted as the "actual" measurement.

4.28 On a few occasions measurement was not carried out immediately after sampling, and in these cases sample bottles were stored in a refrigerator at about 4°C, as mentioned by Beckett & Wilson (1974). Storage did not exceed 36 h, and samples were allowed to equilibrate in the water bath before measurement.

4.29 Occasional check readings with the probe suggested that whether stored at room temperature or in a refrigerator, samples containing about 1.0 mg ℓ^{-1} showed a maximum variability of about 5% in measured value over a period of about 8 h. (Kutty (1972) found no trend in change of ammonia content of samples for up to 4-5 h.) Whilst refrigerated samples maintained this record over about 24 h after sampling, those kept at room temperature changed by up to 25% over this time, hence samples were not kept unrefrigerated over periods longer than 1-2 h.

4.30 The Model 7030 meter could be read on the CONC scale to an accuracy of about 5%. Following Beckett & Wilson's advice (1974) that error due to diluent water can be ignored, and bearing in mind the noted similarity between diluent water and inlet samples, the quoted average error on probe readings of about 10% at 0.1 mg ℓ^{-1} (Beckett & Wilson 1974, Midgley & Torrance (1972)) seems to be a fair guide: the sum of the errors described above would agree with this, and a working value for error of 10% was accepted for the study. This is discussed in the context of the major excretory series of experiments in Chapter 7.

4.31 It was considered that of the various methods tried, none was completely satisfactory in accuracy of measurement of total ammonia below 100 μg ℓ^{-1}. The probe was both speedy and satisfactory above this level, and was used for all subsequent measurements from its date of purchase. Since 100 μg ℓ^{-1} was usually exceeded in major experiments, any values below this level were treated as unreliable.

PART 3

AMMONIA PRODUCTION

AND THE TANK ENVIRONMENT

5. INTRODUCTION TO AMMONIA PRODUCTION

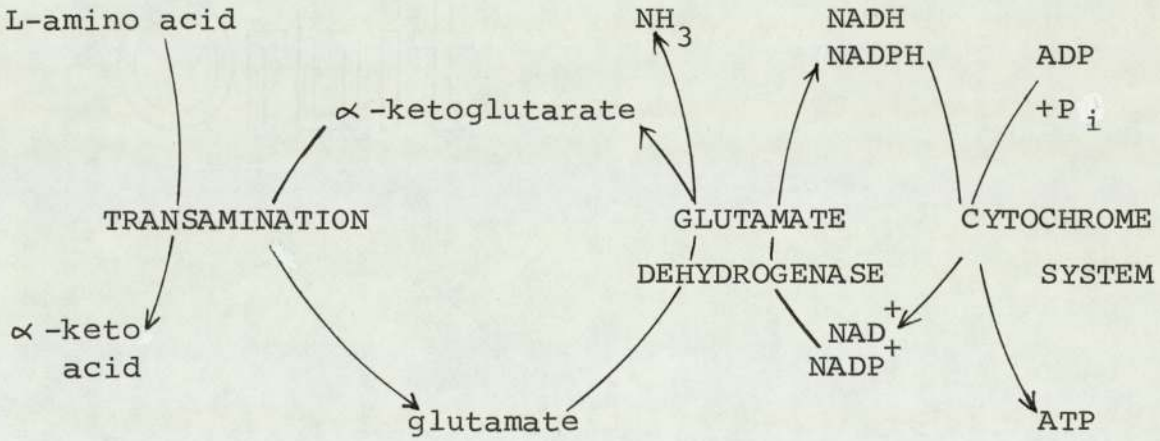
LITERATURE REVIEW

Ammonia as an excretory product

5.1 Excretion of fish is a topic widely surveyed in physiological literature, (e.g. Black 1957, Forster & Goldstein 1969, Goldstein & Forster 1970, Watts & Watts 1974), but rarely from precisely the viewpoint required in this study.

5.2 Much of the work derives from the experiments of Smith (1929) on the accumulation of excretory products in a static tank (see below). Such experiments revealed that the major compound excreted is ammonia.

5.3 Ammonia represents a physiologically "economic" excretory product which requires no energy expenditure in its formation. In fact some of the reactions involved can ultimately lead to ATP generation and the capture of free energy:-



(Forster & Goldstein 1969, Cohen & Brown 1960)

5.4 Ammonia can thus be thought of as a "low-energy" compound, compared with the other common animal excretory end-products, urea and uric acid. These latter products require an energy-consuming synthetic system (Cohen and Brown 1960) and can be thought of as being on a higher energy level; but where water conservation or excretory product storage are important, their less toxic nature makes them more suitable.

5.5 The dissociation of ammonia in solution is described by the relation:

$$NH_4^+ \rightleftharpoons NH_3^o + H^+$$

This equilibrium position is dependent on pH so that, according to the well-known Henderson-Hasselbalch equation:-

$$pH = pK_a + \log_{10} \frac{(NH_3^o)}{(NH_4^+)}$$

In acid solution the equilibrium is in favour of NH_4^+, whilst an alkaline solution will contain a majority of NH_3^o radicals. Below pH 7, the quantity of NH_3^o present is usually negligibly small; at slightly higher pH even the low proportion present may be important in its effects. Free or unionised ammonia NH_3^o readily diffuses into and out of cells because it is lipid-soluble, enabling rapid elimination without water loss. Such diffusion is dependent on the partial pressure (pNH_3) gradient across the membrane. pNH_3 is related to NH_3^o by the equation:

$$[NH_3^o] = \frac{\alpha}{22.1} pNH_3$$

- where α is the solubility co-efficient (see Maetz 1973). Although ammonia is predominantly ionised at body fluid pH (NH_4^+ : NH_3^o is about 100:1), the interconversion is instantaneous and hence would not limit the rate of excretion (Hoar & Randall 1969, Goldstein & Forster 1970).

5.6 Substantial discussion in the literature has been directed at attempting to identify the principal source of ammonia excreted at the gills. Originally Smith (1929) proposed extraction at the gills from ammonia already circulating in the blood, but later modified his opinion in favour of its production <u>in situ</u> at the gills. Later workers (e.g. Goldstein & Forster (1961), Goldstein et al (1964)) have argued in favour of both views and various combinations.

5.7 De Vooys (1969) points out that although mostly in the ionic form (at pH about 7.4), ammonia in fish blood would

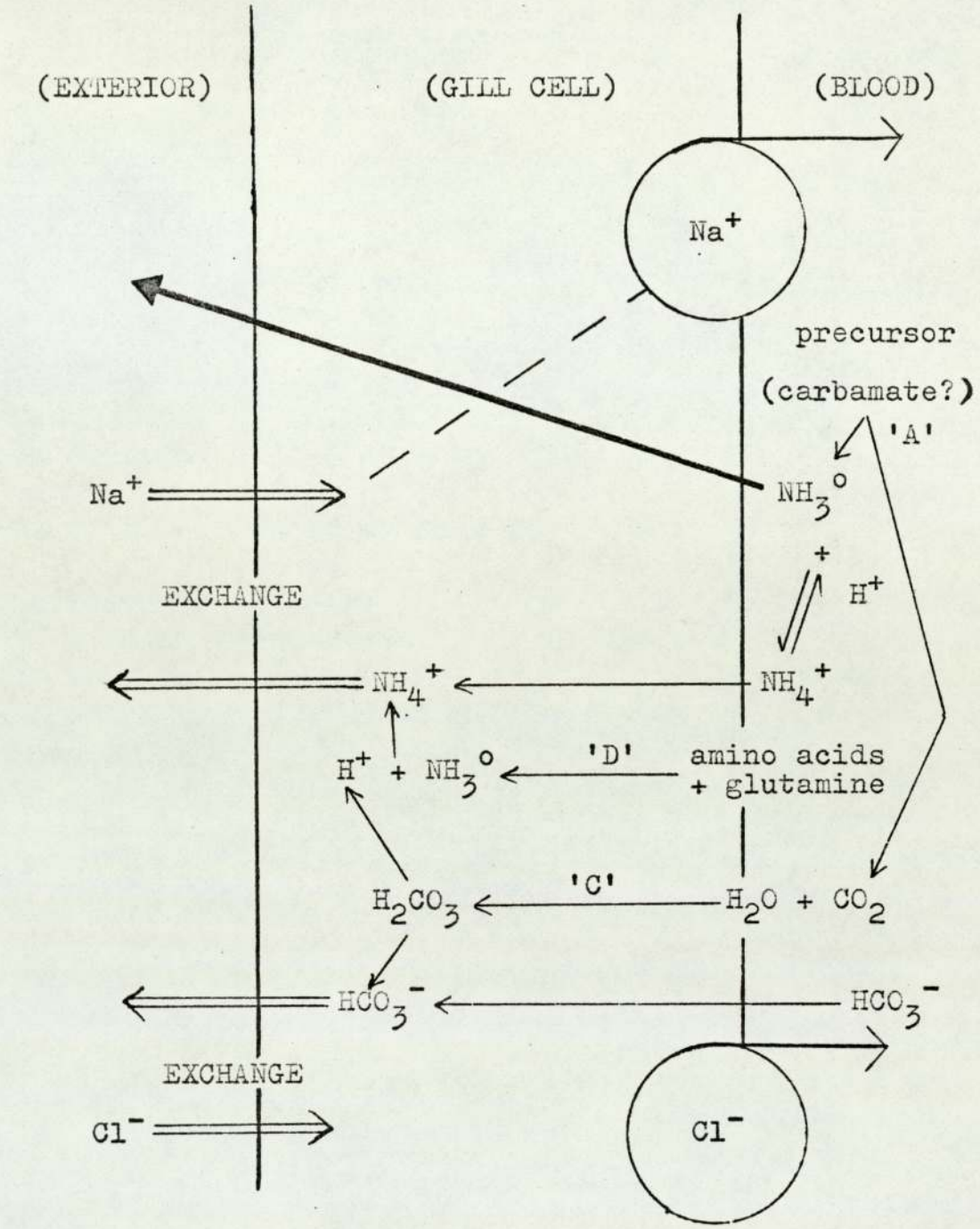
nevertheless continuously furnish NH_3^o molecules, which would act as a nerve poison. He suggests that ammonia occurs in the blood, is transported in a protein-bound carbamate complex and is released when CO_2 is liberated at the gills.

5.8 Thus both transport from other sites of formation (e.g. liver), and peripheral formation at the gills, seem to be possible routes of ammonia arrival at the gill surface. There is also controversy about the passage of ammonia across the gill membrane into the surrounding water; ionic ammonia could be immediately transformed to NH_3^o which could pass freely out, but another possibility also exists.

5.9 Maetz and Garcia Romeu (1964), in studies of ion transfer across the gills of the fasted goldfish Carassius auratus, postulated an exchange of NH_4^+ for Na^+ ions actively taken in. Romeu and Motais (1966) repeated this with the eel Anguilla anguilla. However, Kerstetter et al (1970) using Salmo gairdneri postulated a Na^+/H^+ exchange rather than exchange for ammonia, and De Vooys (1968) also failed to support a Na^+/NH_4^+ exchange, using carp.

5.10 More recently, Maetz (1972, 1973) has fully discussed the exchange across Carassius gills, and indicated that according to pH and available external sodium, ammonia can move either as NH_3^o or as NH_4^+, and that both H^+ and NH_4^+ can be excreted. Excretion is probably limited to NH_3^o only when there is no external sodium.

5.11 All of this work has necessarily been with individual experimentally fasted fish, and the importance of the relative occurrences of ion exchange and passive NH_3^o transfer are unknown for fish in a feeding culture situation. Under these conditions there would be a higher requirement for ammonia elimination, and hence it could be supposed that passive NH_3^o transfer would account for the greater proportion, much in excess of the amount of ion required for Na^+ exchange. Figure 5.1 summarises some possible mechanisms involved in gill



'A' = carbamate deaminase 'C' = carbonic anhydrase

'D' = deamidation/deamination enzymes

= active transport mechanism

Figure 5.1 Some possible ionic pathways in fish gills (modified from Maetz & Garcia Romeu 1964)

ammonia clearance.

Excretion in fish

5.12 Homer Smith's pioneering work (1929) on fresh-water fish in divided chambers established gill excretion as the major route of nitrogen loss, and that most of this was ammonia (60-90%), with a lower proportion of urea. Later studies have confirmed this (Wood 1958, Fromm 1963).

5.13 Kidney excretion via the urine was found to be relatively small (Denis 1913/1914, Smith 1929, Grollman 1929), and to consist of creatine, creatinine, uric acid and traces of other compounds including trimethylamine oxide (TMAO) and a very little ammonia.

5.14 Absolute values of nitrogenous excretory products seem at first sight to be relatively rare in the literature. The difficulty is clarified when the usual requirements of physiological work are taken into account:
a) to make valid samples over a period of time (usually requiring enclosure in a small tank for 12 or 24 hours);
b) to keep urinary and gill measurements separate (usually requiring anaesthesis, operation on the animal and catheterisation of the urinary papilla);
c) to exclude faecal contamination and variability due to feeding (usually requiring the use of fish which have been fasted for several days). Complying with these requirements is a pre-requisite for ionic balance work, but the penalty is paid in divorce of the experimental situation from "real life", and the necessary imposition of stresses which accompany experimental procedure. Such conditions are here designated "physiological".

5.15 Experiments have been performed under these kinds of conditions by several investigators (e.g. Smith 1929, Gerking 1955, Wood 1958, Goldstein & Forster 1961, Fromm 1963, Thornburn & Matty 1963, Fromm & Gillette 1968, Olson & Fromm 1971) in addition to those investigating ion transfer. Information

from these sources is included in Table 5.1 for comparison, although the more important data for this study are those tabulated from fish culture situations, especially those featuring salmonids. Table 5.1 also shows how often the important supportive information has not been recorded (e.g. nutritive status and temperature, see Fromm (1963) and Brockway (1950)). In the table specific excretory rate (SER) (mass of ammonia produced per kg per hour) is calculated from data from the authorities quoted.

5.16 Table 5.1 shows that under physiological (P) conditions of starvation, which lead to a fairly steady low excretion level, referred to here as the endogenous nitrogen excretion, ammonia output from fish is usually <10 mg kg^{-1} h^{-1} and frequently <5 mg kg^{-1} h^{-1}. (Endogenous excretion may be defined differently in nutritional studies (Gerking 1955)).

5.17 Culture situations (C) show a wider variety of values, usually higher. The data for salmonids are interesting in their diversity. Whilst Burrows (1964) quotes figures giving about 3-6 mg kg^{-1} h^{-1} for chinook salmon, presumably on production feeding schedules, Liao's (1970) survey of operating salmonid farms seems to indicate much higher values. Saeki (1958) and Shirahata (1964) roughly agree (between 10 and 20 mg kg^{-1} h^{-1} for rainbow trout), but Gigger and Speece (1970) found values about three times higher.

5.18 Clearly in the culture situation many factors can come into play, and temperature and feeding rates are likely to be particularly important. Liao's survey gives some indication of just how different feeding rates can be (see below), and it is not surprising that under these conditions the measured ammonia output is extremely variable.

5.19 Attention has been focussed on ammonia output, but it is necessary to examine briefly the role of the other excretory products, chiefly urea. The work of Olson and Fromm (1971)

Table 5.1 Ammonia excretion in freshwater fish

FISH	SIZE (g)	TEMP (°C)	NU. ST.	SER (mg $kg^{-1}h^{-1}$)	AUTHORITY	TYPE	COMMENTS
Cyprinus carpio (Carp)	420	-	S	1.36	Smith 1929	P	
	600	-	S	1.82			
	1500	-	S	1.15			
	1400	-	S	2.07			
	530	-	-	4.69			-starved?
	315	-	S	3.97			
	530	-	-	3.93			
	500	-	-	5.06			} -starved?
	500	18.5	-	4.05			
	368	18.5	-	5.46			
	1500	-	-	0.55			
	250	15	S	1.00	De Vooys 1968	P	10% of excreted N is urea
	-	19	-	14.5 T	Pora & Prekup 1960a	C	fed?
	30	16-25	F	4.17-8.33	Saeki 1958	C	
Carassius auratus (goldfish)	235	-	-	3.85	Smith 1929	P	-starved?
	255	-	S	1.61			
	220	-	-	3.84			} -starved?
	330	-	-	0.65			
	10-15	21	S	4.17 7.50 10.42 T	Thornburn & Matty 1963	P	
	80-330	18-23	S	1.05-7.00	Maetz & Garcia Romeu 1964	P	
	12	25	S	5.00	Cocking 1967	P	semi-starved (48 h)
	2	14.5	F	5.21	Saeki 1958	C	
	2	15.5	F	8.75			
	2	25	F	5.00			
	8-9	25	F	10.00			

Table 5.1 continued

FISH	SIZE (g)	TEMP (°C)	NU. ST.	SER (mg mg^{-1} h^{-1})	AUTHORITY	TYPE	COMMENTS
Lepomis Macrochirus (Bluegill sunfish)	34	20	S	9.15 T 10.55 T 8.37 T 9.42 T	Savitz 1973	P	after mild handling stress
	-	25	S	1.62 T	Gerking 1955 (quoted in Fromm 1963)	P	endogenous excretion of fish fed dextrose as energy source
	50	25	S	6.42 T	Gerking 1955	P	
	144	25	S	3.96 T			
Ameiurus nebulosus (Freshwater catfish)	-	-	-	1.05-4.90	Wolbach Heinenmann & Fishman 1959	P	starved?
Tilapia mossambica	8-30	30	S	upto 30.0	Kutty 1972	P	
average for several species	-	-	F	20.8 T	Saeki 1958	C	working figure for filter proposals
Salvelinus fontinalis	-	7.20	F	20.8 T	Saeki 1958	C	
(Brook or speckled trout)	16-30	10.50	S	4.79 1.29	Phillips Brockway et al 1954 (quoted in Fry & Norris 1962)	P	probably semi-starved
Oncorhynchus tschawytscha (Chinook salmon)	f	13	F	6.37	Burrows 1964	C	@loading (kg min^{-1} l^{-1}) 0.52
		11	F	4.97		C	0.61
		13	F	3.41-5.12		C	0.98

Table 5.1 continued

FISH	SIZE (g)	TEMP (°C)	NU.ST.	SER (mg kg^{-1} h^{-1})	AUTHORITY	TYPE	COMMENTS
Salmo gairdneri (Rainbow trout)	129	13	S	5.67 T 3.40	Fromm 1963	P	
	-	12-13	S	10.42 T 5.42	Fromm & Gillette 1968	P	controls for experiment
	150-350	13	S	1.68-4.20	Kerstetter et al 1970	P	
	50-100	13	S	6.67 T 3.54 1.25 U	Olson & Fromm 1971	P	at low ambient ammonia
	60	15	S	6.25 T	Phillips Brockway et al 1954 (quoted in Fry & Norris 1962)	P	
	-	20-25	F	12.50	Saeki 1958	C	
	4	13	F	7.92 18.75 T			
	f	-	F	17.0	Shirahata 1964	C	
	36	19	F	48.0	Gigger & Speece 1970	C	@stocking (g l^{-1}) 0.96
	36	18	F	31.5			2.39
	±150	11	F	14.90	Bozeman 1970	C	
salmon & trout generally	ALL	7-18	F	12.9-168.3 (mean 69.2)	Liao 1970	C	from questionnaire on hatchery practice

Notes:-

a) NU.ST. is nutritional status; S indicates starved to point of endogenous nitrogen excretion; F indicates fed

Table 5.1 continued

b) TYPE is the type of experiments providing data; P indicates physiological (similar to text description in para.5.14); C indicates culture (fish fed for growth)

c) SER values are for ammonia except where marked T (total nitrogen) or U (urea)

d) Sizes are by weight except where marked f (indicates fingerlings of unknown size) or ALL (indicates wide range of sizes up to maturity)

confirmed earlier suggestions (Fromm 1963, Fromm and Gillette 1968) that ammonia makes up 50-60% of the excreted nitrogen compounds under physiological conditions, while suggesting about 19% urea. However, it is important to note the difference between these conditions and those of the culture situation. Although Saeki (1958) also quotes about 50% as the proportion of ammonia, there is no evidence that this is based on measurements in the culture situation. There is no certainty that what may be true for starved fish is true for actively feeding and growing fish, although Fromm (1963) found ammonia at about 60% of total-nitrogen excreted after only two days of starvation.

5.20 In this context the evidence of Burrows (1964) is interesting, and suggests that, at least in chinook salmon, urea is a significant, even dominant, excretory product at low fish loading levels. Consideration of some measure of fish density in the water in connection with their excretion is a helpful concept. and in this study some of the various possible parameters are used as arbitrarily defined below:-

LOADING -- the fish mass per unit flow of water (measured in kg per unit flow rate; the resultant units are kg min ℓ^{-1})

STOCKING -- fish mass per unit volume of water (units: g ℓ^{-1}, kg ℓ^{-1})

DENSITY -- number of fish per unit volume of water (units: fish ℓ^{-1})

5.21 Burrows' results are shown in Fig.5.2. The ammonia levels are surprisingly low in the light of the work of Liao (1970), and Gigger and Speece (1970). Some question must be raised over the sampling technique used (from the effluents of large raceways) and the kind of feeding regime employed, which is not discussed. (It has since been shown that the ammonia profile varies in different parts of a stocked raceway and so effluent values may not be reliable excretion indicators (Bozeman 1970)).

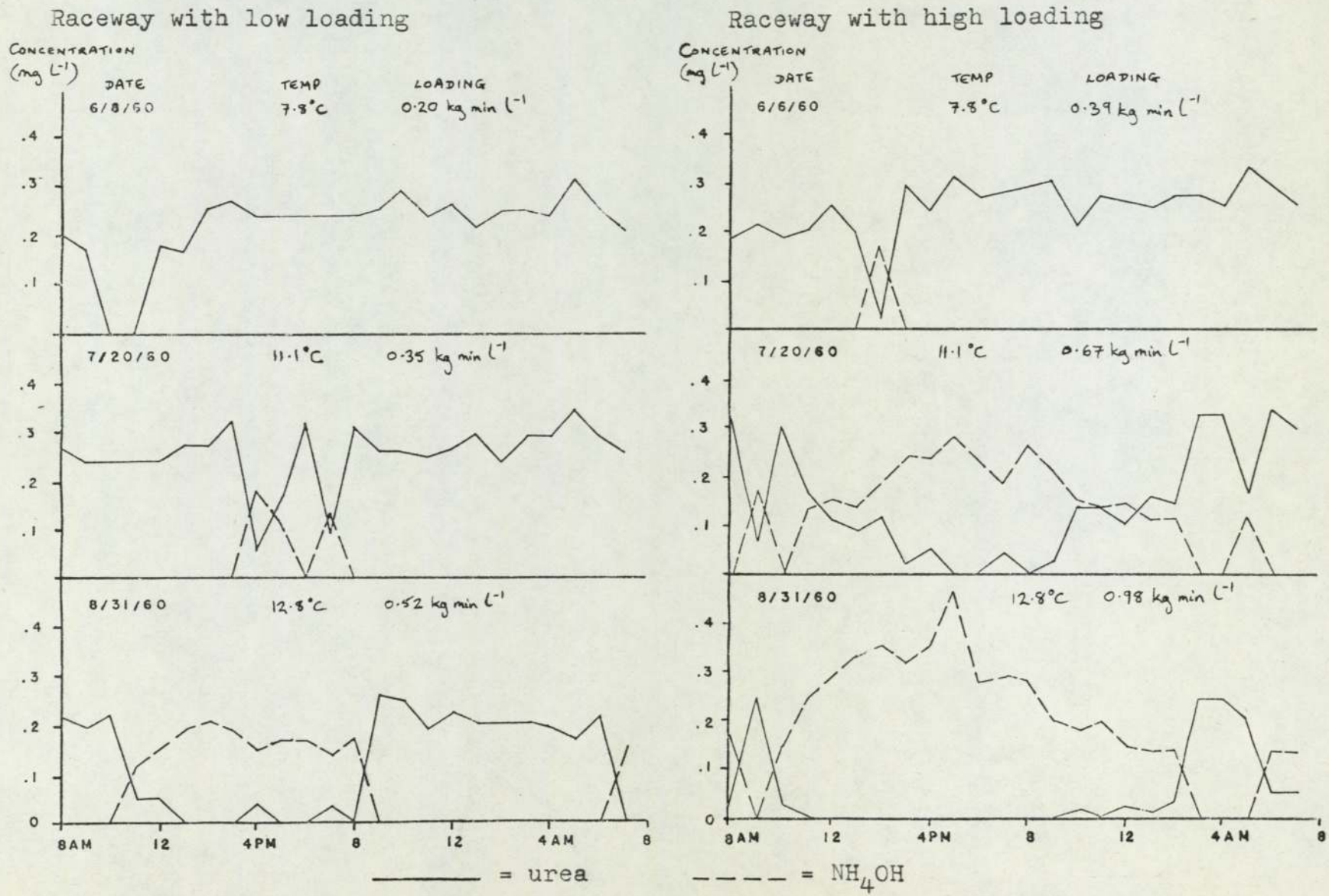


Figure 5.2 Patterns of chinook salmon excretion in raceways (modified from Burrows (1964))

5.22 Gigger and Speece observe that many bacteria can hydrolyse urea to form ammonia;

$$CO(NH_2)_2 + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

and appear to base their work on the assumption that this will happen in any biological filter employed, and that urea is consequently not important enough to measure. This seems illogical, because if a filter system is dealing with (among others) two reactions,

i.e. ammonia → nitrate

and urea ⟶ ammonia

- then the output ammonia level must depend on both the ammonia and the urea in the input. If, however, the urea proportion can be regarded as remaining approximately constant, and in particular if it is low, then a case can be made out for the dismissal of urea as a separate measurement variable, with minimal error caused thereby. Burrows' work suggests that at higher loading levels (more typical of intensive fish-farming situations) ammonia is greatly dominant, and so it was felt justified in the current work to assume for working purposes the relationship described above i.e. that urea is a very low and approximately constant proportion of excretory output.

5.23 Urea is a relatively unimportant teleost excretory product, compared to ammonia. Its production, and the evolutionary aspects of its occurrence, have been discussed by Cohen & Brown (1960, 1963), Prosser & Brown (1961), Huggins et al (1969), and Forster & Goldstein (1969). Although alternative pathways may also exist, the poor development of the common ornithine cycle route to urea formation in fish would support the contention that urea production is likely to be overshadowed by ammonia in a normal growth situation. Certainly Burrows' results do not seem to have been confirmed by later work. Bearing these things in mind, it has seemed reasonable to leave urea, and to concentrate solely on ammonia for the purposes of this study, recognising, however, that

urea ought also to be examined in the future.

5.24 Liao's (1970) work is particularly interesting in the ammonia production context. He sampled several salmonid hatcheries to measure pollutant production, especially in relation to feeding rates and stocking levels. His relationship-line derived from ammonia production data plotted against feed rates (the latter measured in % BWD) is somewhat unconvincing, and when examined statistically the graph (Fig.5.3) has a correlation coefficient of only 0.42 ($P>5\%$,NS). If the arrowed point is ignored, as the line invites, it can be seen that below 4.5%, %BWD seems to have a negligible effect on ammonia excretion, all values being below about 46 mg kg^{-1} h^{-1}. The graph suggests rather that above 4.5 %BWD (i.e. with very small fish below about 5g) the ammonia excretion rate is markedly higher, with almost no transition stage between the two zones.

5.25 Liao's data for ammonia output and stocking levels are plotted in Fig.5.4. The result is not similar to Liao's own figure, and the derivation of his plotted data is not given. Liao's contention is that the relationship between stocking and ammonia output is a downward curve. This would not give a satisfactory explanation of Fig.5.4, which does not lead to any definite statement.

5.26 Liao's work nevertheless introduces several very useful points. Emphasis is laid on the nutritive state and a measure of fish density in the water (in this case stocking), and the multi-variable conditions in hatchery practice are to some extent taken into account. Nevertheless, the approach is only the beginning of such a technique, and only treats a few of the variables. Size is not taken into account, nor temperature; and in the very varied feeding conditions likely to be involved, these factors are probably only roughly expressed through the feed rates (hence the inconclusive nature of Fig.5.3). It is clearly important and desirable to evaluate

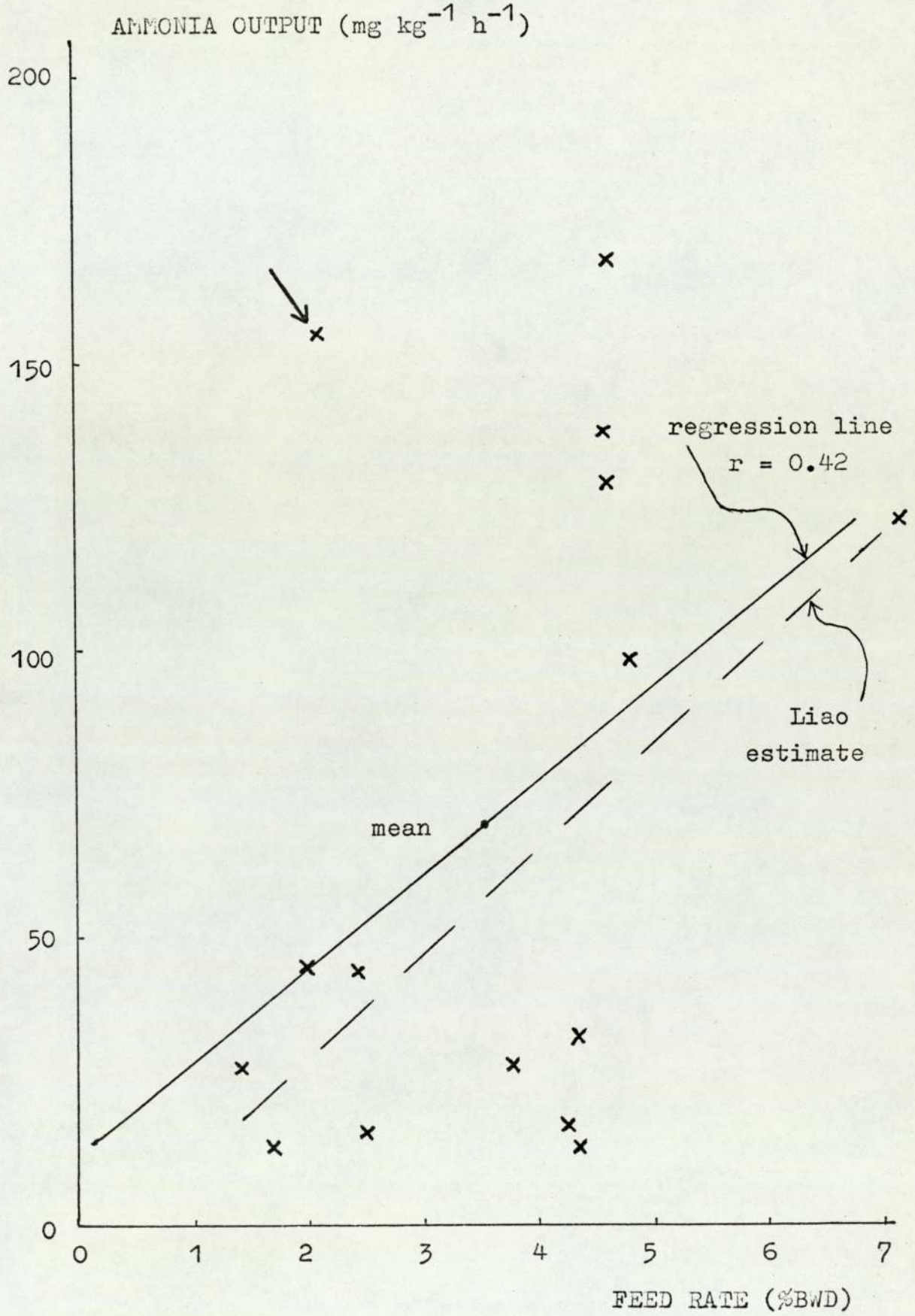


Figure 5.3 Ammonia production and feed rate

(from Liao 1970)

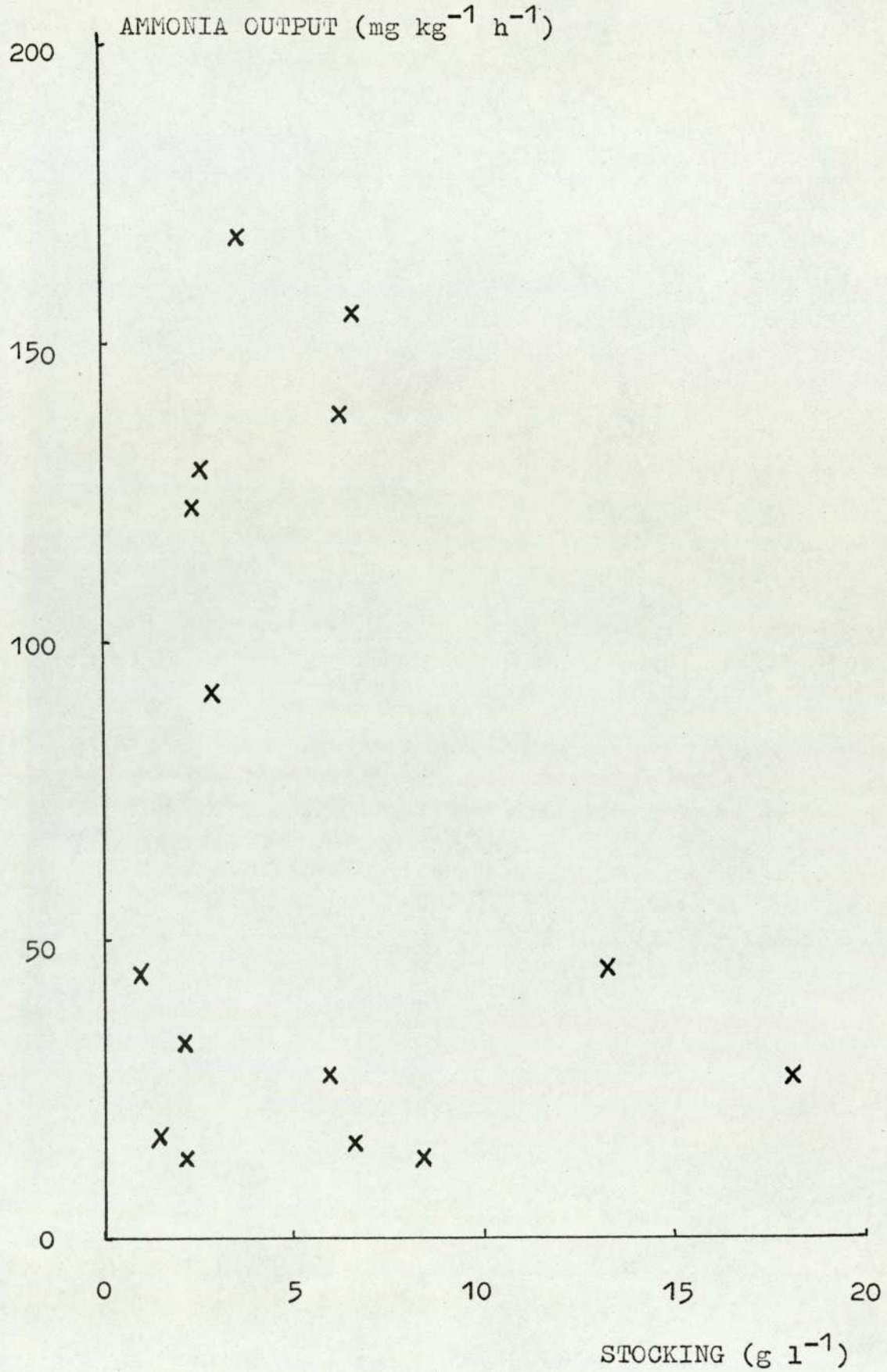


Figure 5.4 Ammonia production and stocking

(from Liao 1970)

the influence of environmental and culture factors on ammonia output.

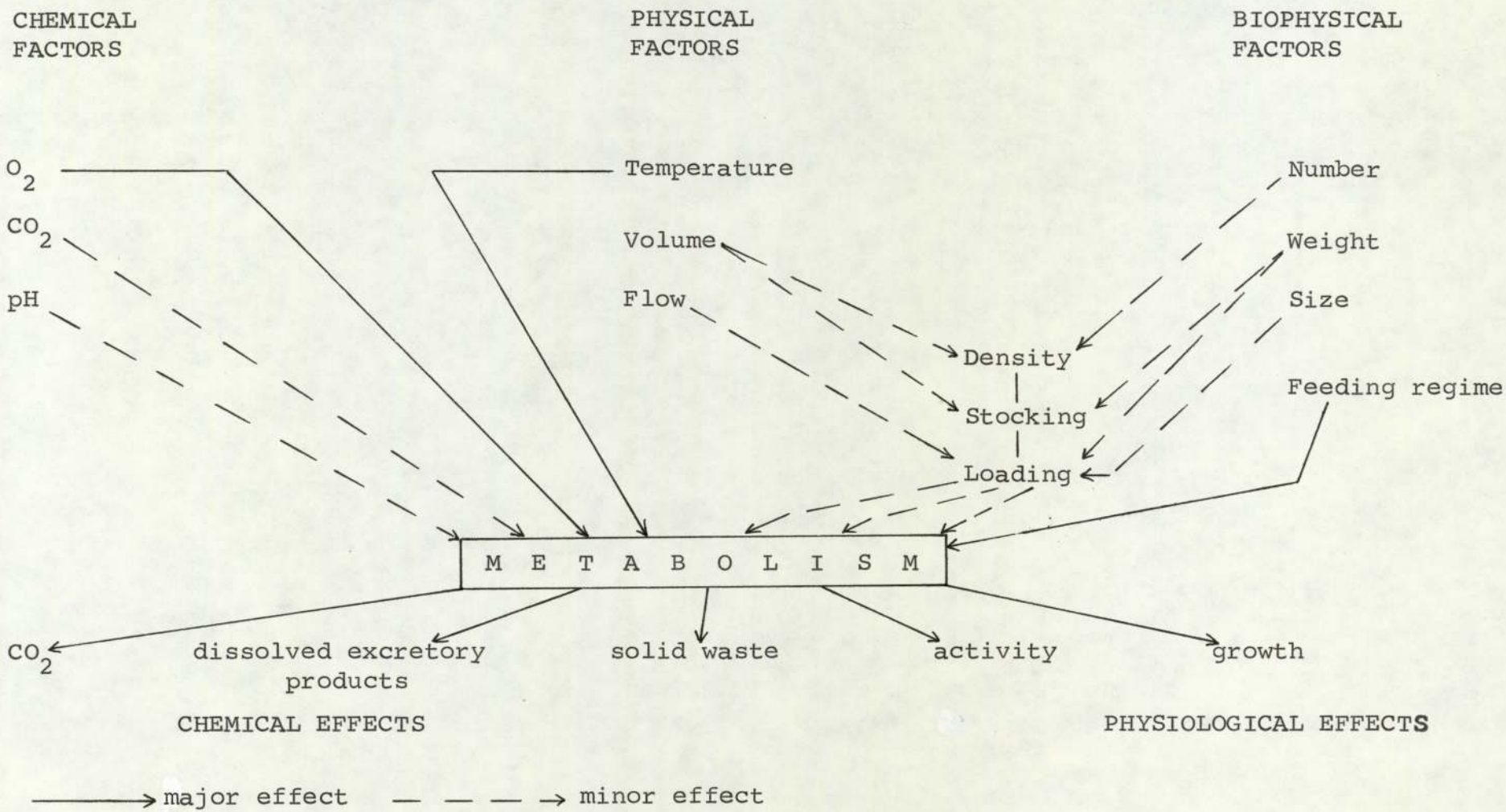
Influence of environmental factors on excretion

5.27 The excretory output of a fish can be considered, in a similar way to oxygen uptake, as a generalised measure of at least some aspects of the metabolism. Thus it is to be expected that factors which influence metabolic rate as determined by oxygen uptake, might also influence excretory, and in particular ammonia, production.

5.28 A much-simplified model of some of the conditions which could be involved in fish culture situations is given in Figure 5.5, where all internal changes, reactions and pathways are contained within the box labelled Metabolism. Metabolism is thus likened to a "black box", and is analogous to the position of Organism in simple stimulus-response behavioural theory.

5.29 Oxygen is clearly of extreme importance; this is a "supply" quantity which the fish draws upon constantly to service its energy requirements. Kutty (1972) reports experiments on Tilapia mossambica which indicate that when oxygen is plentiful (above a concentration of 5 mg ℓ^{-1}) the ammonia quotient (AQ) is about 0.23. (AQ is the ratio of volume of ammonia produced to volume of oxygen taken up). This value is quite close to 0.27, the theoretical value of AQ for 100% aerobic protein metabolism. At low oxygen, there is increased utilisation of protein as an anaerobic energy source, and the liberated ammonia is much higher in proportion to oxygen uptake. This anaerobic stage Kutty (1968) found at about 3 mg ℓ^{-1} oxygen in rainbow trout. As was necessary for his experiments, Kutty used starved fish, and hence the relationship of this work to cultured fish is not clear since energy in the culture situation will be provided by carbohydrate substrate. Whilst it is known that low dissolved oxygen can lessen growth

Figure 5.5 Simplified model of some environmental factors in culture



(Herrmann, Warren & Doudoroff 1962), the effect on ammonia excretion does not seem to be clear, and is probably influenced by the growth effect anyway. Clearly it would be advisable in ammonia production studies in culture situations to preserve growth and rule out complications by maintaining oxygen at non-limiting concentrations.

5.30 Temperature also has a fundamental effect on ammonia production. In early experiments Phillips et al (1947, 1949) detected rises in ammonia excretion with temperature increase in small-scale trials with brook trout. A ten-fold rise was associated with a temperature increase from 47 to 60°F (13 deg F; or 7.2 deg C from 8.3 to 15.5°C). These frequently quoted data (e.g. Brockway 1950, Coates 1962) give rise to a "Q_{10}" value of about 13.9, which is considerably higher than that deriving from Pora & Prekup (1960b) or Maetz (1972); 2.3 and 3.9 respectively. These authors were however using different fish (carp and goldfish) and those of Maetz were starved.

5.31 Nutritional effect has already been mentioned; it is important to note in this context that the feeding rate for salmonids in most culture situations is governed largely by temperature and size of fish (e.g. see Table 1.3 in Chapter 1). Thus effects said to be due to feeding rate might indirectly be ascribed to these quantities. Whilst Speece (1973) takes into account a rather different way of determining feeding rates (see Chapter 7), he also relates ammonia production by trout to the amount fed, quoting data from Bozeman. Ammonia is expressed as a proportion of the weight of food fed, and on this basis, with only two quoted data, Speece goes on to assume a definite positive relation between temperature and ammonia production, and further assumes it to be linear.

5.32 Despite repeated attempts, it has been impossible to obtain from Bozeman Center the original data on which these assumptions are made (Speece's reference does not contain

them); hence it must be assumed that only two points were originally defined. This must leave some doubt over the validity of Speece's argument, and his subsequent system of designing filter units to cope with the ammonia. More information is urgently needed on the effects of temperature and size, as expressed through feed rates.

5.33 Forster & Goldstein (1969) quote a pH effect on excretion, stating that acid or neutral water favours NH_3^o output as compared to alkaline water. Wolbach, Heinenmann and Fishman (1957) showed that internal pH changes could affect ammonia loss, and especially that internal acidosis decreased the gill concentration gradient for free ammonia diffusion to the outside. This would be equivalent to an external rise in pH; the external equilibrium would be shifted in favour of NH_3^o and the NH_3^o concentration gradient would be decreased, hence a decrease in ammonia output. However, it is likely that the pH changes involved in such comparisons are relatively large compared to the acidification caused by output of CO_2 by fish under culture; and since the latter, at high oxygen levels, will be dependent on some measure of body size or weight, it is unlikely that measurements of ammonia output under culture conditions need to involve a direct measure of pH effect on excretion, at least in a first analysis.

5.34 It is now necessary to examine the "biophysical" factors of fish biomass in culture situations as a series of factors which may affect excretion. Certainly stocking, loading and density (see para.5.20) will affect the ammonia concentration in the water, given a definite excretion rate, but it is also important to know whether they have feedback effects on the actual excretion rate itself. Early small-scale experiments by Phillips et al (1947) suggested that at constant flow, ammonia excretion fell with an increase in water volume in the container, and at constant volume ammonia excretion fell with an increase in flow. However the same group of workers (1948) later cast doubt over these experiments

and the difficulty of scaling up results for a raceway production situation. Pora & Prekup (1960a) quote a reduction in nitrogen excretion by carp when the stocking rate is increased, suggesting a feedback prevention leading to auto-intoxication, but a reasonable body of data is not found until the somewhat uncertain assertions of Liao as discussed in para.5.25.

5.35 The important topic of stress will be discussed in Chapter 8, but it should be pointed out that "feedback" effects such as those discussed above can be viewed as a kind of stress. Gigger & Speece (1970) have suggested that factors which increase activity (e.g. higher feed rates or mechanical stress in the water such as excessive fish-chasing while netting, causing over-excitement) can be viewed as major sources of increased excretion, whilst disease and presence of toxic materials would be negative in their effect on excretion by way of decreased activity.

5.36 Putting much of this past work into the context of fish culture, it is clear that there is a great shortage of reliable information on which to base prediction of excretory rates. Whilst many of the effective variables have been identified, their measurable effects have been only loosely quantified, and in a variety of ways, so that comparison between studies is difficult or confusing. With this in mind, a fuller investigation of ammonia excretion by rainbow trout in a circular-tank culture situation has been attempted.

INTRODUCTION TO E-SERIES EXPERIMENTS

Tank excretory theory

5.37 The relationship between flow, ammonia production and ammonia concentration in a constant volume of water can be described at a crude level in terms of a mathematical model governed by simple physical laws, if it is assumed in the first instance that fish produce ammonia at a constant rate

(Brookes 1971).

5.38 Let

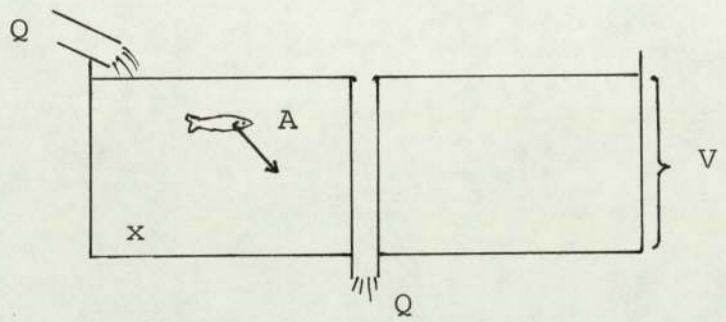
x = mass of ammonia in tank at time t

$x + \delta x$ = mass of ammonia in tank at time $t + \delta t$

V = volume of water (constant)

A = mass of ammonia produced per unit time (assumed constant)

Q = water flow rate



Then

mass ammonia produced in interval $\delta t = A\delta t$

Assuming inflow = outflow = Q

mass ammonia flowing out in interval $\delta t = \frac{Qx\delta t}{V}$

Since δx is the increased mass of ammonia in the tank,

$$\delta x = A\delta t - \frac{Qx\delta t}{V}$$

$$:- \quad \frac{dx}{dt} = A - \frac{Qx}{V}$$

$$:- \quad dt = \frac{dx}{A - \frac{Qx}{V}}$$

$$:- \quad t + \text{constant} = \int \frac{dx}{A - \frac{Qx}{V}}$$

$$= -\frac{V}{Q} \ln \left(A - \frac{Qx}{V}\right)$$

Now at time $t = 0$, mass ammonia $= x = 0$

$$:- \quad \text{constant} = -\frac{V}{Q} \ln A$$

$$:- \quad t - \frac{V}{Q} \ln A = -\frac{V}{Q} \ln\left(A - \frac{Qx}{V}\right)$$

$$:- \quad t = -\frac{V}{Q} \ln \left(1 - \frac{Qx}{AV}\right)$$

$$:- \quad 1 - \frac{Qx}{AV} = e^{-\frac{Qt}{V}}$$

$$:- \quad \frac{Qx}{AV} = 1 - e^{-\frac{Qt}{V}}$$

$$:- \quad x = \frac{AV}{Q}\left(1 - e^{-\frac{Qt}{V}}\right)$$

Under constant conditions, the bracketed function will increase to 1 as the exponential function disappears (at asymptotic value) (see Table 5.2) and so

$$\text{(maximum) } x = \frac{AV}{Q}$$

Now since the measured quantity (ammonia concentration)

$$= c = \frac{x}{V}$$

$$:- \quad c = \frac{A}{Q} \quad \text{or} \quad A = cQ$$

Thus the excretion can be calculated knowing ammonia concentration and water flow at equilibrium conditions, independent of volume.

5.39 In practical terms, the assumption that fish excrete at constant rate is unjustified, as previously discussed. Further, if the excretion rate is variable, then the bracketed function mentioned above may not be unity in all cases of measurement. This should always be borne in mind when calculating excretory rates from water concentrations. Nevertheless for the purposes of this study, the apparent specific excretory rate (SER) will be utilised, defined as the product of ammonia concentration and water flow per unit weight of fish.(Units employed: mg kg^{-1} h^{-1}.) The error involved in this approximation will depend on equilibration time, and the relative magnitudes of volume, flow and ammonia output. In circular tanks, mixing is usually good (Larmoyeux, Piper & Chenoweth 1973), so that creation of zones of different ammonia concentration is much less important than in large raceway production (Bozeman 1970) (see para.5.21); hence effluent sampling will be more reliable for excretion esti-

Table 5.2 Example equilibrium relationship between ammonia production and time

(Ref. para.5.38)

Let $A = 20 \text{ mg } h^{-1}$ (1 kg of fish excreting ammonia at 20 mg kg^{-1} h^{-1})

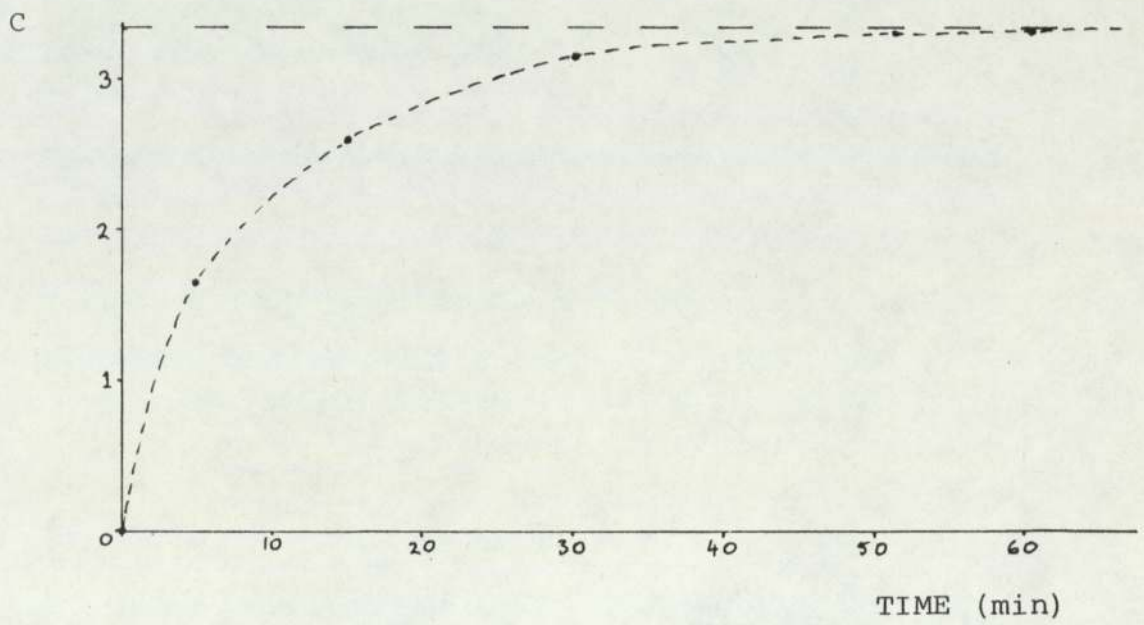
$V = 10 \ell$

$Q = 1 \ell \text{ min}^{-1} = 60 \ell \text{ h}^{-1}$

and

$$C = \frac{A}{Q}\left(1 - e^{\frac{-Qt}{V}}\right)$$

t (min)	e FUNCTION	C (mg ℓ^{-1})
0	1.0000	0.0000
5	0.5000	0.1667
15	0.2231	0.2590
30	0.0498	0.3167
60	0.0025	0.3325
∞	0.0000	0.3333



mates. From the point of view of the operating experimental system, the ammonia concentration in the water remains the only measurable parameter for estimating ammonia output, and hence apparent SER is in practice a more useful quantity than the true excretory rate in a situation where the latter may be fluctuating. All subsequent references to SER indicate apparent SER, as defined above.

5.40 Burrows (1964) found evidence of diurnal fluctuations in levels of excretory products, and it is possible that the considerations of the preceding paragraph, under his particular raceway conditions, could account for the low values of SER which his data suggest. Liao (1970) took no account of such fluctuations in presenting his work, and more recent work at Bozeman Center (Smith 1972, Larmoyeux & Piper 1973) has been geared to ammonia concentration readings over very long intervals (two weeks). This necessarily does not take account of fluctuation between different days, although readings were standardised to "mid-afternoon".

5.41 If the diurnal fluctuation is accounted for by taking daily measurements at the same point in the fish cycle, then values ought to be reasonably comparable. It is then important to analyse the various factors which have contributed to the measured ammonia level. By reference to Figure 5.5, and with suitable additions, it seems that the inter-relationships involved in ammonia excretion can be summarised as in Figure 5.6. The ammonia SER will depend on various influences on metabolism, whereas total ammonia concentration or (TOA) and unionised ammonia concentration (or UIA) are more simply controlled (and hence simply calculated from measurements).

5.42 The design of the E-series experiments which are documented in succeeding chapters was such as to investigate the effects of most of these variables as fully as possible. (E-series designates those experiments on the effect of

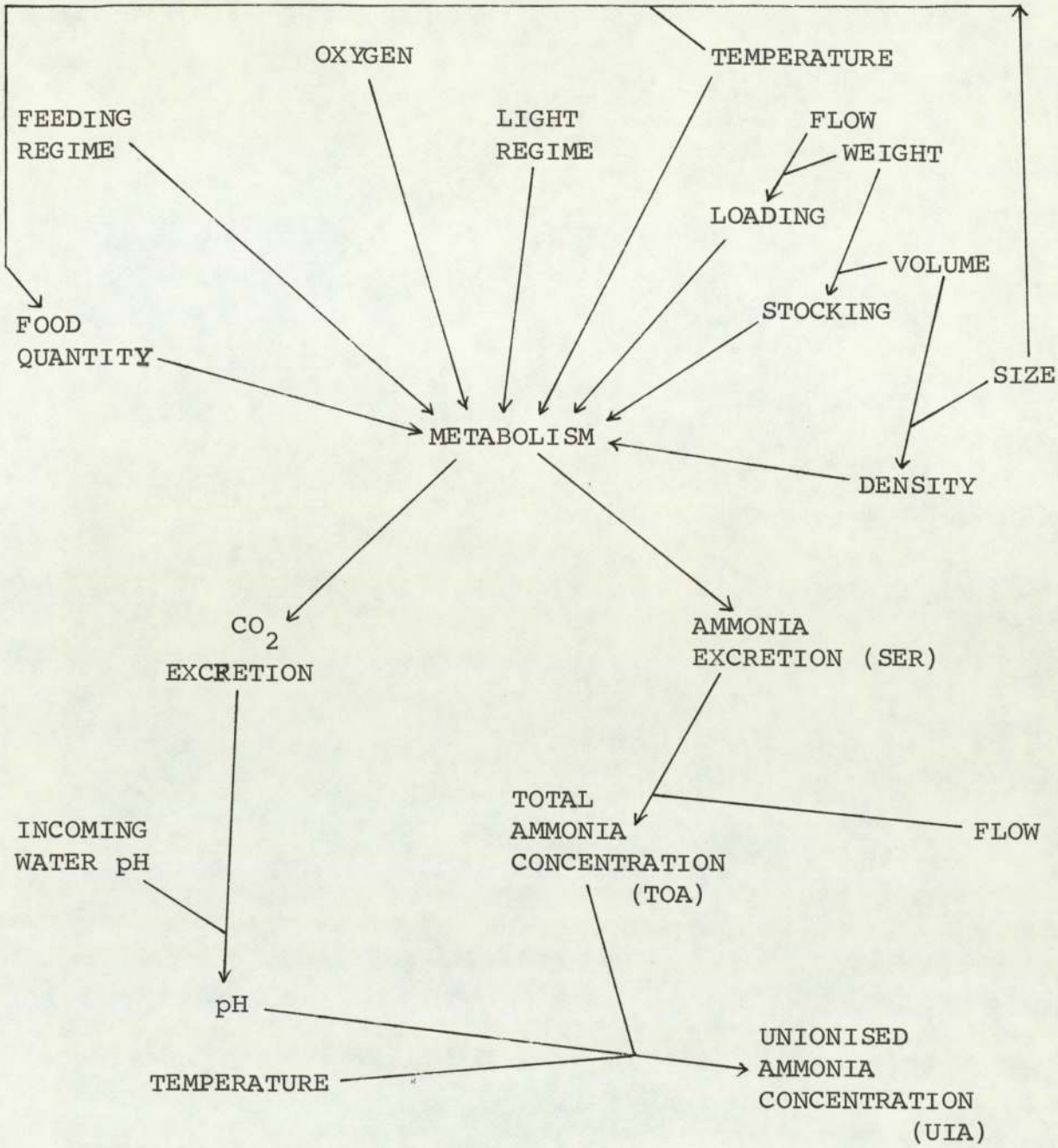


Figure 5.6 Relationships in ammonia excretion

environmental factors on ammonia excretion). However, with the considerations of para.5.29 in mind, all experiments were designed to operate in non-critical conditions of dissolved oxygen. Thus oxygen should be removed as an effective variable. Further design targets were to produce predictable and regular lighting and feeding regimes; so that under stipulated timing of light and food presentation, the major effects of temperature, loading, stocking and density could be studied. Food quantity is a major direct effect, but depends primarily on temperature and size; its treatment as a variable is complex, and requires ongoing explanation during the description of experiments.

5.43 Apart from initial pilot experiments (see below), the approach adopted was to allow a multivariable situation in which the varying factors and ammonia production were simultaneously measured in a series of similar experiments. This approach was used for two major reasons;

a) the apparatus and experimental conditions involved did not allow for temperature control, thus this important variable would have been able to fluctuate in any case;

b) a multivariate treatment, with suitable analysis, allows much more ground to be covered in a limited period of time than an experimental series where each trial has set controlled conditions, with just one variable. This latter situation is in any case extremely difficult to contrive in fish work, since attempting to keep all tanks the same except for one environmental variable is almost impossible. At the end of a single experiment under full control, the experimenter has a series of points to plot on one graph; these points are only reliable for those particular conditions, and to account for any new variables, the experiments required to gain a reasonable amount of information mount up in a geometrical fashion. If a full treatment of each variable is envisaged, the number of experiments involved rapidly becomes excessive. A multivariate approach gives up the precision of set levels of

independent variables in favour of allowing them to vary arbitrarily over their ranges, with simultaneous measurement of all of them. As all independent variables are free to vary, the number of experiments performed need only be enough to encompass a representative range of values for each one, together with enough replication to provide a reasonable number of points. The operational drawback is that all independent variables must be measured in every instance.

5.44 With such an experimental design it is important to prevent variables that cannot be measured from operating, if this is at all possible. A particular example is disturbance of the fish by people or other nearby movement; since the effect would be to increase activity, there could be an unmeasured effect on ammonia production. In this case the remedy is relatively simple; curtains surround the tanks and cut off the fish from disturbance or uncontrolled lighting.

5.45 In order to provide sufficient flow and to remove problems of filtration or ammonia accumulation in deriving data in the E-series experiments, an open-flow system was used, with all effluent water from the tanks run to waste. This system also ensures a continual supply of fresh aerated water to the fish, and conserves space in organising and building tank systems.

5.46 Although experience proved pH to be low enough to keep UIA to extremely low values, it is necessary to be aware of the probable changes in the nature of ammonia in a tank containing fish as compared to the influent water. Incoming water will contain a very low value of TOA (TOA_1) of which a small proportion is UIA (UIA_1). In the fish tank it could be postulated that this will undergo change as shown in Figure 5.7. The fish excrete ammonia, raising the TOA by ΔTOA to TOA_2. Since pH is somewhat lowered in the presence of the fish, the proportion of UIA due to influent falls to UIA_2, but added to

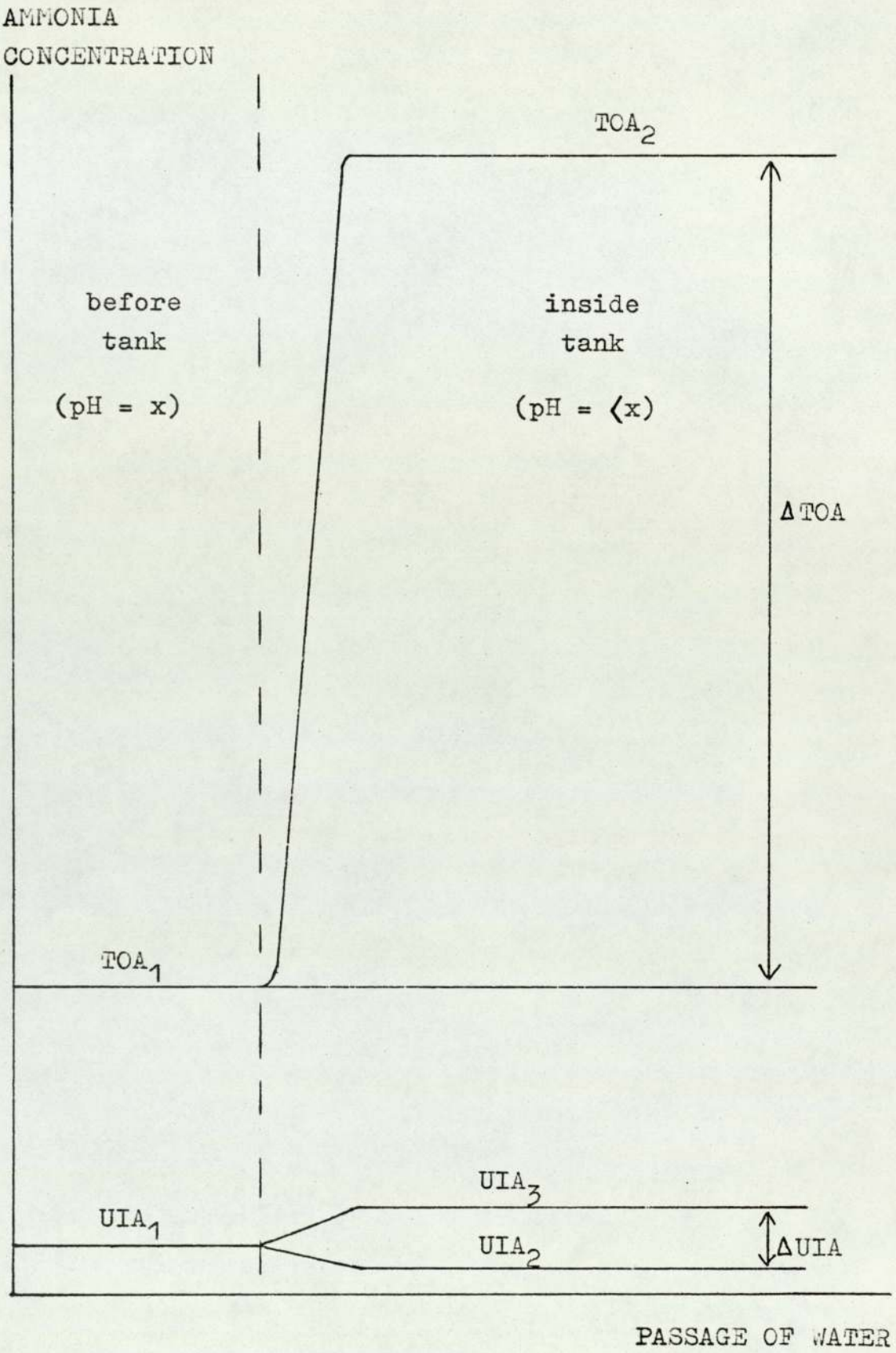


Figure 5.7 TOA and UIA in water passing through a fish tank

this is the UIA due to excretion, raising the overall UIA to UIA_3. By measuring ammonia in the water the experimenter observes the final TOA value (TOA_2, due to both excretion and influent), and by pH measurement and calculation arrives at UIA_3 (also due to both excretion and influent). In practice TOA_1 was found to be so low that the measured value of TOA_2 could be assumed to be equivalent to ΔTOA (due to excretion only), with a minimal error (see Chapter 4). Similarly UIA_3 would approximate ΔUIA. This model assumes complete mixing of water prior to measurement, a requirement which is normally fulfilled, if the tank has a reasonable flow and contains actively swimming fish, when dealing with circular tanks. Provided that pH is low enough (in practice below about pH7), the proportion of UIA present is so low that its concentration does not even approach 25 $\mu g\ l^{-1}$. This is the level at which Lloyd and Orr (1969) estimated UIA to have no diuretic effect on rainbow trout, and this is probably the most sensitive short-term indicator of adverse effect found to date (see Chapter 8). In all E-series experiments UIA was referred to this standard, and if below it (in nearly all cases) was ignored. Thus SER is envisaged in terms of TOA only.

Introduction to pilot E-series experiments

5.47 Before proceeding to experiments for a full multivariate analysis, it was decided to undertake several pilot experiments. These were necessary to formulate a reference framework for the later experiments both in terms of apparatus and experimental facilities, and in terms of methodology.

5.48 Using a pilot experimental system (see Chapter 2) three pilot experiments were carried out. The major objectives were as below:-

a) to test the feasibility of measuring ammonia concentrations under flowing fish culture conditions,

b) to evaluate methods and techniques required,

c) to evaluate apparatus and experimental system,

d) to check the behaviour and well-being of fish in the imposed conditions,

e) to obtain preliminary information on the levels of ammonia to be expected,

f) to obtain preliminary evidence on the effect on SER of some different stocking and loading conditions,

g) to briefly assess any diurnal rhythm in excretion and estimate optimum parts of such a cycle for later analysis.

5.49 For these pilot studies, the influence of "crowding" as a possible stressor was not assessed except in terms of stocking and loading. The concept of crowding is difficult to pin down in this kind of work. Fish might or might not react to any or all of the following quantities:-

a) stocking (weight per volume)

b) loading (weight per flow)

c) size (length)

d) combination of (b) and (c)

e) number of fish

f) combination of (c) and (e)

g) density (number per volume)

h) mean free path ($\sqrt[3]{\text{volume/number of fish}}$)

i) combination of volume and (b)

j) hierarchical effects due to size differences

k) pheromones

There are other possibilities also; for the purposes of the E-series in full, several of the likeliest quantities and combinations are tested in the multivariate analysis of data, but for the purposes of the pilot experiments, only stocking and loading are treated.

5.50 To set this in context, a list of stocking and loading values from various authors is given in Table 5.3, converted from the units used to those of this study.

Table 5.3 Salmonid stocking and loading from culture systems

AUTHOR(S)	YEAR	STOCKING ($g\ l^{-1}$)	LOADING (kg min l^{-1})	COMMENTS
Phillips et al	1948	3.2 - 47.8	-	quoted raceway usual values
		47.8 - 79.8	-	hatchery troughs
Tanizaki et al	1957	200.0	-	during transport in aerated container on truck (short term)
Saeki	1958	-	1.10	series of aquaria
De Witt & Salo	1960	-	0.21	combination of raceways and ponds
Burrows	1964	-	0.52	exptl. 4' x 40' raceways
		-	0.98	
Parisot	1967	0.3	0.10	4' diam. circular tanks
Burrows & Combs	1968	-	1.00	17' x 75' rectangular circulating ponds (projection)
Robinson & Vernesoni	1969	34.0	7.20	20' diam. circular pond
Bridges et al	1969	25.5	1.50	4') diam.
		25.0	3.33	6')- circular
		25.0	3.96	8') tanks
Liao	1970	(1.1 - 18.2 (mean 5.6	0.06 - 3.11) 0.51)	various US hatcheries
Bozeman	1970	55.9	1.69	6' x 60' exptl. raceway
Buss Graff & Miller	1970	505.0	1.40	jar) vertical
		125.4	1.80	drum)- unit
		136.9	1.66	silo) culture
FSC	1971	35.8	0.04	25' diam circular pond
Scott & Gillespie	1972	46.9	1.30	exptl. circular tank

Table 5.3 continued

AUTHOR(S)	YEAR	STOCKING ($g\ \ell^{-1}$)	LOADING ($kg\ min\ \ell^{-1}$)	COMMENTS
Bardach Ryther & McLarney	1972	-	2.20	market size finger-lings) US trout farm race-ways*
		-	1.50	
Prewitt	1972	-	11.98	) series of cir-cular ponds
Michalek	1972	-	7.57	
JR/PS	1974	48.0	-	circular ponds on Scottish trout farm

* quoted for Snake River trout farm, world's largest trout producer

6. PILOT SYSTEM EXPERIMENTS

METHODS AND MATERIALS

Fish

6.1 Young rainbow trout for the three pilot experiments (PO1, PO2, PO3) were sampled from an original batch (Lot O1) of about 480 fish purchased as fish of less than 5 cm length (FCAT 1 see Chapter 1). Brought to Aston in May, 1972, they were fed progressively on Salmon No.2 and 3 diets and held in HCl conditions (see Chapter 1). For experiment PO1 40 fish were selected; for PO2: 39 fish; and for PO3: 84 fish; the only selection criteria being healthy appearance and required size (FCAT 4 for PO1, FCAT 6 for PO2 and PO3) to make up the desired fish weights for the experiments.

6.2 Since PO1 did not begin until early September 1972, all fish had at least a four-month history of growth and maintenance in Birmingham tap-water prior to experiments. Fish were changed to Trout No.4 floating pellet diet in November 1972, between PO1 and PO2. PO2 began in early January 1973 and PO3 in late January /early February.

Tanks

6.3 ST type 1 tanks were employed in PO1, ST type 2 in PO2 and PO3. In each case four tanks were used within the water bath. Flow through the tanks was maintained in the region of $1\,\ell\,min^{-1}$ for each tank in all experiments.

Feeding

6.4 During experiment, fish in the tanks were fed on a regime calculated to spread the physiological effects of feeding as much as possible over the photoperiod. This was done by feeding at approximately 2 h intervals. For the purposes of PO2, an 8 h photoperiod was used, in deference to the shorter light period experienced during the winter in the wild, but for a number of reasons (see Chapter 1) including the removal of one further

variable from consideration, all subsequent experiments were performed using a 12 h photoperiod.

6.5 Feed rates were calculated according to temperature from Table 1.3 and adjusted to the nearest 0.1 g each time food was weighed out (just prior to each feeding). Food was given by hand in one lot per tank at each feed. Table 6.1 summarises the conditions of feeding.

Stocking and loading (see Chapter 5)

6.6 Since one of the aims of the pilot experiments was to test measured ammonia concentrations at different fish loadings, the approach used in pilot studies was to apportion fish to tanks in such a way as to create particular stocking and loading conditions.

6.7 For PO1, an arbitrary exploratory range of values was chosen so that by combinations of two fish batch-weights and two tank volumes, four different sets of circumstances were set up. These approximated to the target values given in Table 6.2. As TOA levels proved to be fairly low in all these circumstances, greater batch-weights of fish were used in PO2 and PO3, generating higher stocking and loading (Table 6.2).

6.8 In PO1 weighing of fish prior to the experiment was only performed with the points of para.6.5 and 6.7 in mind, and an accurate weight determination was not made until the end of the experiment. In PO2 and PO3 accurate weights were taken both before and after the experiment.

6.9 Fish were weighed by a solution balance technique; a 5ℓ beaker half-full of water was placed on a support on the weigh-pan of a Gallenkamp BC/110 sliding-weight balance and the weight tared by counter-balancing on the other pan. Fish were added to the beaker until one tank batch was weighed to the nearest gram. A standard time of 15s was used as the drainage interval while fish were in the net between holding tank and weighing beaker.

EXPERIMENT	FCAT	TEMP (°C)	APPROX %BWD	FEED (g) L	FEED (g) H		TIME + 0730 0800 0830 0900 0930 1000 1030 1100 1130 1200 1230 1300 1330 1400 1430 1500 1530 1600 1630 1700 1730 1800 1830 1900 1930 2000
PO1	4	16-17	3.2	1.8	6.0	PHOTO-PERIOD:	← →
						FEED:	× × × × × ×
PO2	6	8-9	1.4	4.0	8.0	PHOTO PERIOD:	← →
						FEED:	× × × ×
PO3	6	8-	1.4	14.0	17.5	PHOTO PERIOD:	← →
						FEED:	× × × × × ×

L = low loading tanks H = high loading tanks + BST for PO1; GMT for PO2, PO3

Table 6.1 Feeding conditions in pilot experiments

EXPERIMENT	TANK (ST)	No.of FISH	TARGET FISH WEIGHT (g)	TARGET VOLUME (ℓ)	TARGET STOCKING ($g\ \ell^{-1}$)	TARGET LOADING* ($kg\ min^{-1}\ \ell^{-1}$)	ACTUAL INITIAL FISH WEIGHT (g)+
PO1	O1	4	50	2	25	0.05	61
	O2	5	50	8	6.25	0.05	57
	O4	13	200	8	25	0.20	170
	O6	18	200	2	100	0.20	211
PO2	O9	7	300	4	75	0.30	311
	10	6	300	8	37.5	0.30	324
	11	13	600	8	75	0.60	618
	12	13	600	4	150	0.60	618
PO3	O9	18	1000	6	166.67	1.00	998
	10	18	1000	8	125	1.00	1025
	11	24	1250	6	208.33	1.25	1241
	12	24	1250	8	156.25	1.25	1257

* assuming 1 $\ell\ min^{-1}$ flow

+ post-experiment values for PO1 (see para.6.8)

Table 6.2 Stocking and loading conditions in pilot experiments

Procedure

6.10 Table 6.3 outlines the procedure followed in the three pilot experiments. In PO1 and PO2 readings were obtained for background water levels of TOA prior to the introduction of fish, and subsequent sampling followed on every 4 h where possible; a full record of samples proved impossible to attain due to operator fatigue. In PO3 sampling was limited to 2 h intervals during the photoperiod.

6.11 Each sample was taken just prior to a feed, to prevent any disruption associated with feeding from affecting the water. Samples were partially immersed in a container holding water from the water-bath in order to conserve heat during transfer to the pH meter; temperature readings (by mercury-in-glass thermometer accurate to 0.1 deg C) were taken prior to pH measurement. During PO2 and PO3 the inlet water supply was also sampled each time from the by-pass tube.

6.12 A "dummy run" (without fish) was performed one week prior to PO1 in order to test timetabling, measurement speed, and coordination of activities. This was particularly important for PO1, where samples had to be treated in preparation for spectrophotometric measurement within a limited space of time.

6.13 Accurate volume measurements were taken at the end of each experiment by simultaneous closure of tank inlet and outlet (thus arresting water flow) and drainage of tank contents into suitable measuring cylinders. During experiments PO2 and PO3 volume was periodically checked by means of a previously calibrated dipstick, and if necessary adjusted back to target values. Flow measurements were periodically taken; overflow water was collected in a wide-necked 500 ml volumetric flask, being timed to "full" with a stopwatch, for each tank. After each flow measurement session, adjustment was made if required toward the target value of $1\ \ell\ min^{-1}$ in each tank.

EXPT	PHASE	DATES	TIMES* AND EVENTS (unless otherwise stated, feeds as in Table 6.1)
PO1	1	4/9/72	PRE-FISH SAMPLE:0925/INAUGURATION:1400+/POST-FISH SAMPLE:1725/FEEDS:1800 2000
		5/9	SAMPLES:1200 1600
	2	6-8/9	SAMPLES:10000 1400 1800 2200 (-) 0600 1000 1400 1800 2200 (-) 0600 1000/TERMINATION:1100+
PO2	1	9/1/73	PRE-FISH SAMPLE:1000/INAUGURATION:1100+/FEED:1500/SAMPLE:2200
	2	10-15/1	SAMPLES:0200 0600 1000 1400 1800 (-) (-) (-) 1000 1400 1800 2200
			0200 0600 1000 1400 (-) (-) (-) (-) 1000 1400 1800 (-)
			(-) (-) 1000 1400 (-) 2200 0200 0600 1000/TERMINATION:1100+
PO3	1	2-3/2/73	(2/2) INAUGURATION (NO SAMPLES) (3/2) NO SAMPLES
	2	4-6/2	SAMPLES:1000 1200 1400 1600 EACH DAY
		7/2	(NO SAMPLES) TERMINATION

*BST for PO1; GMT for PO2, PO3 (-) indicates samples missed from 4 h regime

Table 6.3 Pilot experiment procedures

6.14 In between taking samples and measurements, periodic checks were made of experimental conditions e.g. apparent variations in volume or flow; correct functioning of lights, tank drainage and aeration systems; behaviour of fish in case of apparent stress.

6.15 During PO1 and PO2 it was assumed that all food offered to the fish was being consumed. The results of PO2 indicated that this might not be so, and so for PO3, an attempt was made to quantify the food consumed by netting off and counting any uneaten pellets. By relating this "net index" to the number and weight of pellets offered, the amount of food taken could be estimated.

RESULTS

6.16 For a full tabulation of results see Appendix A1.

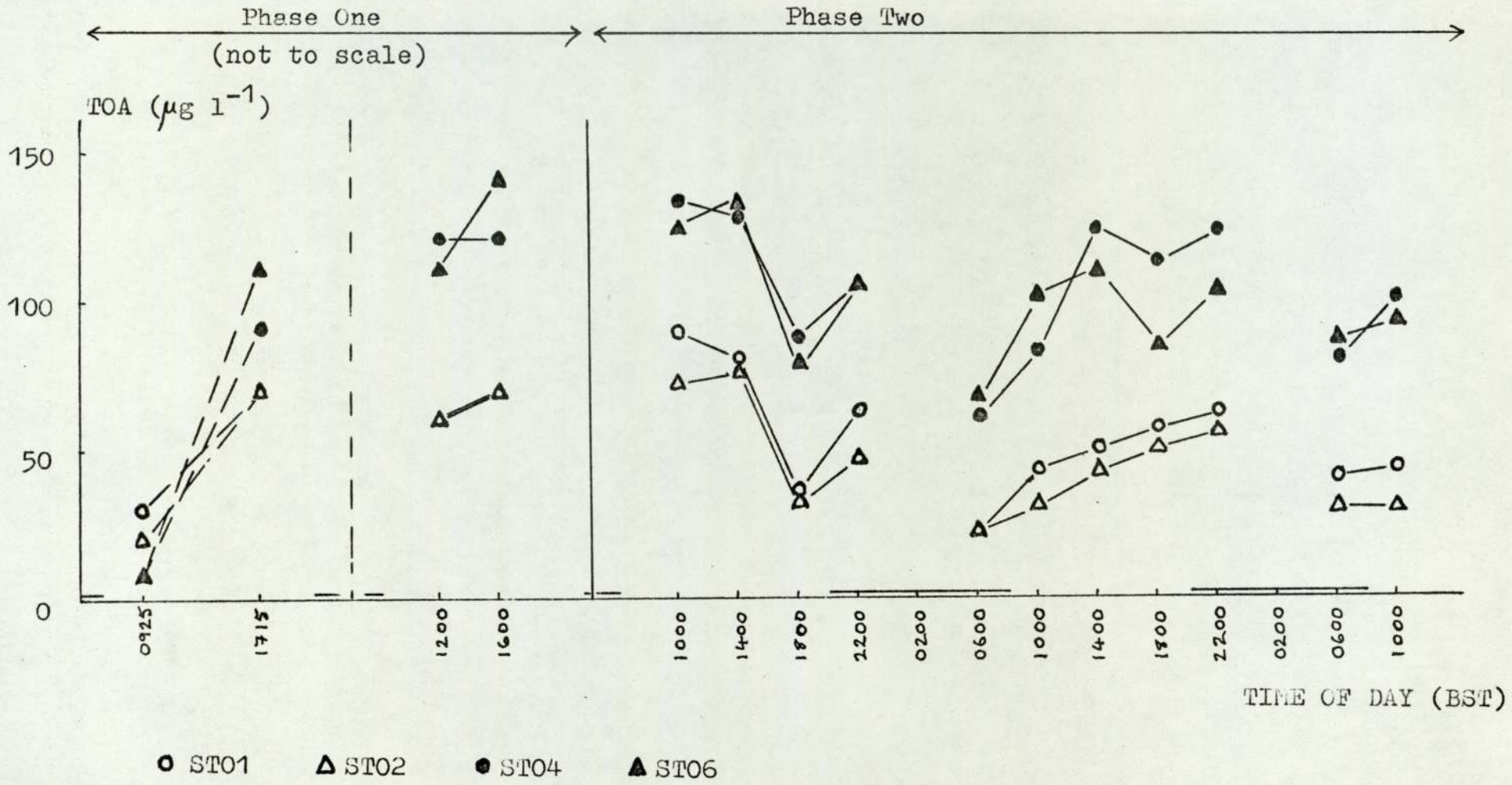
6.17 TOA results for PO1 are plotted against time on Graph 6.1 and show several points of interest:

i) a clear rise in TOA upon the introduction of fish;

ii) a clear separation of TOA effects between higher and lower loading levels, irrespective of stocking;

iii) some indication of a diurnal rhythm in TOA effects, with (after two days of settling down after inauguration) a fall during the dark period and a rise during the photoperiod (i.e. wavelength of 24 h).

6.18 As indicated in Chapter 4, TOA measurements below 100 $\mu g\ l^{-1}$ are subject to doubt, and as the majority of PO1 values fell in this category, it would be unwise to make further deductions from the data obtained. However, it should be mentioned that by calculation from TOA, pH and temperature readings, the UIA present would at all times have been below 1.0 $\mu g\ l^{-1}$ (even allowing for the error in TOA). Comparing this to the diuresis criterion of 25 $\mu g\ l^{-1}$ reported by Lloyd and Orr (1969) (see Chapter 8), it is probably that UIA effects were negligible.



Graph 6.1 P01 TOA results

6.19 The fish used in PO1 were previously held in conditions where average TOA was approximately 450 $\mu g\ l^{-1}$ (estimated by twice-daily measurements for several days preceding PO1). At prevailing pH values the UIA in the holding tanks would be no more than 0.2 - 0.3 $\mu g\ l^{-1}$.

6.20 In experiment PO2 stocking and loading were higher but measured TOA was in the same range as in PO1 (Graph 6.2). Similar results were obtained, with a rise in TOA on fish introduction, eventual separation of TOA values in high and low loading tanks, and more evidence for a daily rhythm (rising values during photoperiod and low values during the dark period).

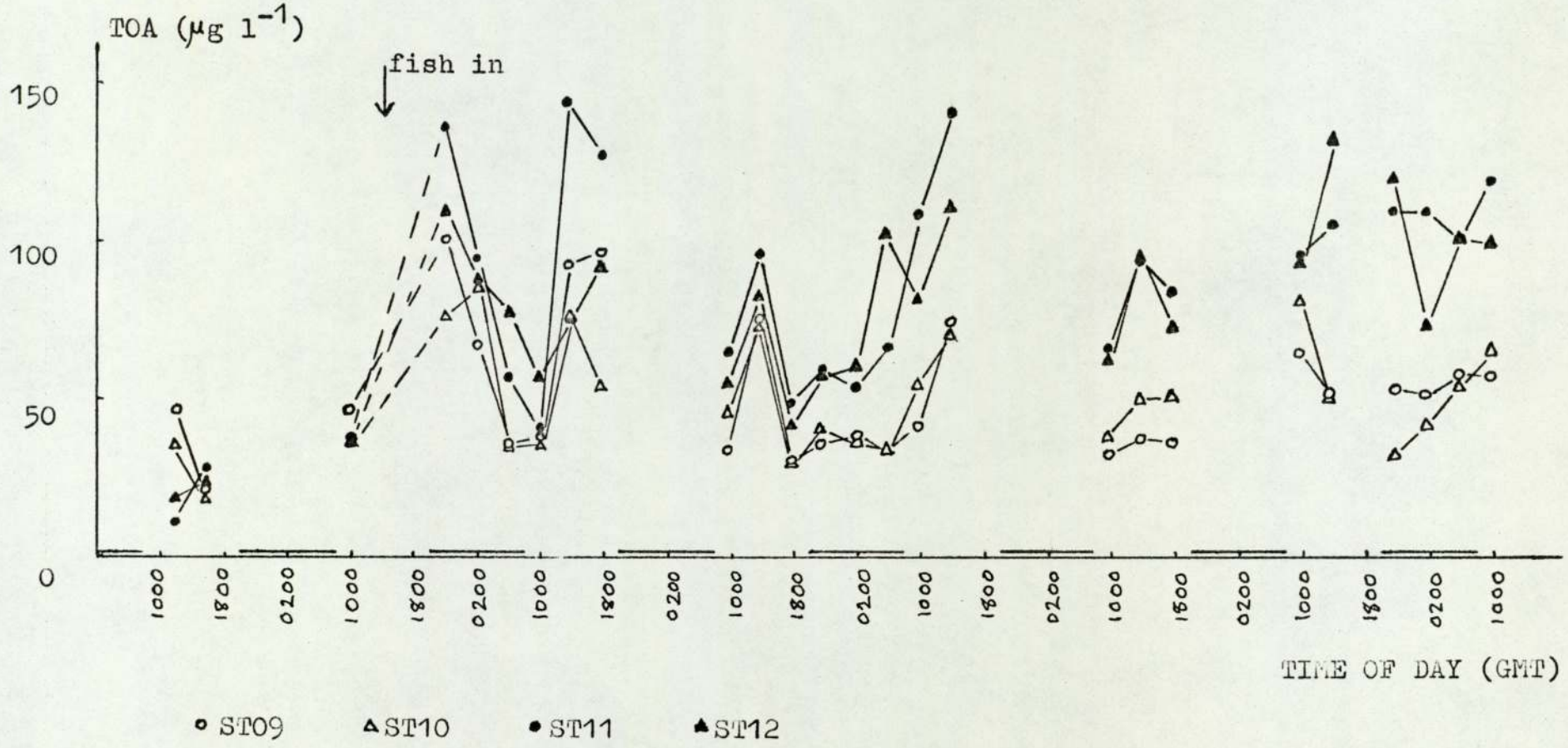
6.21 The results for PO2 also show a fall in pH associated with fish loading; this is more marked than in PO1, with an average fall of 0.4 - 0.5 pH unit in the low loading tanks and about 0.6 unit in the higher loading tanks. The pH pattern reflects that of TOA in following a diurnal rhythm (Graph 6.3).

6.22 PO2 is similar to PO1 in that calculated UIA is probably negligible, at 0.1 $\mu g\ l^{-1}$ or less.

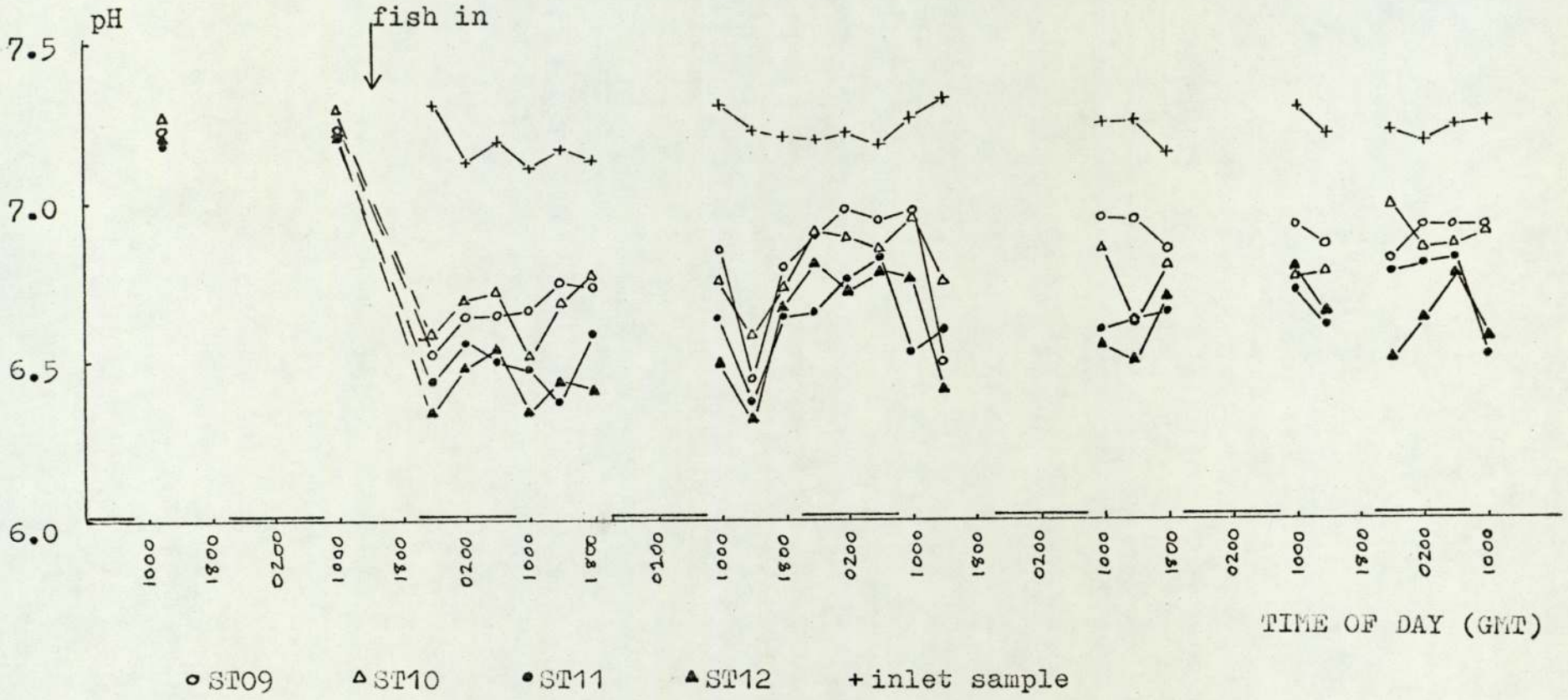
6.23 In most cases, the results for TOA in PO2 show a peak at 1400 during the day, with lower values at 1800. Bearing in mind that the photoperiod ended at 1530, the highest values of TOA are associated with the latter hours of the photoperiod (feeding period).

6.24 Regarding the weight results for PO2, (Table 6.4), a fall in weight was noted for tanks 09, 11 and 12. This lack of growth over a 6-day period has an important bearing on the results (see Discussion section).

6.25 Fish used in PO2 and PO3 were held prior to each experiment in approximately the same holding conditions; the average TOA was estimated to be in the region of 730 $\mu g\ l^{-1}$ (corresponding to about 0.4 $\mu g\ l^{-1}$ UIA).



Graph 6.2 P02 TOA results



Graph 6.3 P02 pH results

EXPERIMENT	TANK (ST)	No. of FISH	WEIGHT (g)				
			TANK INITIAL	TANK FINAL	TANK CHANGE	FISH MEAN CHANGE	TANK MEAN
P01	01	4	-	61	-	-	61
	02	5	-	57	-	-	57
	04	13	-	170	-	-	170
	06	18	-	211	-	-	211*
P02	09	7	311	301	- 10	- 1.4	306
	10	6	324	327	3	0.5	325
	11	13	618	601	- 17	- 1.3	609
	12	13	618	564	- 54	- 4.1	591
P03	09	18	998	931	- 67	- 3.7	965*
	10	18	1025	1045	20	1.1	1035
	11	24	1241	1121	-120	- 5.0	1181
	12	24	1257	1216	- 41	- 1.7	1237

* see Appendix A1 re these data

Table 6.4 Weight results from pilot experiments

6.26 In experiment PO3, TOA values were measured only during the photoperiod. Graph 6.4 shows how values gradually rose over the four days of measurement, and the clear rise over the course of the photoperiod during each day, as suggested in PO1 and PO2. Three days after inauguration, the separation between high and low loading tanks is apparent, but subsequently the effect is altered, and higher TOA is found in tanks with lower stocking rather than higher loading.

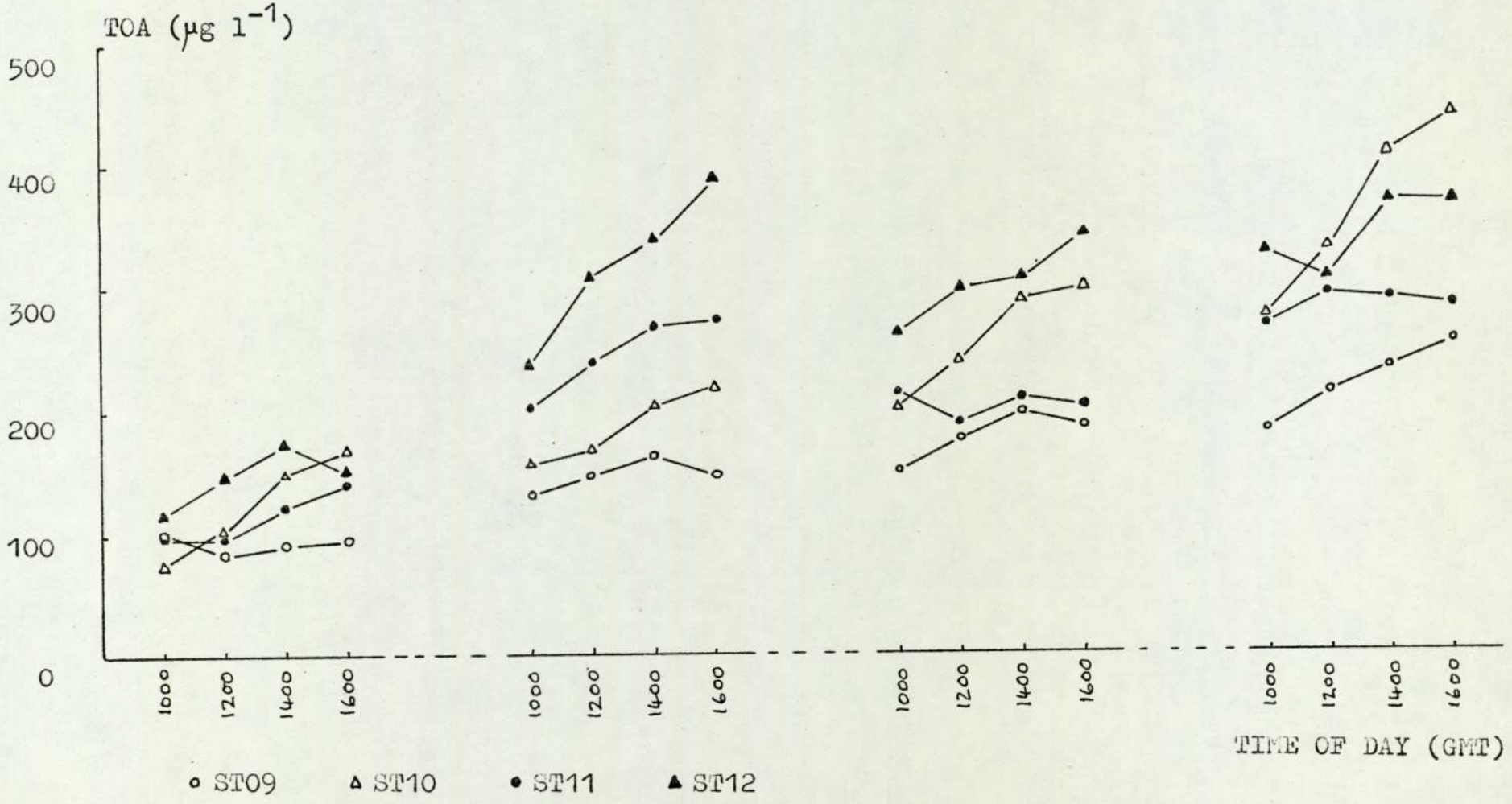
6.27 Most TOA values in PO3 were above 100 $\mu g\ l^{-1}$ and so their values are more reliable than those of PO1 and PO2, but due to other difficulties with PO3 (see Discussion) further inferences are not made directly from the results.

6.28 pH records for PO3 indicate a fairly steady difference of about 1.0 pH unit below the inlet water supply value with little indication of a diurnal pattern. Temperature records indicate a similarly steady difference of about 0.5 to 1.0 deg C rise within the tanks, an effect also present in PO2.

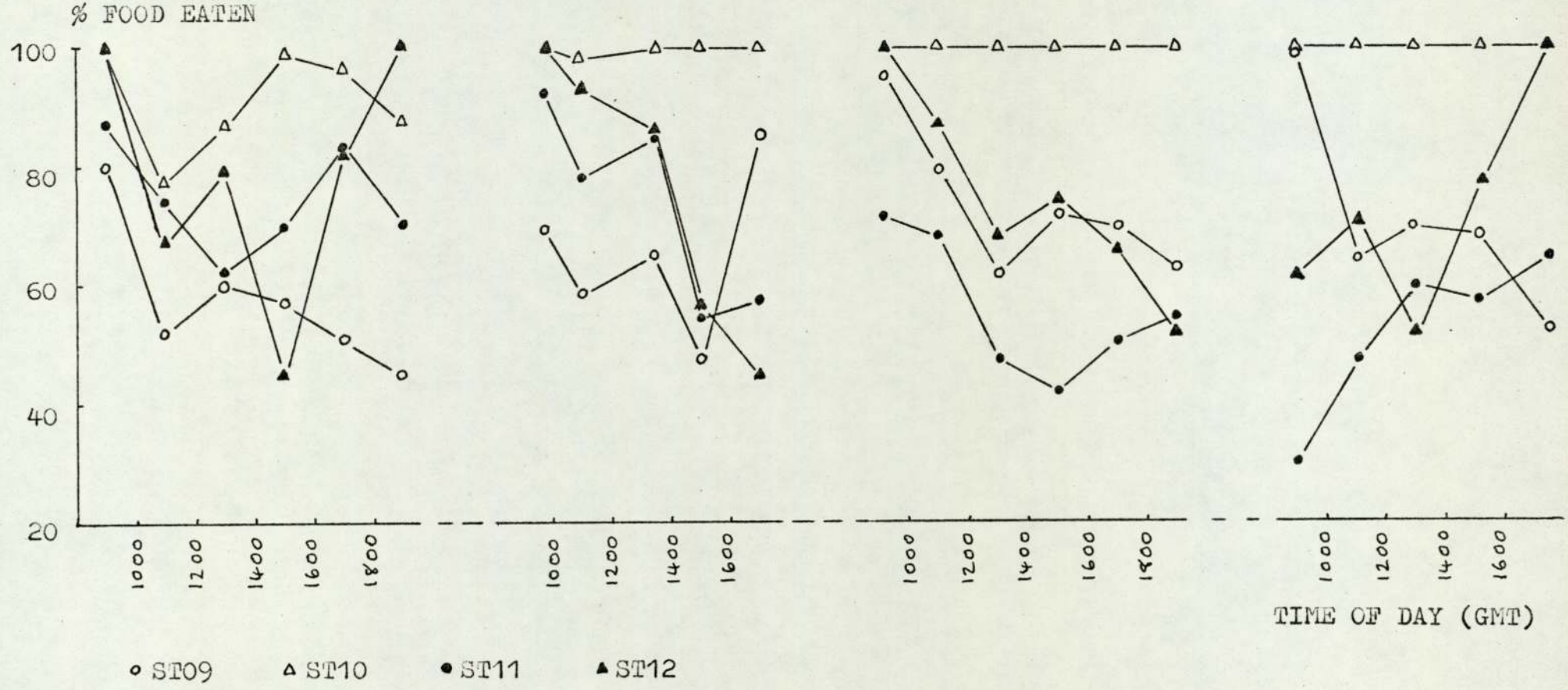
6.29 The stocking and loading in PO3 were the highest in the pilot series of experiments, and were associated with rather higher TOA than those found in PO1 and PO2. Values of UIA were around 0.2 $\mu g\ l^{-1}$ and below.

6.30 In most cases, TOA in PO3 continued to increase throughout the photoperiod. However, in several cases measurements taken at 1600 (the last reading) showed either very little rise, no rise, or a fall compared to TOA values at 1400. In the case of ST 10 (see below), the steepest rise each day occurred between 1200 and 1400.

6.31 In examining PO3 weight results a loss in weight is again noted in tanks 09, 11 and 12, with a little growth in tank 10. Reference to Graph 6.5 shows that ST 10 was the only tank with a consistently high feeding record as measured by the net index of uneaten food. By the last day of experiment, ST 11 had the



Graph 6.4 P03 TOA results



Graph 6.5 P03 food consumption

worst feeding record (even early in the photoperiod), and this corresponds to the greatest loss in weight over the experimental period. On the second day after inauguration (the first day plotted on Graph 6.5), the lowest feeding levels shown by ST 10 are associated with the lowest TOA levels for that tank in Graph 6.4.

6.32 Fish behaviour in PO1 was active (good feeding, some jumping) but not indicative of stress. In PO2 some sign of bullying by the largest fish was seen, with otherwise quiet behaviour and some reluctance in feeding. These signals of stress were magnified in PO3, especially in terms of very poor feeding behaviour; fish became dark and inactive apart from sudden darts frequently associated with bullying.

DISCUSSION

6.33 The three pilot experiments, besides yielding valuable information on method, gave a preliminary outline of the micro-environment situation inside the tank.

6.34 Despite doubt surrounding absolute values of TOA in PO1 and PO2, and a developing stress situation in PO3 (see below), clear indications were obtained that TOA rises during the photoperiod and falls during the dark period, and that when food consumption is at or near the maximum (limited by the amount offered), high loading, as opposed to stocking, is associated with high TOA (together with a drop in pH and a slight raising of temperature).

6.35 Statistical procedures were not employed for the data (bearing in mind the doubts about absolute values), but calculated mean values of TOA support the above contention (when diurnal rhythm effects are roughly balanced out).

6.36 It would seem fair to conclude that TOA in the water is

directly affected by the food consumption of the fish during the course of the photoperiod, although the exact "process time" (the time taken for the effects of one meal to be measurable in terms of TOA) is not clear.

6.37 In all conditions of the three experiments UIA never exceeded 1 $\mu g\ l^{-1}$ and was thus considered to be negligible.

6.38 Fish used in the experiments were previously subject to higher TOA (in holding tank) than encountered in the experiments; to define a level to which they were acclimated would however be difficult due to the probable diurnal fluctuation of TOA in the holding tank. Two or three days seemed to be necessary for fish under experiment to surmount the stresses associated with experiment inauguration, settle in experimental conditions, and reach maximum feeding; this period was characterised by TOA measurements which did not conform to the final pattern. As all fish were from the same batch and holding tank, acclimatory effects should have been similar for both high and low loading.

6.39 Values of TOA, fish weight and flow taken from Table 6.4 and Appendix A1, are used in Table 6.5 to calculate approximate mean values and ranges for stocking, loading and specific excretory rate (SER). It can be seen that SER is roughly similar in all tanks within the confines of each experiment and the limits of experimental error. Overall mean values of SER are 36.99 mg $kg^{-1}\ h^{-1}$ for PO1 and 7.84 mg $kg^{-1}\ h^{-1}$ for PO2, whilst the value of 8.51 mg $kg^{-1}\ h^{-1}$ for ST 10 in PO3 is probably the most reliable datum for that experiment.

6.40 It thus seems probable that the specific excretory rate (which determines TOA in the tank) is inversely related to loading (Graph 6.6) but it is important to recognise the differences between experiments PO1 and PO2, the most important being temperature. Thus the hypothesis lines on Graph 6.6 would be one possible way of interpreting the situation in the light of the

Table 6.5 Pilot experiments: stocking, loading, SER

EXPT	TANK (ST)	MEAN STOCKING (g ℓ^{-1})	FLOW (ℓ min $^{-1}$) MEAN MIN	MAX	LOADING (kg min ℓ^{-1}) MEAN MIN	MAX	TOA (μg ℓ^{-1}) MEAN MIN	MAX	SER (mg kg^{-1} h^{-1}) MEAN MIN	MAX
P01	01	26.52	0.896		0.068		53		46.70	
			0.882	0.909	0.067	0.069	22	89	19.08	79.58
	02	6.95	0.913		0.062		44		42.31	
			0.909	0.923	0.063	0.062	22	76	21.05	73.79
	04	19.77	0.917		0.185		102		33.01	
			0.896	0.937	0.181	0.190	60	132	18.98	43.66
	06	81.15	0.912		0.231		100		25.93	
			0.896	0.923	0.229	0.236	67	132	17.07	34.65
P02	09	85.00	0.819		0.374		54		8.67	
			0.805	0.833	0.367	0.380	31	100	4.89	16.34
	10	41.67	0.827		0.393		53		8.09	
			0.816	0.845	0.385	0.398	30	84	4.52	13.10
	11	79.09	0.821		0.742		92		7.44	
			0.811	0.833	0.731	0.751	40	145	3.20	11.90
	12	155.53	0.828		0.714		85		7.14	
			0.805	0.851	0.695	0.734	42	133	3.43	11.49
P03	09	160.83	0.638		1.513		137		5.43	
			0.622	0.667	1.447	1.551	83	196	3.21	8.13
	10	136.18	0.638		1.622		230		8.51	
			0.504	0.706	1.466	2.054	74	435	2.16	17.80
	11	207.19	0.642		1.840		200		6.52	
			0.622	0.674	1.752	1.899	93	292	2.94	10.00
	12	154.62	0.622		1.989		275		8.30	
			0.594	0.649	1.906	2.083	115	385	3.31	12.12

Table 6.5 continued

Notes:-

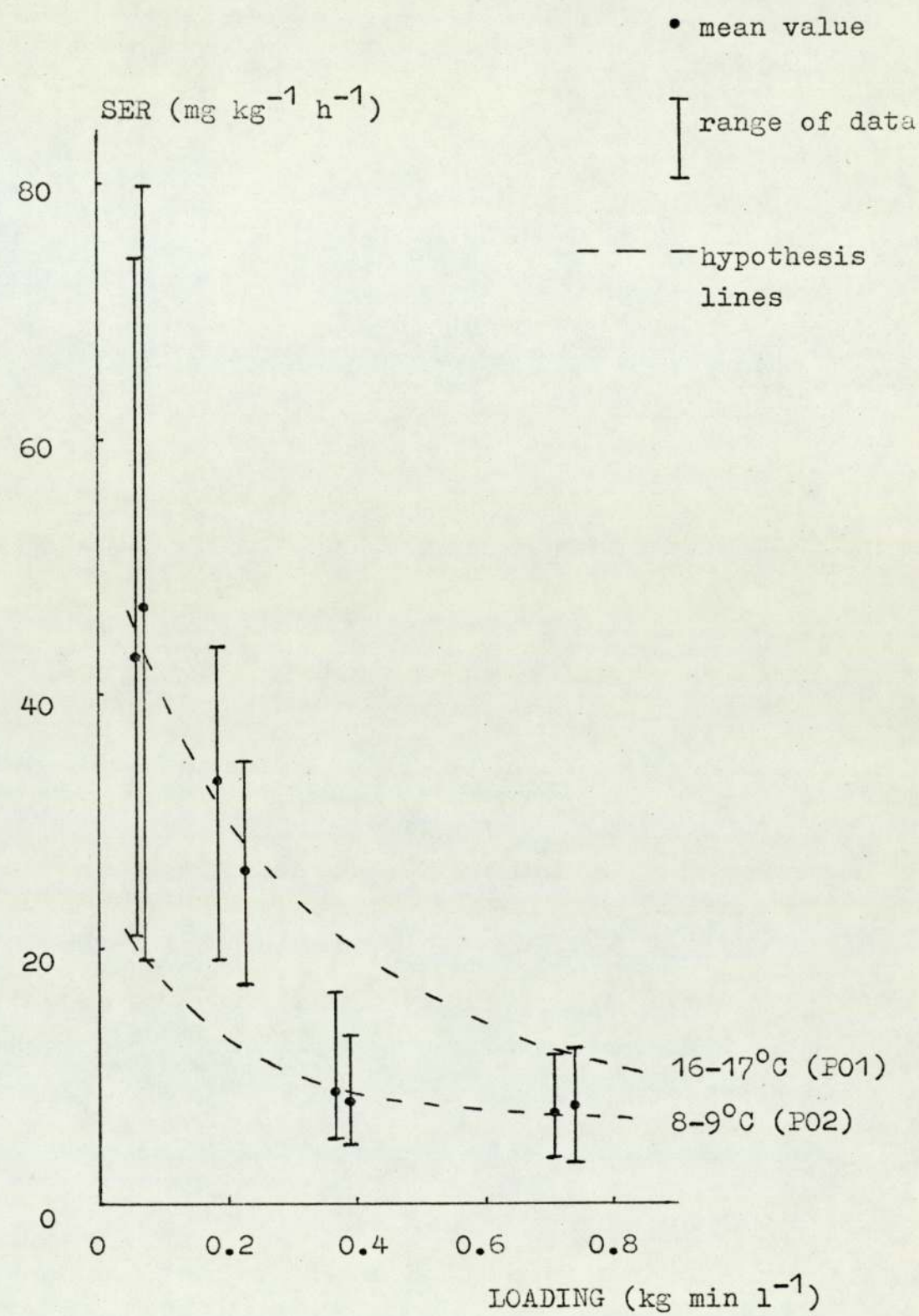
a) MIN = minimum value, MAX = maximum value
b) Stocking is only expressed by the mean, as only one volume determination was made in each case.
c) SER is calculated thus:-

$$SER_{MEAN} = \frac{0.06 \times TOA_{MEAN}}{LOADING_{MEAN}}$$

$$SER_{MIN} = \frac{0.06 \times TOA_{MIN}}{LOADING_{MAX}}$$

$$SER_{MAX} = \frac{0.06 \times TOA_{MAX}}{LOADING_{MIN}}$$

(0.06 is the conversion factor for units)



Graph 6.6 Hypothetical relationship between loading and excretory rate

temperature difference.

6.41 Graph 6.7 shows a similar plot for stocking levels; here the possible relationship is much less clear.

6.42 A further speculation not incompatible with the data is that SER could be related to loading whilst stocking is low (e.g. below 100 g ℓ^{-1}), but that at higher stocking SER is depressed even at lower loading.

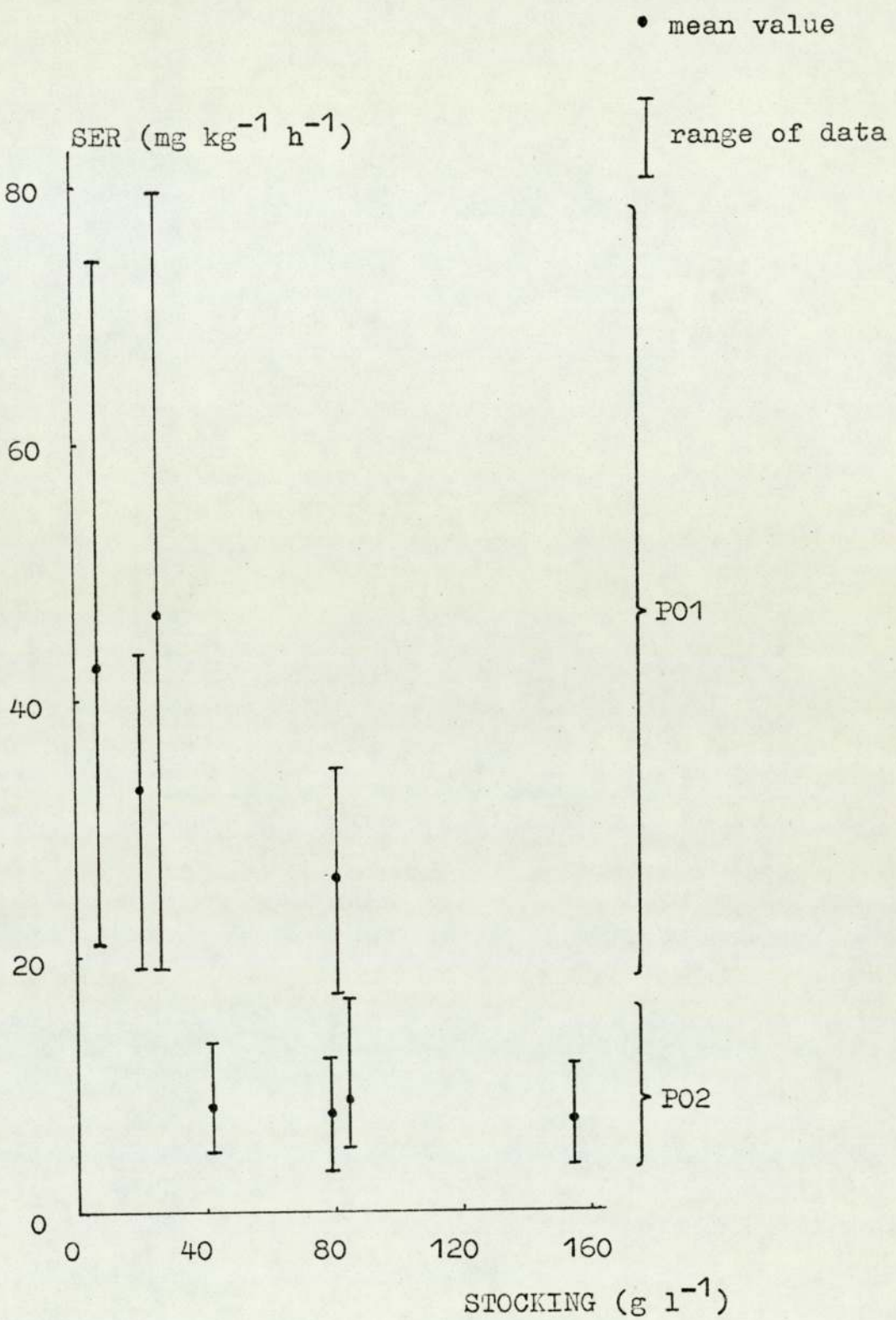
6.43 Testing of such hypotheses arising from the pilot experiments clearly depends on accumulation of a greater body of data, especially in regard to different levels of stocking, loading and temperature, and subsequent experiments were designed to provide such data.

6.44 However, several other aspects of the pilot experiments were relevant to the design of subsequent experiments, in particular the state of feeding, the estimation of weight, and the choice of representative TOA values.

6.45 It is clear that fish were not feeding properly in P03. This led to lack of growth and casts all other results of this experiment into doubt. It would seem from observation of fish behaviour in P02 that similar conditions may have occurred there to a lesser extent (para.6.32). A general explanation for this effect would be to assign it to "stress", but it is necessary to define more closely which aspects of the situation were involved in creating a stress whose symptom was reduced feeding.

6.46 By inspection, it is likely that the following factors were involved in creating such a stress:-

a) Inauguration routine. The method of weighing prior to placing fish in tanks was naturally stressful in requiring handling of fish, but this was enhanced by the small size of the weighing container (see para.6.9) and the time that the fish spent in it while balance was achieved. Fish were close to over-



Graph 6.7 Stocking and excretory rate in pilot experiments

turning from lack of oxygen in the case of PO3, though they were subsequently revived in the experimental tanks by extra aeration.

b) Photoperiod change. In PO2 the length of photoperiod was not drastically changed from the holding condition, but it was moved to about half an hour earlier. In PO3, the photoperiod length was 12 h (extra light all occurring at the end of the day), as compared to around 9.5 h under holding conditions. This sudden acceleration and relative displacement could be involved in the fish stress response.

c) "Crowding". Probably the most likely effects would be those due to the relative sizes of fish and tanks and the number of fish used, in experiment PO3. The difficulties involved in assessing these factors are mentioned in Chapter 5, but it is not surprising that 24 fish averaging 16 cm length enclosed in a tank of about 28 cm diameter holding less than 6ℓ of water (the case for ST 11 in PO3) should produce evidence of stress. Replacement of ST type 1 tanks (used in PO1) for tanks of larger diameter was probably still insufficient for fish of the size employed in PO3.

6.47 From the experience of PO3, it was clear that for future experiments, fish of this size would require larger tanks, and that experiments in tanks near to ST size (about 10ℓ) could only employ small fish, for example up to FCAT 4 (as used in PO1). It was also clear that inauguration stress should be reduced to a minimum by means of a better weighing method, and that photoperiod shock should be avoided.

6.48 A longer period of measurement was also indicated, to be sure of getting clear of any early confusion due to inauguration effects (para.6.38). This, however, would mean magnification of an error already built-in to pilot experiments: that of growth effects over the period of the experiment. In pilot experiments a simple mean was taken from the starting and finishing weights.

For measurements over a longer period, a method would be required to ascertain weight at different times in the experiment; it would also be desirable to measure flow and volume frequently,

6.49 The clearest result of the pilot experiments, in terms of TOA, was the extreme variability of the values measured. Postulating a diurnal cycle in TOA (i.e. wavelength 24 h), the amplitude of the oscillation seemed to depend on loading in PO1 and PO2, being greater in higher-loading tanks. The evidence of PO2 and PO3 points to a maximum value at the end of the photo/feed period, and a minimum at the end of the dark/fasting period. (The actual positions of the extremes may be at the beginning of the subsequent period rather than within the ones indicated. It also seems likely from PO3 that the quickest change in value on the 12 h photoperiod regime is between 1200 and 1400, with lesser changes both before and after (see para.6.30 and Graph 6.4). Thus an asymmetrical, slightly "saw-tooth" type of waveform is suggested. Figure 6.1 shows the possible form of such an oscillation, with maximum TOA rise from 1200-1400, and the assumption made (in the absence of any definite evidence) that TOA fall during the dark period is the reverse of the photoperiod pattern (with peaks and troughs at the light-changes); a smooth curve has been drawn to connect hypothetical points plotted in this fashion.

6.50 With these points borne in mind, it becomes necessary to select a time reference for subsequent experiments so that inter-experiment TOA comparisons are valid and minimise error due to position on the waveform.

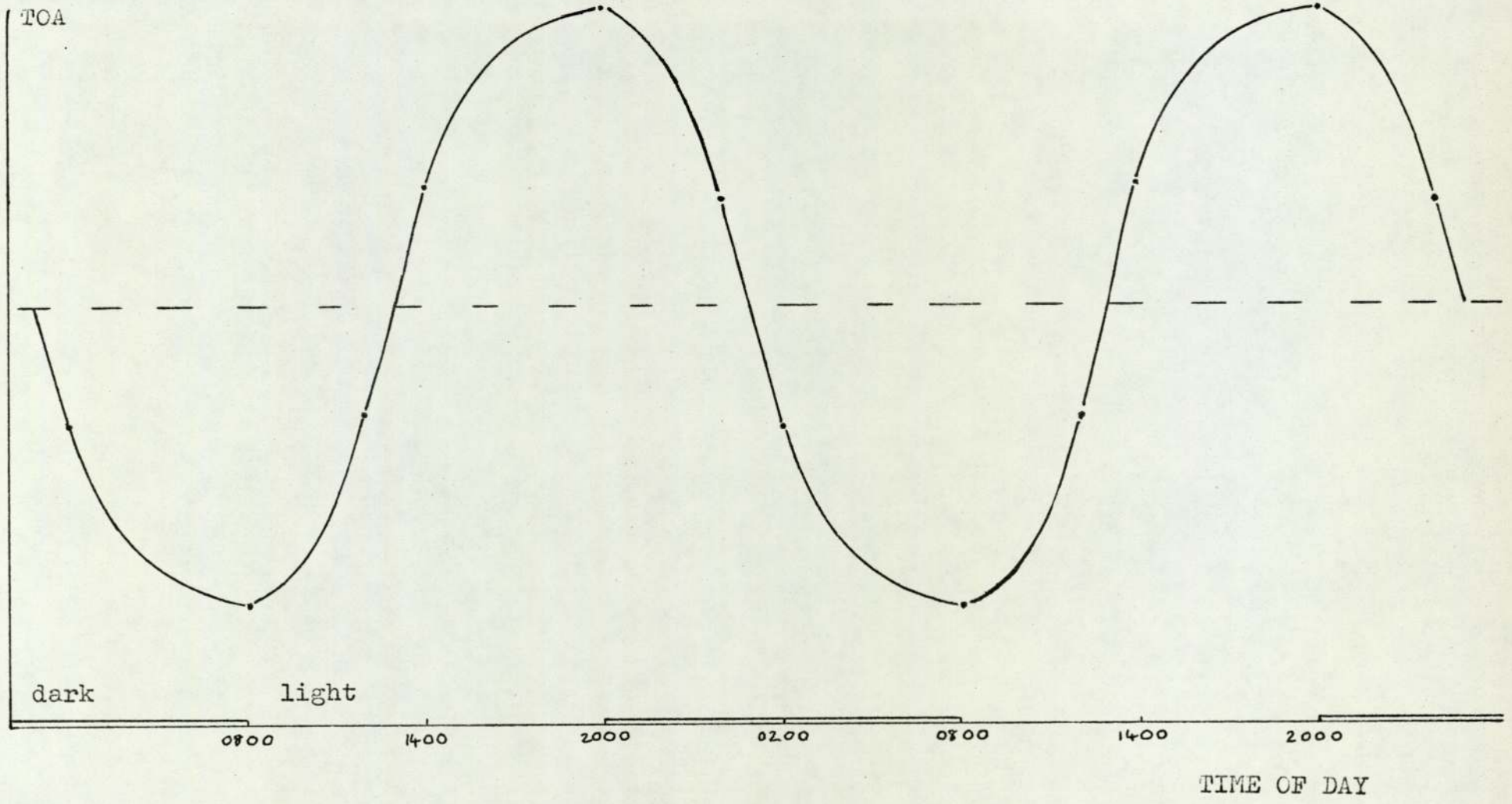


Figure 6.1 Hypothetical oscillation of TOA under 12 h regime

7. FULL SYSTEM EXPERIMENTS

PRELIMINARY WORK

Background monitoring program

7.1 From early October until early December 1973 Lots 01 and 02 (pooled) were employed in refining a weight-estimation technique and in testing experimental procedure for the following E-series, so that fish were treated as if on experiment, except for the measurement of ammonia. This invaluable experience was termed the background monitoring program (BMP); larger fish were used than could be accomodated in the actual E-series; these were fed equal amounts five times per day, and the total fed was adjusted according to temperature difference or fish mortalities.

7.2 The BMP was divided into four 2-weekly periods beginning on a Wednesday, so that each alternate succeeding Wednesday was a grading day. On the grading day, fish were weighed individually after anaesthesia, their lengths (to nearest 0.5 cm) noted, regraded according to FCAT attained by growth in the previous two weeks, and then returned to tanks (LTS) for the next two weeks. Full records were kept of weights, lengths, food fed, temperature, mortalities, and tank flow and volume.

7.3 Since BMP was a design and testing exercise, the results are relatively unimportant; they are given in Appendix A2. Graph 7.1 shows that over the spread of fish size (halfway through BMP) the fish population was fairly homogeneous; the condition factor (K) (relating length and weight) at this point averaged 1.47 (values below 1.00 indicate emaciated unhealthy fish), and had risen from 1.44 two weeks previously, indicating healthy growth.*

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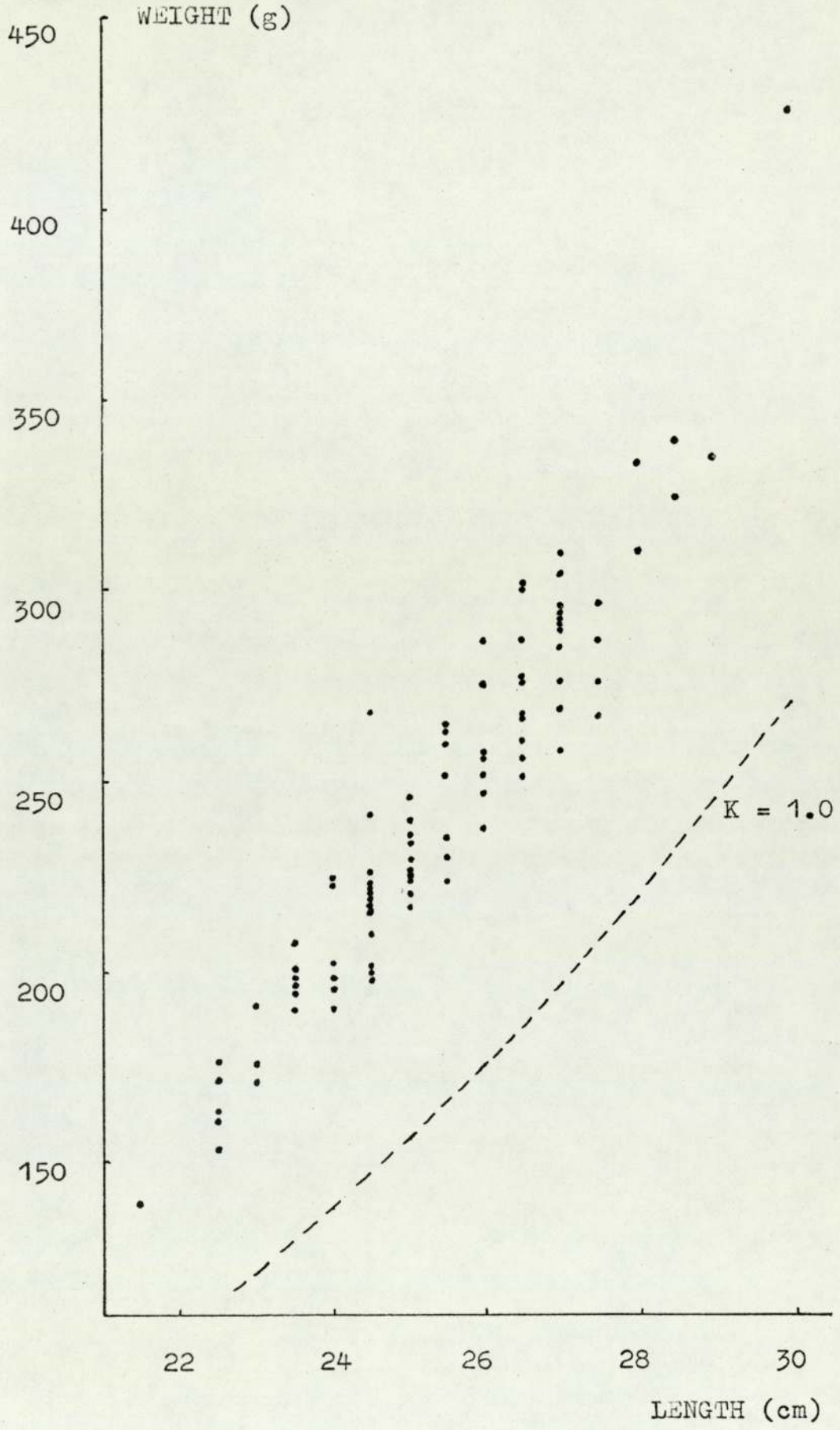
* (condition factor is given by: $K = \frac{\text{weight} \times 100}{\text{length}^3}$ (Brown 1957)



Graph 7.1 Length and weight of fish during BMP

Units in this case would be g cm^{-3}, but:

a) this differs from density in that the cube of length is a different quantity from fish volume;

b) units are not usually quoted in literature references to condition factor; when other units are used instead of cm and g, the resulting factor is related to this one by a constant coefficient.

7.4 Weight estimation data treatment is explained later, under DATA ANALYSIS.

Pilot experiment P04

7.5 Although out of chronological order, it is appropriate to mention at this point the fourth pilot experiment P04 which was undertaken between 30/8/74 and 13/9/74, and thus performed under the E-series routine of method. Only one tank was used, to test the feasibility of experimenting on large fish (FCAT 6) in the MTS, with temperatures of about 14 to 16°C. Although some growth was achieved, conversion (see METHODS section) was poor, fish showed clear signs of stress including dark colouration, and bullying and cannibalisation were severe enough to cause 37% mortality, thus invalidating the intermediate weight estimation technique.

7.6 P04 results are given in Appendix A2. They indicate that at high temperatures fish of FCAT6 were unsuitable for MTS experimentation, and subsequently only one MTS E-series experiment featured fish as large as this, at a lower temperature. (FCAT 6 was used successfully in LTS E-series experiments.)

Rehearsal experiment E00

7.7 Just prior to the E-series full experiments, a one-week rehearsal trial was undertaken with two MT from 5/3/74 to 12/3/74. Each tank contained 30 fish from Lot 03 of FCAT 3 and 4. The full E-series routine was used except that volume was not monitored due to a delay in equipment delivery. Ammonia readings were taken.

7.8 Temperature at this time was at the lowest of the year (see Chapter 1) hence excretion rates were expected to be very low. This is reflected in the low TOA measured.

7.9 Appendix A2 gives a summary of EOO results. These were disqualified from further consideration on four counts:-

a) the experiment only lasted half the standard period,
b) this short period magnifies the error due to mortalities in weight estimation (see DATA ANALYSIS section),
c) a fall in condition factor in MT8 showed poor feeding and growth,
d) the measured TOA tended to be low, and in MT8 was below 100 $\mu g\ l^{-1}$ (see Chapter 4).

EOO was nevertheless a successful rehearsal in terms of time-tabling, procedure and feasibility, and was followed almost immediately by the first of the full E-series experiments, EO1.

METHODS AND MATERIALS

Fish

7.10 Fish Lots O3 and O4 were used for E-series experiments. Lot O3, originally 1350 fish of FCAT 1, arrived at Aston in early December 1973 and was maintained under HC2. This Lot was sampled for experiments EOO to EO5 inclusive and for PO4; EOO began in early March 1974. Lot O4, 1500 fish of FCAT 2, arrived in late August 1974 and was held separately under HC2 until early November; Lots O3 and O4 were then pooled and the combined population sampled for experiments EO6 to EO9.

7.11 Selection criteria for experiments were, simply, required FCAT and healthy appearance. Fish were sampled by netting out of their holding tank into buckets; in most cases the majority of fish from one holding tank would be used as several experimental tanks would be involved. Each experimental tank was allotted a population by number, no attempt being made to attain target weights of fish.

Tanks

7.12 All MT were employed in one experiment or another, although all eight were rarely in operation together, due to cleaning, repairs or limitation on fish stocks of required size. On two occasions the LTS was employed, allowing larger fish and different stocking and loading conditions to be used.

Feeding

7.13 Both Lots 03 and 04 progressed during holding from Salmon No.2 diet through No.3 to Trout No.4. All experiments were conducted using Trout No.4 floating pellets.

7.14 Feeding routine during experiments was automatically controlled (see Chapter 3) with food dispensed five times per day. Photoperiod lasted from 0800 to 2000 (GMT or BST according to time of year), with feeds at 1000, 1200, 1400, 1600 and 1800. 1400 marked the "mid-day" point. This regime was calculated, as in pilot experiments, to spread physiological effects as much as possible over the photoperiod.

7.15 Feed rates were calculated rather differently from pilot experiments, in an attempt to feed by a predictive method worked out by American fishery scientists over a number of years (Haskell 1959, Freeman et al 1967, Buterbaugh & Willoughby 1967). The method attempts to prevent food wastage, and is based on a theory of growth and temperature interaction originally formulated by Haskell (1959) and since refined.

7.16 The background for this method (and the CNP feeding chart which derives from similar origins) rests on the use of hatchery records to define terms of reference for individual trout farmers, and whilst this is an excellent pragmatic approach in the field situation, it lacks the feeling of ability to derive from first principles which ought to characterise a truly scientific method. Nevertheless, in any experiment which is designed to reflect the field situation to a reasonable extent, this kind of approach to feeding, a basic factor in the complex, must be accounted for. Hence the use of this method as explained below.

7.17 Haskell (1959), from studies of US trout hatchery records, proposed that fish growth under production conditions could be described thus:-

$$\text{daily \% weight gain} = \frac{3 \times \Delta L \times 100}{L}$$

where L = fish length and ΔL = daily gain in length.
Since both L and ΔL could be estimated from individual hatcheries' records (or L calculated from weight records by means of a generalised relationship), then daily weight gain could be easily related to daily amount to feed by means of a suitable feed conversion.

7.18 The conversion referred to here is that known to nutritional literature as the gross conversion ratio, defined thus:-

$$\text{gross conversion} = \frac{\text{weight of food fed over a certain period}}{\text{weight of animal flesh produced over that period}}$$

Both weights measured are "raw" or "wet" weights of the material, as opposed to dry weights which are the results of oven treatment to remove all water. In the case of fish, the weights are of fish food (pellets) from the supply bag, and of fish immediately as harvested. Commercially, the lower this conversion is, the more fish the farmer obtains per quantity of food required, and hence per cost incurred.

7.19 Haskell's relationship can thus be rephrased:-

$$\text{\% body weight to feed daily (\%BWD)} = \frac{\text{conversion} \times 3 \times \Delta L \times 100}{L}$$

Determination of conversion seems to depend on one of the following:-

a) appraisal of past conversion achieved and its assumption for the future,
b) choice of a target conversion which seems economically desirable,
c) calculation of a reasonable conversion from estimates of trout requirements and feed contents. It is clear that these considerations may not all give the same answer, and it is not

always obvious in any given situation whether one can choose a desired conversion or can only accept the conversion which experience dictates. The room for manoeuvre between these two states will depend greatly on feeding practice and general husbandry. In the present situation, with no records on which to rely, a target conversion was assumed for purposes of feeding. This assumption followed Phillips (1970) in presuming an average trout production requirement of about 16590 kJ per kg of fish produced, and in presuming that a modern pellet diet contains about 11060 kJ per kg of feed. Thus the conversion ratio implied is 16590/11060 or 1.5.

7.20 Assuming that water temperature stays constant, Buterbaugh and Willoughby (1967) consolidated the derived formula to:-

$$\%BWD = \frac{H}{L}$$

- where H = conversion x 3 x ΔL x 100 and is termed the hatchery constant.

7.21 However, for situations where water temperature varies, it is necessary to estimate ΔL by using Haskell's temperature unit theory of growth. This maintains that a definite rate of growth can be predicted for any temperature between 38.6^{o} and 60^{o}F, and the temperature unit (TU) is defined as the average Fahrenheit temperature, for the period over which it is wished to calculate (usually one month), minus 38.6. In converting this process to metric units, one may take note of Speece's recent statement (1973), quoting Bowen (1971), that 32^{o}F can be substituted for 38.6^{o}F. This means that TU in metric units is numerically equal to the temperature in ^{o}C. Having derived TU for the required predictive period from hatchery records, these records must further be consulted for the TU usually required per unit length increase, in which case:-

$$\Delta L = \left[\frac{\text{TU expected in next month}}{\text{TU required per unit length growth}}\right] \div 30$$

Thus ΔL can be derived for the future month, H can be calculated (assuming a conversion) and%BWD finally estimated.

7.22 In the current work there were no suitable records on which to rely for source information, with the exception of water temperature records maintained since the beginning of fish holding (however change of HC rendered these of only limited help). Under these conditions, several assumptions were made:-

a) conversion would be 1.5;

b) TU required per cm of length increase would be 7.9 (calculated from Speece (1973) as a reasonable usual value);

c) that the process was equally applicable over a shorter period i.e. 14 days;

d) that the assumed (expected) temperature for the 14 days (FPT)* would be reasonably estimated by reference to previous records and the current temperature, and arbitrarily selecting a likely value. *Forward projected temperature.

7.23 It follows that, in order to estimate the food required for the duration of an experiment (14 days) it was necessary to assume the FPT and to measure the total weight and average length of the fish. Thus feed calculation could not be performed until fish had been measured and sorted to experimental tanks. Use was made for feed calculation of Piper's (1970) slide rule technique, suitably modified.

7.24 After feed calculation, feeders were individually calibrated (ensuring no feed reached the fish) as in Chapter 3 to dispense one-fifth of the daily food requirement per stroke of the feeder piston.

7.25 No food was delivered during the afternoon of sorting, since usually the full afternoon was required, and feed calculation could not be performed until sorting was completed. This time, followed by the dark period, allowed fish to settle down before the first feed the following morning. As fish were weighed with empty guts during the initial sorting period (they were not fed during the preceding morning), it was necessary to

maintain comparability, to starve them on the last morning (14th day) prior to weighing and sorting on the final afternoon.

7.26 Since any mortality during an experiment would affect the total fish weight in the tank, it was necessary to re-calculate the daily feed in the case of mortality, having taken into account the change in total weight. As the weight and length of the dead fish were also necessary data for the process of intermediate weight estimation, dead fish were always immediately weighed and measured for length when removed from the experimental tank.

7.27 Feeder calibration (and re-calibration after mortality) was, as explained in Chapter 3, a process of "zeroing-in" on the target value. After sorting and feed calculation on the first day, each feeder would be calibrated by means of several non-delivery trials to dispense the correct amount of food. After the first full day's feeding, the amount dispensed would be compared to the target value, and the calibration nut slightly adjusted in respect of any excess or deficiency. Over-compensation would be corrected for on the following day, and so on. There also occurred, at times, gradual slippage of the calibration nut over 24 h, which would necessitate re-adjustment on the following day. Thus the overall effect was for the actual amount of feed dispensed to vary around the target value. This introduced a certain variability into the feed data supplied for analysis, expressed as "noise" over the target amounts. It is not unreasonable to suppose that similar considerations might apply in a full-scale fish production situation.

7.28 Using this system of feeding, it was found in the great majority of cases that food consumption was virtually total. It was never necessary to net out uneaten food, and although a very small proportion of food may have been swept out of the tanks before fish could consume it, the situation never reached the acute conditions of PO3. Thus in general it was safe to

assume total food consumption; any tank failing to achieve this would furnish poor growth indication data (see DATA ANALYSIS section) at the end of the two-week period. This would disqualify data from further treatment.

Sorting

7.29 Unlike the pilot experiments, the E-series experiments were not designed around target values of stocking and loading. Instead, a reasonable number of fish of the desired FCAT were accommodated in each experimental tank so that the tank was well populated but not overcrowded (by eye appreciation) bearing in mind the volume and flow available. Since crowding is such a difficult phenomenon (see Chapter 5), it was felt that this was an acceptable method of setting up. With the variations of volume and flow which occurred, a fairly wide range of loading and stocking values were obtained for the multivariate analysis used.

7.30 Initial conditions of all experiments are given in Table 7.1. Experiments were performed under the 12 h photoperiod, five-feed regime previously discussed; in these respects there was no abrupt change between holding conditions and experiment.

7.31 The sorting procedure was as follows. Fish were starved in holding tanks during the morning before sorting to enable weighing with empty guts. Commencing at about 1400, the experimental tanks, having been cleaned, prepared and put under flow, fish of the desired sizes were sampled from the holding tanks. For each experimental tank required, fish were netted out in successive batches into a 10ℓ bucket containing water from the holding tank with accessory aeration. Fish were then transferred a few at a time into a second bucket containing aerated anaesthetic (MS.222) at a strength of approximately 50 mg ℓ^{-1}. After 2-3 minutes fish would be sufficiently quiescent for individual length measurement (just at the overturning point).

Table 7.1 Initial conditions of E-series experiments

EXPT	STARTING DATE	FPT (°C)	TANK	FCAT	No.of FISH	%BWD	INITIAL MEAN LENGTH (cm)	INITIAL TOTAL WEIGHT (g)	INITIAL STOCKING ($g\ l^{-1}$)
E01	14/3/74	6.5	MT1	3	30	1.37	9.00	292	(24.3)
			2	4	20	1.09	11.37	382	(31.8)
			4	3	30	1.35	9.12	309	(25.8)
			5	4	20	1.10	11.27	361	(30.1)
			7	3	30	1.36	9.05	297	(24.8)
			8	4	20	1.08	11.47	406	(33.8)
E02	30/4/74	10.0	MT1	3	30	2.10	9.05	283	32.2
			2	4	20	1.75	10.87	321	33.8
			3	3	30	2.12	8.97	280	29.8
			4	4	20	1.68	11.35	386	41.5
			5	3	30	2.15	8.83	267	28.7
			6	4	20	1.68	11.32	355	35.9
			7	3	30	2.16	8.77	257	25.7
			8	4	20	1.65	11.50	390	36.8
E03	28/6/74	16.5	MT1	2	27	4.31	7.28	121	13.7
			2	3	40	3.32	9.44	404	40.0
			3	4	30	2.94	10.65	428	43.2
			4	3	40	3.32	9.44	405	42.6
			5	4	30	2.94	10.65	422	38.4
			6	3	40	3.28	9.55	407	34.5
			7	4	30	2.61	12.02	578	55.0
			8	5	20	2.35	13.35	547	47.2
E04	13/8/74	17.0	MT4	3	30	3.39	9.53	321	29.2
			5	4	20	3.05	10.58	276	22.6
			6	3	30	3.39	9.53	316	30.4
			7	4	20	3.07	10.53	273	22.2
			8	4	20	2.88	11.20	324	26.3
E05	23/9/74	12.5	MT6	5	31	1.71	13.87	866	61.0
			7	5	30	1.72	13.85	878	68.6
			8	6	26	1.40	16.96	1585	115.7
E06	12/11/74	8.0	MT5	4	30	1.32	11.55	530	42.4
			6	4	30	1.28	11.85	573	62.3
			7	4	30	1.30	11.72	571	51.0
			8	4	30	1.31	11.60	547	48.8
E07	14/11/74	8.0	LT2	6	63	0.95	16.14	3226	38.4
			4	5	212	1.09	14.05	6824	70.4
			5	4	147	1.28	11.83	2948	35.1
			6	3	350	1.75	8.72	2932	53.3

Table 7.1 continued

EXPT	STARTING DATE	FPT	TANK	FCAT	No.of FISH	%BWD	INITIAL MEAN LENGTH	INITIAL TOTAL WEIGHT	INITIAL STOCKING
EO8	29/11/74	6.5	MT5	4	40	1.05	11.72	816	65.3
			6	4	40	1.03	11.95	889	90.7
			7	4	40	1.06	11.59	781	67.9
			8	4	40	1.04	11.86	835	73.2
EO9	6/12/74	6.5	LT2	6	93	0.76	16.17	4728	53.1
			4	5	235	0.89	13.95	7671	69.7

Notes:-

a) Stocking values for EO1 are estimates, based on a tank volume of 12 ℓ. Volume measurement equipment was not available and so stocking figures are not reliable.

b) In EO3, MT1 was stocked with fish of FCAT 2, fed on Salmon No. 3 diet, for comparison with fish of larger FCAT. Because of these differences, MT1 is not included in any further analysis of EO3 data.

For this, each fish was placed against a wetted length guide (see Figure 7.1) and length (from snout to tail-fork) read off to the nearest 0.5 cm and noted. The fish was then transferred to an appropriate aerated recovery bucket according to FCAT. Working at speed, the length measurement process (including anaesthesia) could be reduced to an average of about 3.5 min for each fish (the times overlapping as fish were continuously dealt with), and thus the stress of the procedure was minimised. When the desired number of fish for a batch had accumulated in a recovery bucket, that batch was weighed.

7.32 For weighing, a weigh-bucket containing between 5 and 8 ℓ of aerated water was placed on the pan of the 10 kg balance and its weight tared off. The fish batch was poured from the recovery bucket into a suitably-sized net and held steady for a standard draining-time of 15 s. The batch was then gently transferred into the weigh bucket and the batch weight read off to the nearest 1g from the direct-read scale, and noted (Fig. 7.1). Fish were then immediately transferred to a further aerated bucket to settle down, and then by small hand-net to their experimental tank. Weighing of a batch could be accomplished in about 1 min from pouring to settling-bucket and hence stress was minimised (most fish were still slightly under the anaesthetic influence in any case). Fish batch size varied according to fish size and water temperature. Maximum values were; FCAT 3:50; FCAT 4:40; FCAT 5:30; FCAT 6:20. It was estimated from repeated trials that the maximum error of the process would be about 1% to account for variations in drainage, and a further 1% in the accuracy of the balance. Sorting at the end of the experiment followed a similar process.

7.33 After the required number of batches had been sorted in successive buckets, the fish superfluous to experimental requirements were restored to their holding tank. In all cases, fish in both holding and experimental tanks were found to consume food as normal when presented with their first feed after sorting.

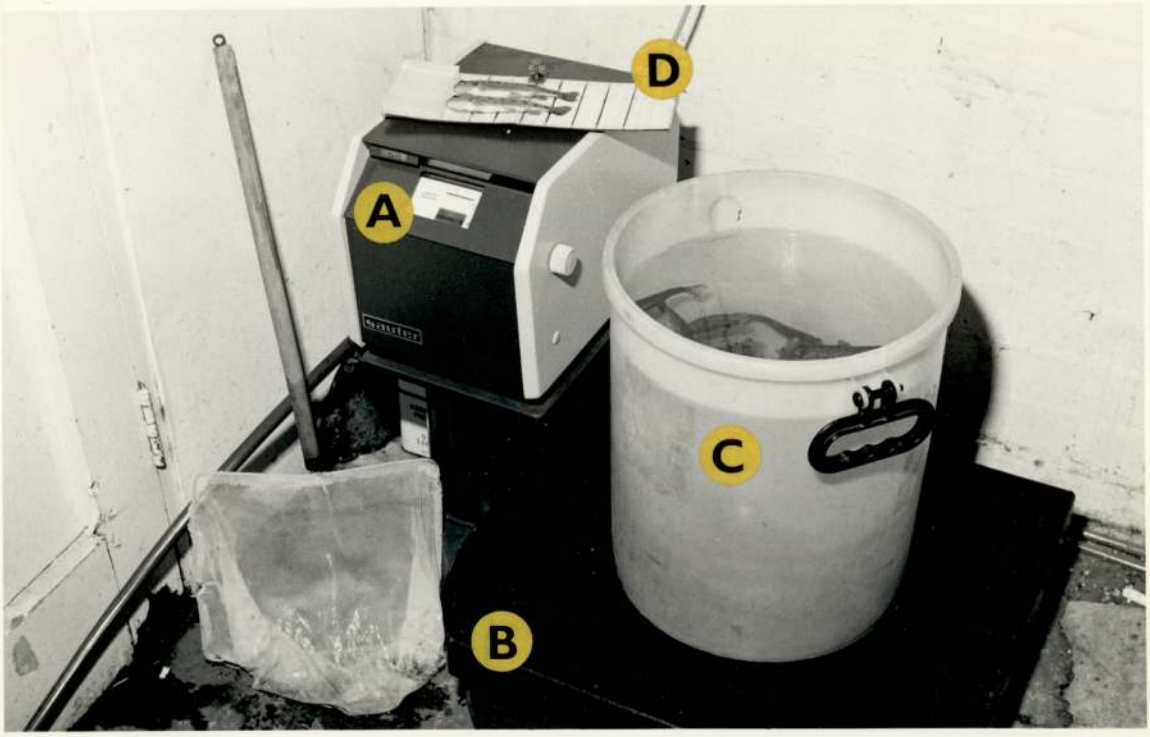


Figure 7.1 Sorting equipment

A Direct-read scale

B Balance platform

C Weigh-bucket with fish

D Length measure

This indicates that the low handling stress involved, followed by a dark period for recovery, was quite adequate to condition the fish for feeding.

7.34 Several authors have commented on the influence of various stresses in setting up physiological experiments for fish. Two main areas of effect are involved, anaesthetic and handling. In the case of this work, the MS.222 concentration used, 50 mg ℓ^{-1}, is within the "useful" range recommended by Bell (1964) and is the same as quoted by Larsen and Snieszko (1961) in their efforts to avoid partial asphyxiation (which may occur at higher concentrations) and hence prevent disturbance in blood parameters such as haematocrit, and possibly deeper-seated effects. 50 mg ℓ^{-1} is also below the value (80 mg ℓ^{-1}) which Wedemeyer (1970) found to be increasingly effective, after up to 12 min exposure, in disturbing ACTH production (indicating hormonal stress) in soft water. His recommended remedy, of preventing pH falling to about pH 4.0 by buffering, was unnecessary in the present work, since exposure was so short (3 min) and the concentration lower. Hunn and Wilford (1970) reported a requirement of 24 h for the diuretic effect of anaesthesis and catheterisation to clear; in the present study stress would be much less severe since no catheterisation was involved. Effects of handling stress have been commented on by several authors, usually at rather higher levels of handling activity than in this study. Wedemeyer (1972) reported effects on sugar, chloride and calcium levels in steelhead trout blood when, using water chemically similar to that of this study, he netted fish into a bucket and "transferred them 25 metres". He estimated that 24 h was necessary for normalisation of blood parameters; it is possible that steelheads react differently from rainbow trout, but this seems a good guide. Savitz (1973) reported no effect on the nitrogen excretion of starved bluegill sunfish over four days following rather heavier handling stress involving hand-catching from a

bucket; and McKim (1966) found that even severe handling stress on individual fish only lasted in its effect on corticosteroid production for a few days. The stress associated with batch drainage in the weighing procedure is unknown, but Eisler (1957) used the same drainage time without reporting lasting effects, and in the present study some degree of anaesthesia was frequently still present.

7.35 It is concluded that handling and anaesthetic stresses were almost minimal, certainly much less than during pilot experiments. In all cases, fish were left to settle overnight before feeding (which was always successful) and usually 72 h were allowed to elapse before ammonia measurements were begun. This should have allowed ample time for general metabolism to settle down, going by the guidelines of Wedemeyer (1972).

Measurements

7.36 Details of all systems and apparatus used are given in Chapter 3. Bearing in mind the comments of para.6.50 on the necessity of selecting a time-reference for comparability between experiments, and the findings of pilot experiments that the midpoint of the 12 h photoperiod (1400) seemed a suitable sampling time (since it occurred after the steepest rise in TOA in the diurnal pattern), it was decided to standardise on an approximately "mid-day" time for TOA sampling during experiments. Since there was a feed at 1400, the time chosen was 1330. This allowed all tanks to be sampled, and temperatures taken, (also pH readings when required) in good time before the 1400 feed. As 1½ h had elapsed since the previous feed (1200), all disturbance effects associated with feeding would have subsided well in advance of the sampling time. Sampling at lunchtime allowed TOA measurement to take place in the afternoon, and time was available during the morning for making ready sampling bottles and measuring equipment.

7.37 Flow and volume of the tanks were measured prior to

sampling. Since these quantities would affect the resultant TOA at 1330, they were measured well before (while "in operation"), usually at about 1230 to 1245. Flow was measured by timing with a stopwatch, how long it took to fill a 500 ml or 1000 ml volumetric flask from the overflow; choice of flask was dictated by speed of flow, and measurements were taken to the nearest quarter-second. Volume (except in E01, see Table 7.1) was measured with the calibrated indicator tubes for MTS, or by measuring water height for LTS (see Chapter 3). Volume was read off calibration graphs to the nearest 0.1 ℓ for MTS, and to the nearest 1.0 ℓ for LTS.

7.38 The 60 ml bottles for TOA sampling (see Chapter 4) were rinsed out twice each in water from the appropriate tank overflows, before being overfilled. They were then removed to the water-bath prior to TOA measurement. It was important to avoid faecal contamination (a possible source of excess ammonia) and so after flow measurement at 1230-1245, any accumulated debris in the tank U-tube was removed. The effects of this process on flow and volume were only momentary, and negligible.

7.39 The other major measurement was of food fed. Each morning, before the first feed, at 1000, the feed hoppers were emptied into weighing cups and the food weighed to the nearest 0.1 g. By difference between successive days' measurements, with topping up when necessary, the amount delivered over five feeds per day could be measured.

7.40 Thus for each day of the experiment, and each tank, readings were collected for: TOA, flow, volume, temperature, and food consumed over the previous 24 h. For the full 14-day period, readings were collected at beginning and end of the period for number, total weight, and individual lengths of fish.

7.41 Periodically, check-readings were taken of dissolved oxygen (DO) content of the tank water (both inflow and outflow) and pH of the outflowing water. In nearly all cases DO was

close to saturation values in inflowing water and only 20 to 30% depleted in outflow, while pH rarely rose above 7.0 or fell below 6.3. While these values were maintained, no further checks were carried out and these parameters were ignored. Values never departed substantially from the ranges quoted except for some oxygen levels in one of the high-temperature experiments during the middle of summer. Data from tanks affected were disqualified from further treatment. Oxygen was measured from water samples either by a modified Winkler's method (Spotte 1970) or by oxygen electrode and meter (Simac Model 65). pH was measured from water samples from tank overflows. At the pH levels recorded, UIA would not have been of any importance.

Experimental timetable

7.42 Due to the requirement for TOA, etc. readings at 1330, the food consumption of interest was that for the preceding 24 h i.e. since 1330 the previous day. Thus the experiment, although taking place under the normal photoperiod regime of 0800 to 2000, was divided for data treatment purposes into 14 "experimental days", each commencing at 1400 on one date and finishing at 1400 on the next. Thus readings were taken at the end of each "experimental day", which, as thus defined is referred to as DAY in order to avoid confusion. Table 7.2 gives a summary of the events from DAY 1 to DAY 14.

7.43 Between other activities detailed in Table 7.2, and in particular at the beginning of each photoperiod, systematic checks were made of experimental conditions, including water supply, lighting system, tank drainage, waste gutter and pipe maintenance, feeding system operation, feeding behaviour, and general fish behaviour.

DATA ANALYSIS

Basic data

7.44 There are three main sources of data in the E-series experiments. The first is the information gathered at the

	1400	1500	1600	1700	1800	1900	2000	DARK PERIOD	0800	0900	1000	1100	1200	1300
Morning prior to DAY 1									Fish not fed in holding tanks Cleaning and preparation of exptl tanks Establishing of required fish number Flow initiation in tanks Equipment preparation					
DAY 1	Sorting: weight and length measurement and allotting to tanks Feed rate calculation/Acclimatisation								Feed preparation		1st feed		2nd feed	
DAY 2	3rd feed		4th feed		5th feed				Feed measurement	Feeding regime continues as explained in text				
DAY 3 to DAY 14	(Feed regime continues) Ammonia measurement for previous DAY								Feed measurement Preparation for sampling		(Feed regime continues)		A	B
DAY 14	(Feed regime continues) Ammonia measurement for previous DAY								Feed measurement	No further feeding				
Afternoon following DAY 14	Final sorting: weight and length measurement and fish returned to holding tanks													

Table 7.2 E-series experimental timetable

Table 7.2 continued

Notes:-

a) During DAY 3 to DAY 14, box labelled 'A' refers to measurement of temperature, water flow and volume (1230).

b) Similarly, box labelled 'B' refers to sampling of tank overflows for TOA measurement, accompanied by pH measurement when carried out (1330).

c) Checks of dissolved oxygen were normally carried out during the morning period, after the 1000 feed.

beginning and end of the experiment (14-day data), which comprises weight, length and number of fish. The second source is the data gathered on each day of TOA measurement (1-day data), comprising TOA, temperature, flow, volume and food. The third source is any data due to mortalities; in each case, the number, weight, and length of the fish concerned, together with the day of death, were recorded.

7.45 The major omission from the 1-day data is the weight and length of the fish corresponding to each set of 1-day data. Since the only measurements of weight and length were taken as 14-day data, it was necessary to estimate intermediate values. Over the BMP period, an intermediate weight estimation process was evolved in preparation for the E-series.

Intermediate weight estimation

7.46 A simple case is considered first in order to clarify the method, and various problems are then accounted for. For a hypothetical tank with no mortality and exactly the same amount of food fed for each DAY, it could be reasonably assumed that growth in weight would be linear when measured on a daily basis over a short period such as 14 DAYs (Fig.7.2). Although in practice the proportions of food used for maintenance and growth will gradually increase and diminish respectively as the fish grow, this change in proportion is unlikely to be of great importance when considering the overall effect on a whole population in a tank over such a period. In order to estimate intermediate weights, it is necessary first to determine the overall conversion ratio (CR). This is given by:-

$$CR = \frac{F_t}{(W_{14} - W_o)}$$

- where F_t = total weight of food fed,
  W_o = initial total fish weight
  W_{14} = final total fish weight

Since food fed for DAY 1 is known (F_1) the weight increase for this DAY (W') is given by:-

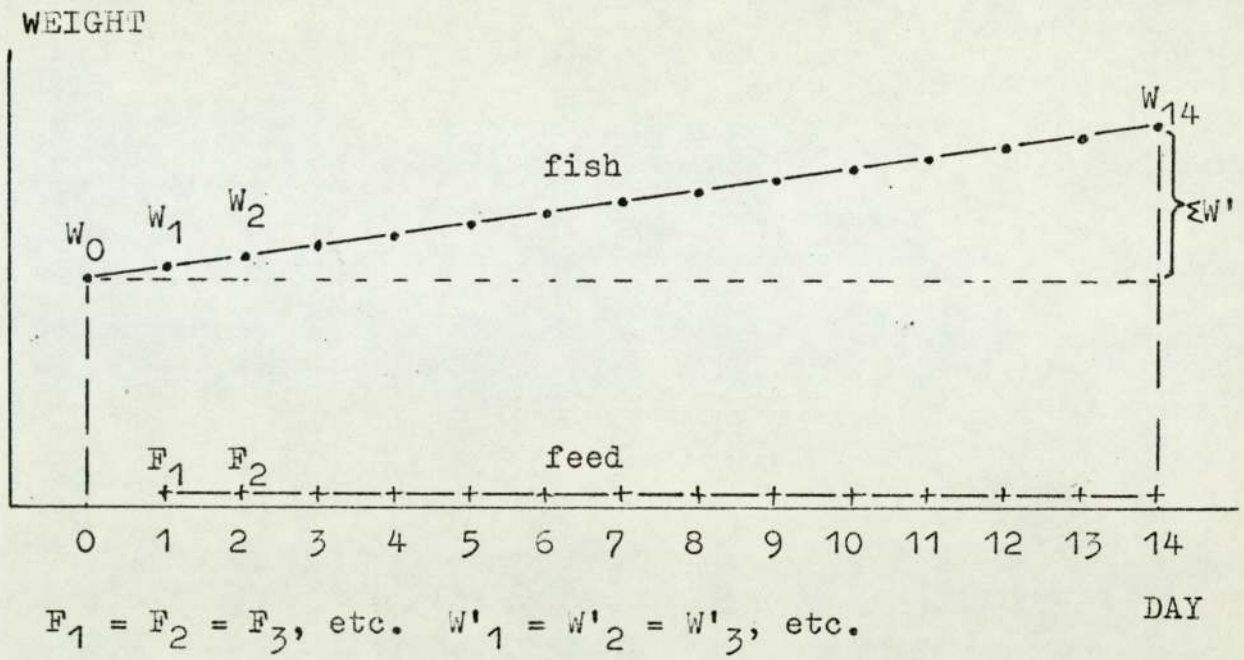


Figure 7.2 Intermediate fish weights (constant feed rate)

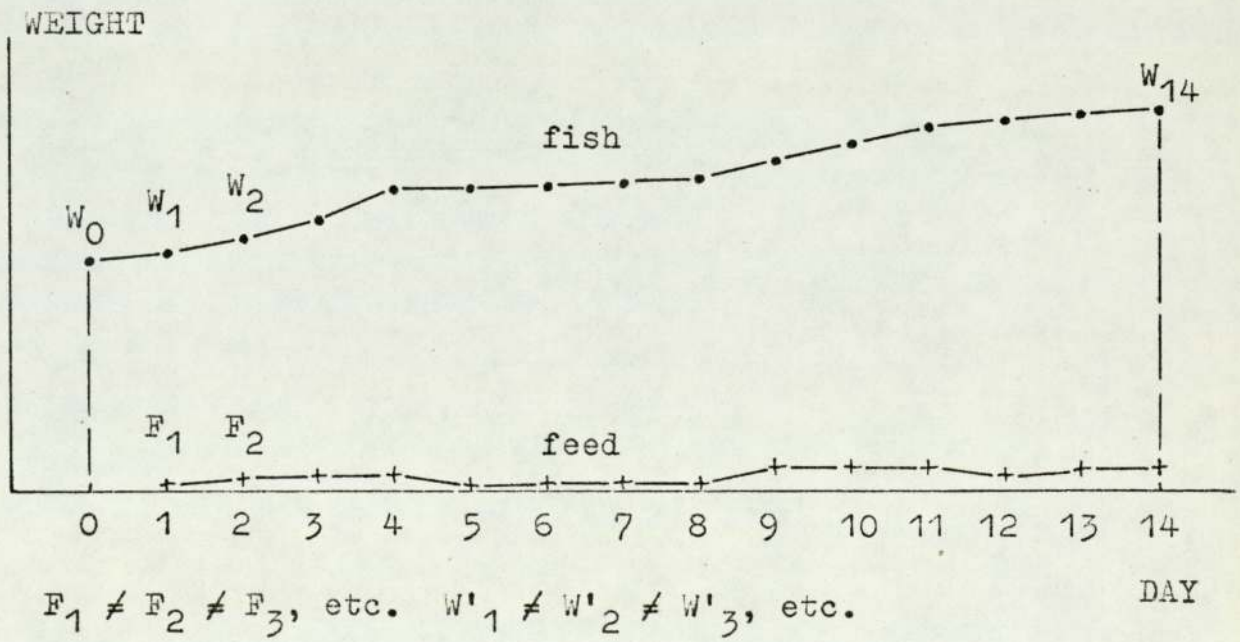


Figure 7.3 Intermediate fish weights (variable feed rate)

$$W' = \frac{F_1}{CR}$$

Hence the current total fish weight at the end of DAY 1 will be $(W_o + W') = W_1$. In the present case, W' remains constant for each day hence $W_2 = W_1 + W'$, $W_3 = W_2 + W'$, etc.

Hence

$$W' = \frac{W_{14} - W_o}{14} \text{ and } W_{14} = W_o + \Sigma W'$$

- since there are 14 DAYs in the experiment)

7.47 If, as is usual, the food fed for each DAY is slightly different, it is assumed that growth in weight will vary in proportion (Fig.7.3). Estimation of each DAY's increase in weight must therefore be independently carried out, thus:-

$W'_1 = \frac{F_1}{CR}$, $W'_2 = \frac{F_2}{CR}$, etc.

Then:-

$W_1 = W_o + W'_1$, $W_2 = W_1 + W'_2$, $W_3 = W_2 + W'_3$, etc.

In the cases referred to so far, estimation of W_{14} by calculation will provide the same answer as the originally measured W_{14}. However, if any mortalities occurred in the tank, this would not be so, and a method was therefore required to account for mortalities.

7.48 To explain this a second hypothetical tank can be considered, in which one fish dies at DAY 7 (Fig 7.4). For simplicity, the food fed per DAY will be assumed constant, since the difference due to variability in this quantity has been explained above. Let the weight of the dead fish on removal be w. Then in order to estimate a conversion for DAY 1 to DAY 7, it is necessary to estimate what the value of W_{14} would have been had the death not occurred (W_F). The best estimate of W_F is the sum of the final mean fish weight and the actual final total weight, i.e.

$$W_F = W_{14} + \frac{W_{14}}{N_F}$$

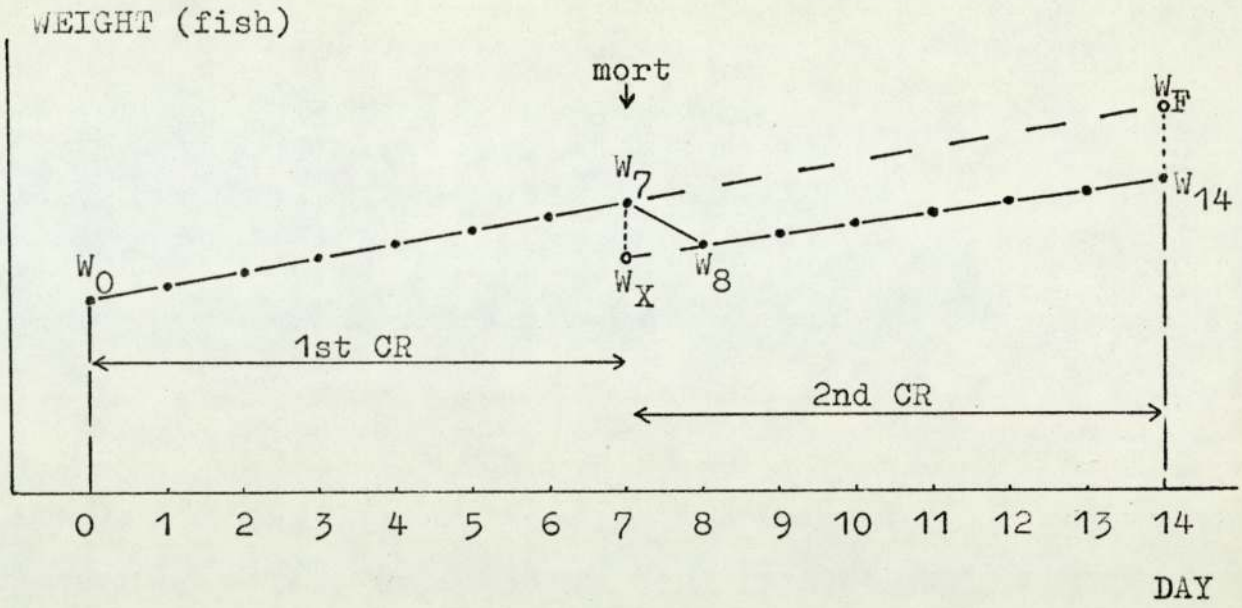


Figure 7.4 Intermediate fish weights (one mortality)

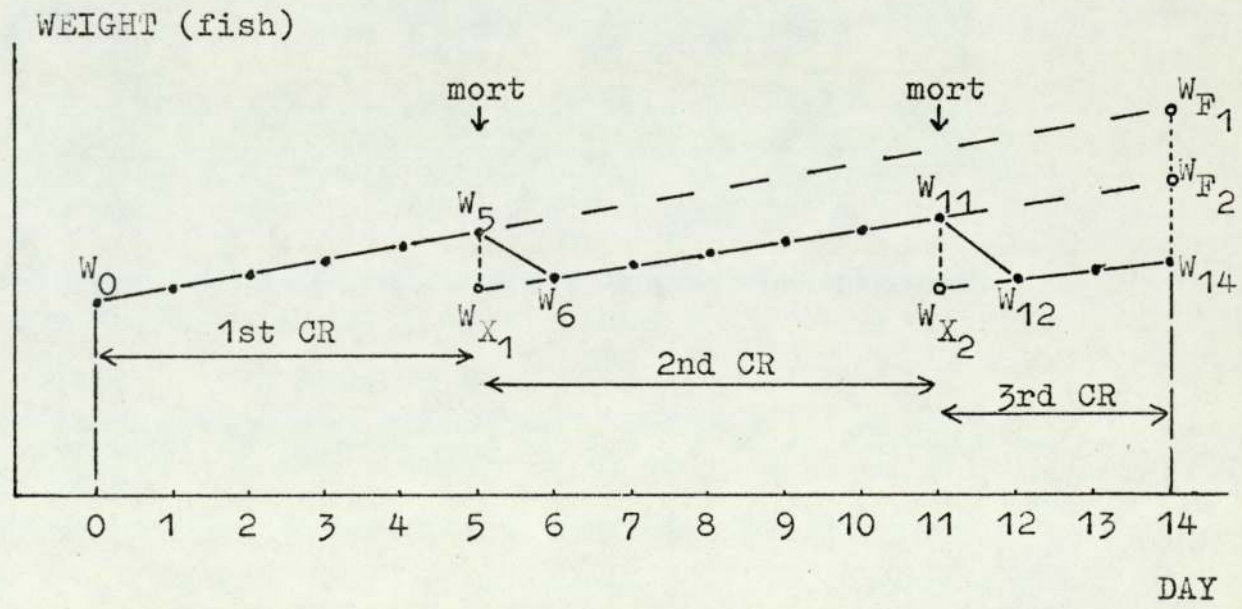


Figure 7.5 Intermediate fish weights (two mortalities)

- where N_F is the final number of fish in the tank. Using W_F in place of W_{14}, CR can be calculated and values for W_1 to W_7 calculated in the normal way. The mortality weight is then brought into use, to estimate a second CR covering the time from DAY 8 to DAY 14. In this case it is necessary to estimate W_X, the DAY 7 total weight less mortality weight, i.e.

$W_X = W_7 - w$; then

$$\text{second CR} = \frac{\Sigma(F_8 \ldots\ldots F_{14})}{(W_{14} - W_X)}$$

Using the second CR, values for W_8 to W_{14} can then be calculated.

7.49 When there is more than one instance of mortality, the process of para.7.48 is repeated each time, and more values of W_F and W_X are required (see Fig. 7.5). With each mortality accounted for, the errors involved (see below) have greater significance.

7.50 A process similar to that used for weight can be followed to estimate intermediate length data. The process corresponds to the weight process in dealing with "total" or cumulative length (i.e. sum of the lengths of all the fish in the tank), and involving a length conversion i.e. ratio of food fed to cumulative length increase. The mean fish length is then simply calculated by division of cumulative length by the number of fish in the tank at the time.

7.51 The processes used for intermediate weight and length estimation are liable to the following sources of error.

a) If feeding is incomplete, then the assumption that growth is directly proportional to feed is probably invalid, unless the same proportion of the food delivered is consumed throughout, which seems improbable. However, feeding was in general excellent, and this error is thus unlikely to be important. In cases where feeding was poor, the growth indication data (see below) would reflect the situation.

In the case of mortalities, several sources of error occur:-

b) The effect of sickening. A fish lost at a certain time (e.g. DAY 7 in Fig.7.3) would almost certainly have been sickening for some time previously, and hence not feeding. Thus the weight loss on its death would probably be underestimated by taking the weight of the dead fish (w). This would make W_X artifically high, affecting the subsequent CR and values of weight.

c) It is quite likely that the fish which die are not of average size, as is implied in the calculation of W_F. In the case of death by bullying, the victim would probably be one of the smallest in the tank (hence W_F would be overestimated); or in the case of death from crowding or oxygen stress, the victim would probably be above average size (hence W_F would be underestimated).

d) The spread of weights for large FCAT fish was greater than for small FCAT; thus errors in averaging (estimation of W_F) would be larger.

e) The first CR calculation involves use of F_t; this is the total food fed in practice. If no mortality had occurred, the fish would have consumed the same total amount but received less each; thus after mortality with more food to go round, growth is probably better. This consideration leads to underestimate of the first CR.

f) The proportions of consumed food which go to maintenance and growth (see para.7.46) may be extensively altered by a number of mortalities especially where the fish which die are particularly larger or smaller than the mean (see below).

7.52 Mortalities are notoriously difficult to deal with in any study involving fish growth (Brown 1957); one possible practice in nutrition work is to ignore the dead fish's effect from the beginning of the experiment. In the case of this study such an approach was not possible, since a fish lost at one point in the experiment could have been affecting its environment, and particularly TOA, quite substantially at an earlier point. So long as the number of fish involved was fairly high, the loss of one

fish would not introduce serious error e.g. for a batch of 20 fish of total initial weight 300 g loss of one fish, would probably have a maximum error of about 0.7%. This is below the accuracy of weighing techniques. However, with a mounting number of mortalities, the error in calculation of W_F becomes larger, especially in the calculation of the first (highest) value, which will depend on the proximity to mean weight of the dead fish. For 5 deaths out of 20 fish, with each one being larger than the final mean weight, the best estimate of W_F is

$$\frac{5W_{14}}{15} + W_{14}$$

$$= \left(\frac{W_{14}}{3}\right) + W_{14}$$

If, however, all 5 lost fish are 2 g heavier in final effect than the final average, then true W_F

$$= 5\left(\frac{W_{14}}{15} + 2\right) + W_{14}$$

$$= \frac{W_{14}}{3} + 10 + W_{14}$$

If the error of 10 g in W_F is related to a starting weight of 300 g, with 5 losses estimated at 15 g each, the error is over 4%; this still seems modest, but the calculation has so far been applied to starting weight only. In practice, the errors of individual occurrences of mortality affect each other, causing error in an increasing proportion over all estimates of weight for which W_F values have been employed. This kind of cumulative effect can lead, after several mortalities, to such a large discrepancy between theoretical and actual values of W_{14}, that the only theoretical solution is to assume no growth, or even loss in weight, after the last mortality. In the actual situation, such a case will be well signposted by the growth indication data after computation (see para.7.58); the loss in growth may well be true, but whether it is or not, such data are unreliable.

7.53 One possible way of avoiding such accumulation errors and non-average mortality effect, would be to have an individual

fish identification and ranking system, since fish often maintain their hierarchical (size) rank position. Unfortunately, in the case of these experiments, the time involved to weigh each fish individually would have been prohibitive, and also profoundly stressful. Stress would also have been involved in any individual marking system, especially with fish of smaller FCAT. The built-in danger signals of error in the process (growth indication data) were felt to be sufficiently good as a guide to selecting reliable data.

Calculated data

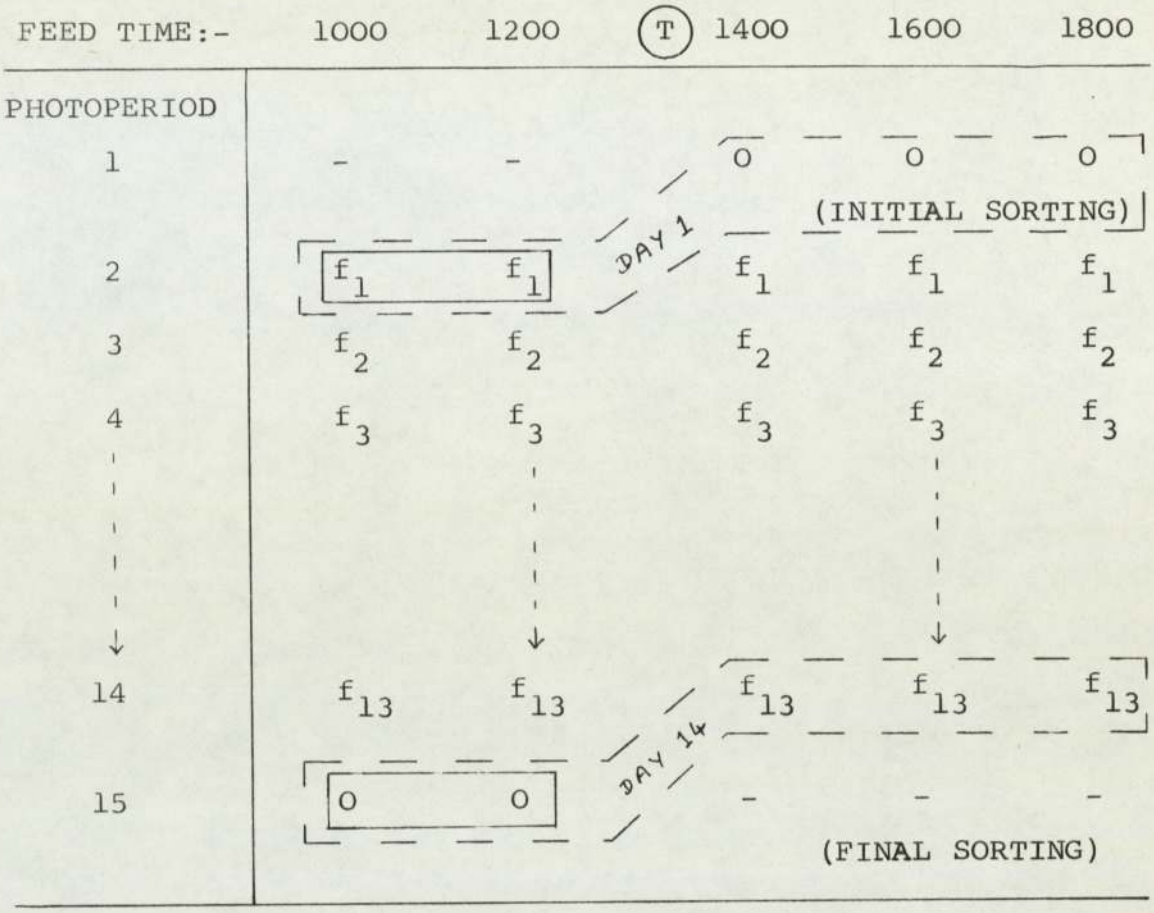
7.54 After the collection of basic data (14-day, 1-day and mortality sets), two stages of computation were involved to generate a second class of data, the "calculated data". Stage 1 was the estimation of intermediate weight and length, as above; stage 2 was calculation of SER, loading, stocking, density and food fed for each set of basic data supplied. Thus for any one tank on any one DAY:-

BASIC DATA:	CALCULATED DATA:
TOA	total weight
flow	mean length
volume	SER
temperature	loading
food fed	stocking
	density
	food fed

It will be noted that food occurs twice. This is because the collected (basic) food data required manipulation before it could be set up as corresponding to the other data.

7.55 Manipulation of food data was due to the system of measurement, which was conveniently performed between 0900 and 1000 (see Table 7.2). Thus the actual measurement was of food fed over 5 feeds during the course of a natural day (photoperiod), and not during an experimental DAY. To calculate the food fed per DAY, it was necessary to divide each food measurement by 5 and group the individual feed amounts as shown in Figure 7.6.

Figure 7.6 Grouping of food data



Notes:-

a) Food calibration / measurement is at beginning of photoperiod (e.g. for photoperiod 2, 5 x f_1).

b) For DAY period, total food fed (F) = last three feeds of one photoperiod + first two of next photoperiod
e.g. $F_1 = 0 + 0 + 0 + f_1 + f_1$, $F_2 = f_1 + f_1 + f_1 + f_2 + f_2$ (dotted box).

c) For DAY period, last 2 feeds (G) = first two of photoperiod
e.g. $G_1 = f_1 + f_1$, $G_2 = f_2 + f_2$, $G_{14} = 0 + 0$ (solid-line box).

d) (T) indicates time of TOA measurement.

For later treatment of data, two quantities were calculated; the food fed in the previous 24 h before a TOA measurement (i.e. food fed per DAY), designated as F; and the food fed over the last two feeds (the morning feeds at 1000 and 1200) prior to TOA measurement, designated as G. Thus G = 2F/5.

7.56 In summary, the following calculations were performed on each basic data set:-

a) estimation of total weight (W) and mean length (L);
b) manipulation of food data to give F and G;
c) loading = W/flow;
d) stocking = W/volume;
e) SER = TOA/loading;
f) density = number/volume

(Each calculation embodied suitable conversion factors in order to attain appropriate units.)

7.57 All basic and calculated data are computer-listed in Appendix A3. Data from P01 to P04, and E00, are included for comparison, although some values are unreliable estimates only.

Data selection

7.58 Before further treatment, it was necessary to screen data to remove unreliable sets. All sets were reviewed, and rejected if they were unsatisfactory in terms of any of the following criteria:-

a) More than 2 mortalities (para.7.52).
b) Fall in condition factor (sign of poor growth).
c) Weight conversion ratio which is negative or in excess of 3.0; these indicate loss of weight or poor feeding.
d) Lack of, or negative, growth in mean weight or length.
e) Errors in sorting (e.g. miscalculation of fish number).
f) Irregular setting-up conditions (e.g. as in E03, MT1; see Table 7.1); this includes pilot experiments and E00.
g) Errors in sampling or measuring 1-day data.

Criteria (a), (b), (c) and (d) comprise the "growth indication data", and were referred to after computation (to produce

"calculated data") and before subsequent data analysis, in order to assist in data selection.

7.59 All screened data, acceptable by criteria (a) to (g), were subjected to further analysis, and are computer-listed in Appendix A4.

Multiple regression analysis

7.60 It is frequently found that biological data can be related, either in raw or in logarithmic form, by equations which describe a straight line when plotted on a graph; thus in standard nomenclature:-

$$Y = a + bX \quad \text{or} \quad \log Y = \log a + b(\log X)$$

The latter can equally be expressed as:-

$$Y + aX^b$$

(this relationship in raw form may be a curve, but is reduced to a straight line in logarithmic form). A common example is the relationship between length and weight for salmonid fish where:-

$$\text{weight} = K \times \text{length}^3$$ (Brown 1957, Bowen & Studdard 1970)

(However, see Ricker (1973) for criticism of usually-quoted values for this exponent.) This relationship forms the basis of the condition factor (K) referred to earlier.

7.61 The above refers to a dependent variable (Y) related to only one independent variable (X). Where more than one independent variable is involved (X_1, X_2, X_3, etc.), the resulting graph must be "plotted" in n-dimensional space where n = number of independent variables. Where $n > 3$, this becomes difficult to comprehend, and impossible to display in a simple visual way.

7.62 The purpose of this study, as explained in Chapter 5, involved a number of X quantities, and their effect on a particular Y quantity identified as SER. For further analysis these X quantities were transformed to give an overall linear relationship. Expressing relationships in an n-dimensional straight-line form allows relatively easy comparison of different relationships, and selection of the one which gives the best-fitting

model, in order to predict SER in terms of the most appropriate X quantities; the technique is that of multiple regression analysis.

7.63 In the case of simple regression (one X quantity), prediction of Y from X values is usually governed by the regression of Y on X (this procedure has been criticised for fish work by Ricker (1973)); this is one of the two best straight lines drawn through the plotted data, calculated in well-known fashion by minimising the sum of squares of deviations from the line in the Y-direction (e.g. as in Bishop 1966), thus for n pairs of data:-

$$\Sigma d_x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n}$$

$$\Sigma d_y^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$$

$$\Sigma d_x d_y = \Sigma xy - \frac{(\Sigma x . \Sigma y)}{n};$$

then

$$\text{regression coefficient, } b = \frac{\Sigma d_x d_y}{\Sigma d_x^2}$$

and

$$r = \frac{\Sigma d_x d_y}{(\Sigma d_x^2 . \Sigma d_y^2)}$$

Now $\bar{x} = \frac{\Sigma x}{n}$ and $\bar{y} = \frac{\Sigma y}{n}$

and the regression equation is

$$y = \bar{y} + b\,(x - \bar{x})$$

Thus having calculated b, $\bar{x}$ and $\bar{y}$, a prediction of y can be made for any given value of x. Substitution of values for b, $\bar{x}$ and $\bar{y}$ allows calculation of the intercept, a, such that $y = bx + a$. When this line is plotted, the slope of the line is b. The quantity r derived above is the correlation coefficient, and expresses the degree of closeness of relationship between X and Y. This quantity can be tested for statistical probability of significance according to the magnitude of n.

7.64 In the case of multiple regression, each X quantity

nominated can be tested to derive similar information with respect to Y; the overall equation for prediction will be (for n X-quantities):-

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \ldots\ldots + b_nx_n$$

and the overall relationship will have a multiple correlation coefficient, R, corresponding in nature to r for simple regression, but of more complex derivation. Thus the procedure of multiple regression analysis involves:-

a) nomination of X quantities and Y,

b) calculation of R,

c) test of significance of R,

d) calculation of a and b_1, b_2, b_3..... b_n to define the equation,

e) test of significance of b_1, etc. by t-test ($t = \frac{b}{SE_b}$)

7.65 Now R can be thought of as a measure of the amount of Y variability explained, or accounted for, by the nominated X quantities. Since R can only take values between 0 and 1, these values can be very loosely translated into % of Y variability explained by the X quantities; thus if R = 0.75, the X quantities can be said, for intuitive comprehension, to explain approximately 75% of the Y variability. (Strictly, R^2 is the proportion of the squared deviations accounted for.) The remaining variability is expressed in a quantity known as the error sum of squares, which allows the setting of limits of accuracy to the multiple regression equation.

7.66 Examples of multiple regression used for fish physiology work can be found in Allen (1974) and Beamish (1974), and their examples illustrate a further complication in multiple regression. Whilst in simple regression the Y quantity can only be affected by X or some power of X, with increasing numbers of X quantities more possibilities occur. Y may be related to:

a) X_1, X_2, X_3, etc.,

b) powers of X (e.g. X_1^2, X_2^2 ; X_1^3, etc.),

c) interactions between X quantities or their powers; e.g.

X_1X_2, X_1X_3, X_2X_3 etc.,

$X_1^2X_2$, $X_2^2X_3$, $X_1^3X_2$, $X_1^2X_2^3$ etc.

Clearly, the more X quantities there are, the more of these possibilities there are, by geometrical progression. In order to deal with such a large range of possibilities, especially when working with a large number of data, it is necessary to use a digital computer.

7.67 Although a large range of possible effectors could thus be tested, it is important to note that many of the quantities generated by interactions as mentioned above are probably meaningless in any environmental sense, e.g. the cube of the temperature multiplied by the square of the number of fish is a valid interaction but somewhat difficult to appreciate or to relate to the situation in the tank. Thus, selection of meaningful quantities was equally as important as identifying significant ones.

7.68 As previously explained, Y for this analysis was SER. The nomination of X quantities was rather complex and several models of analysis were tested, in accordance with various hypotheses of effect. Two overall models (OM) were envisaged.

7.69 OM1 was characterised by deliberate avoidance of consideration of the number of fish in the tank, and hence the density (as earlier defined). Thus a basic question was posed: could the environmental effect on fish in the tank be independent of the actual number or density, and therefore was it capable of description by quantities related to purely chemical or physical effects? This model was as fundamentally mechanistic as possible, given that the organism was occupying a "black box" role (see Chapter 5). In this case the fish population in the tank was likened to a single ammonia-producing machine rather than a collection of them, dependent on variables apprehended by the population collectively rather than individually, and independ-

ent of differences between the fish in any one tank at a particular time. A property of this model would be that quantity of fish was adequately expressed by the weight, so that 50 fish of 2 g each would be equivalent to 2 fish of 50 g each.

7.70 OM2 took the number and density of fish into account, and thus considered a less mechanistic view of the situation.

7.71 Within the overall model, various combinations of possible X quantities could be tested, and analysed, by the use of a suitable package of computer routines. The following combinations were tested under OM1:-

a) temperature, loading, stocking, food fed, length; and squares of these quantities, and first-order interactions i.e. simple multiples (e.g. temperature x length);

b) logarithmic transformations of temperature, loading, stocking, food fed, and length.

Under OM2, the first combination tested was of the simplest quantities measured, i.e. temperature, number, flow, volume, length and food fed; then there was consideration of the calculated quantities such as loading, stocking and density; and subsequently consideration was given to more complex possible effector quantities such as mean free path (see Chapter 5).

7.72 Promising models for explanation of SER were computer-graphed to enable individual quantities to be examined in the context of the group. The first step was to identify in multiple regression analysis those quantities which were statistically significant at the level of $P = 0.05$, and then to calculate partially-corrected values of the dependent and independent variables (see Appendix C).

7.73 If, for example, there were three independent variables, three partially-corrected relationships would be derived; each one could be plotted for a different independent variable. The series of three graphs which such a process would generate is equivalent to a plot of the observations and the regression

equation in 3-D space (or, for n graphs of n quantities of X, in n-dimensional space). Each individual graph is equivalent to an observation of this space from a side corresponding to the particular independent variable, and looking in such a direction that the regression plane appears as a straight line (Aston 1972). Fig.7.7 shows the process involved in partial correction by using a simple model with two independent variables, which is appreciable on paper.

7.74 The graphs are useful for:
a) eye-examination of the closeness of fit to a straight line;
b) picking out of points which are well away from the line, allowing investigation of their particular circumstances;
c) checking whether points are relatively evenly distributed along the axes, and hence suggesting where further measurements may be taken.

COMPUTER PROCEDURES

Introduction

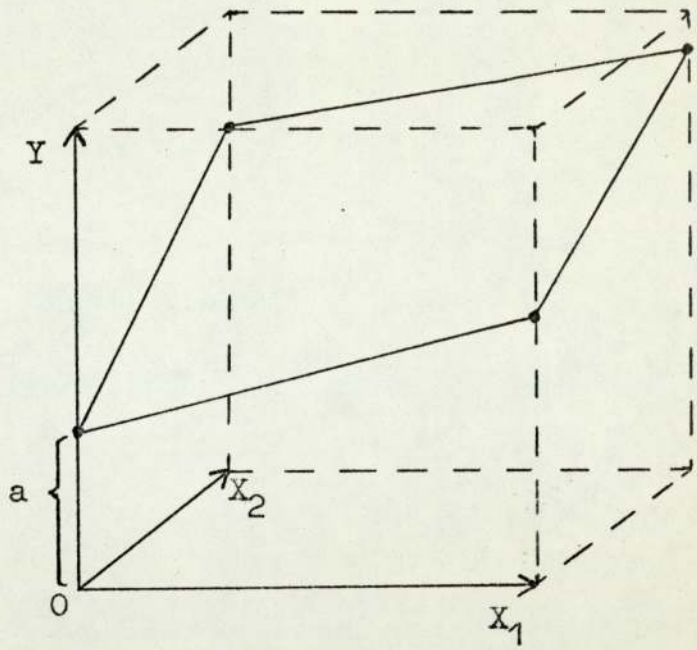
7.75 Handling large data arrays and subjecting them to complex analyses, with the limitations on time available, was a process demanding the use of digital computer time. All work was performed on the University of Aston ICL 1905E (later 1904S) machine using FØRTRAN programming language. A substantial amount of programming was required in early stages, but for multiple regression (analysis and presentation) standard software packages were employed.

7.76 At all times special code names were used for the various quantities dealt with. Those of major importance were required to have 6-character names (for the regression analysis package layout), and a list of these names is given below:-

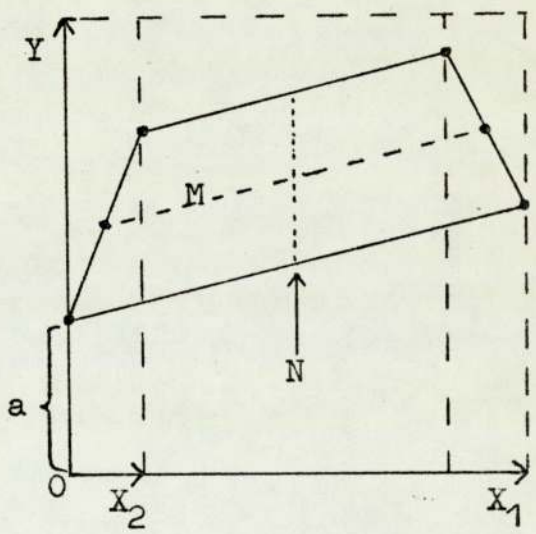
TØTAMM : TOA (concentration)
ENUMBR : number of fish
FLØWRT : rate of water flow through tank

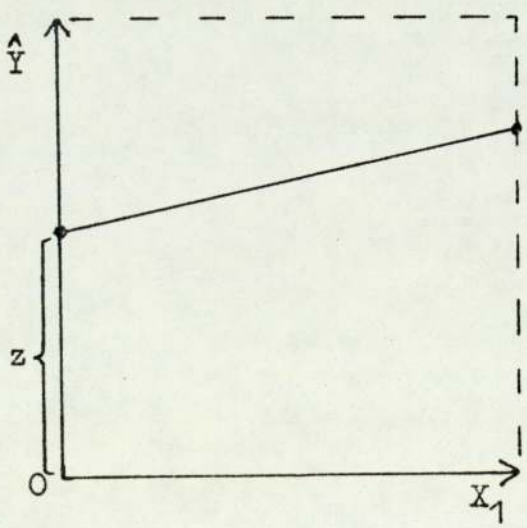
A) 3-D view of a regression plane given by:

$Y = a + b_1X_1 + b_2X_2$



B) Frontal view of (A) with depth perspective retained





C) Frontal view shifted to N in (B) and advanced to M;

$z = a + b_2t_2$

$\hat{Y}$ is partially corrected for X_1

(see Appendix C)

Figure 7.7 Partial correction procedure for Y

VØLUME : water volume in tank

WEIGHT : total mass of fish in tank

SIZELN : cumulative length of all fish in tank (see AVEENU)

AVERLN : mean length of fish in tank

SPEXRT : SER // EXRATE : water exchange rate:flow/volume

TEMPRT : temperature of tank water

DENSTY : number of fish per unit volume of water (density)

DNLØAD : mass of fish per unit flow of water (loading)

DNSTØK : mass of fish per unit volume of water (stocking)

FACTØR : loading per unit length of fish

GRUBFD : food fed in last 2 feeds of experimental DAY (G in para.7.55)

FDMEAL : total food fed in experimental DAY (F in para.7.55)

DNFACR : a loading term relating length to weight via the equation $W = KL^3$ (see para.7.60); quantity should be equivalent in effect to FACTØR above, and is given by: $\frac{\text{weight}^{2/3}}{\text{flow rate}}$ or $\frac{\sqrt[3]{\text{weight}^2}}{\text{flow rate}}$

FREPAT : mean freepath; a term relating the volume and the number of fish thus: $\frac{\text{volume}^{1/3}}{\text{number}}$ or $\frac{\sqrt[3]{\text{volume}}}{\text{number}}$

This is a measure of the theoretical average distance of separation between fish if they are randomly distributed through the tank.

STØLIN : stocking/loading interaction factor; $\frac{\text{weight}}{\text{flow x volume}}$

FDGØVN : feed-governing factor i.e. the quantity which determines the food fed: $\frac{\text{temperature x number}}{\text{mean length}}$

AVEENU : multiple of AVERLN and ENUMBR; same as, and used as alternative to, SIZELN; cumulative length

STKFAC : stocking per unit length of fish

DNSTAC : a stocking term related to STKFAC as DNFACR is to FACTØR (relating length to weight); it is given by: $\frac{\text{weight}^{2/3}}{\text{volume}}$ or $\frac{\sqrt[3]{\text{weight}^2}}{\text{volume}}$

7.77 The importance of these quantities is discussed in the RESULTS and DISCUSSION sections.

Calculation of UIA

7.78 The simplest calculation that was commonly required was the determination of UIA from measured data for TOA, pH and temperature. The relation is as follows:-

$$UIA = \frac{TOA}{1.0 + \text{antilog}\ (pK_a - pH)}$$

where

$$pK_a = \frac{(2835.76)}{T} - 0.6322 + (0.001225 \times T)$$

The latter equation for pK_a determination derives from, and uses, constants given by Robinson and Stokes (1969); T is the temperature on the Kelvin (Absolute) scale.

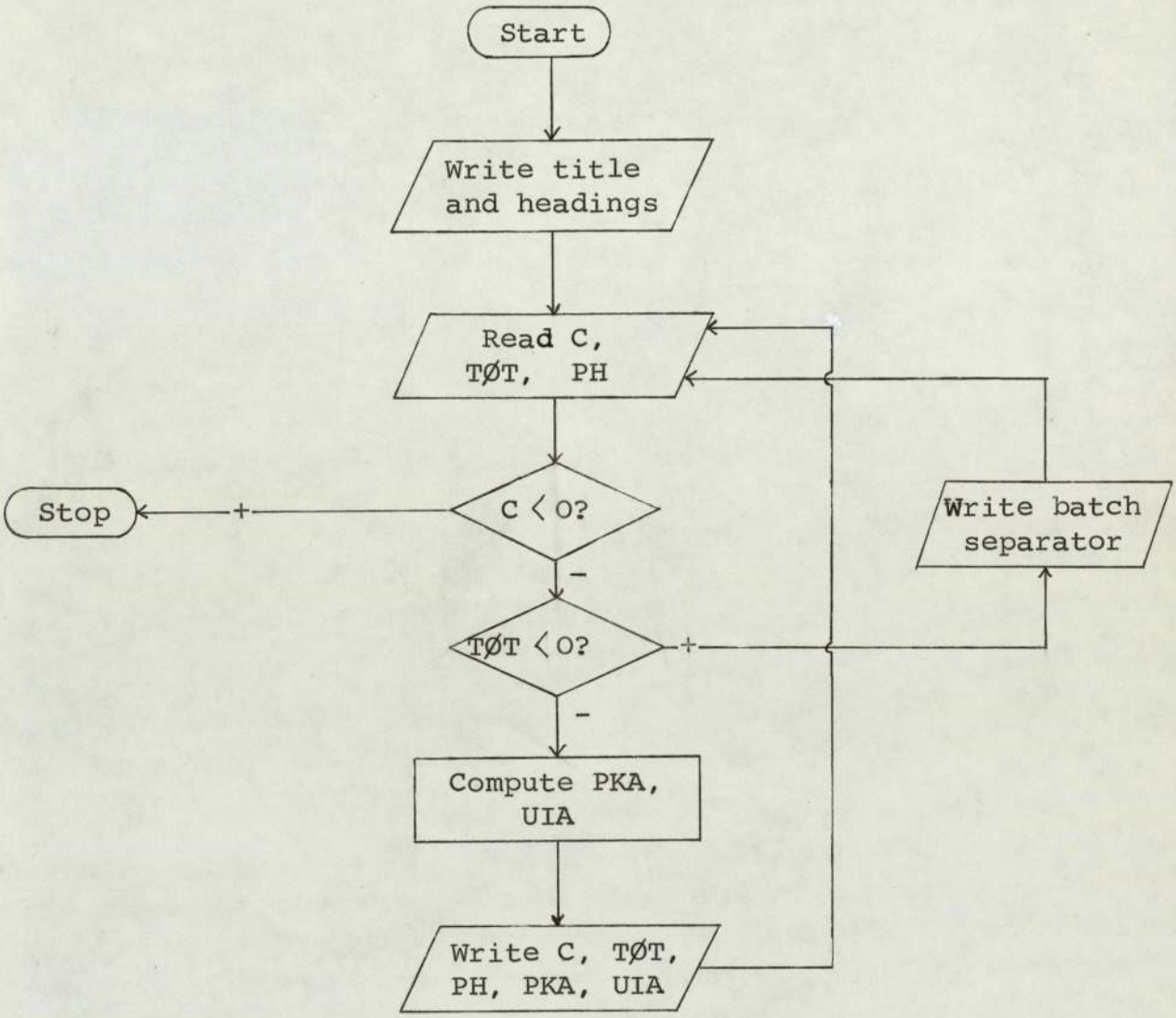
7.79 A simple program codenamed UIACALC was constructed to perform this calculation repeatedly. UIACALC is described by the flowchart of Figure 7.8 and was run under the SØFØR batch system for small FØRTRAN programs.

Calculation of intermediate weight and length data

7.80 As indicated in para.7.45, an estimate of intermediate weights and lengths was necessary to set alongside other 1-day data. To perform this estimation, program GRØWCALC was evolved over a series of trials, using 14-day data and 1-day food data to estimate conversion ratios and intermediate values as described earlier. Data was read in in separate complete matrices and was later accessed by a matrix indexing system as required.

7.81 The basic pattern of the simplest version of the program is given in Figure 7.9. However, in order to contend with mortalities, lack of data for particular tanks or days within the indexing system, or other problems, a complex program was required. The flowchart (Fig.7.10) is similarly complex, and has been reduced to a convenient pattern dispensing with the normal conventions used in Fig.7.8. However, apart from convenient changes in order of logic to allow easier handling of quantities, the flowchart covers the same procedures as are described in para.7.46 to 7.48 and Fig.7.2 to 7.5.

Figure 7.8 Flowchart for program UIACALC

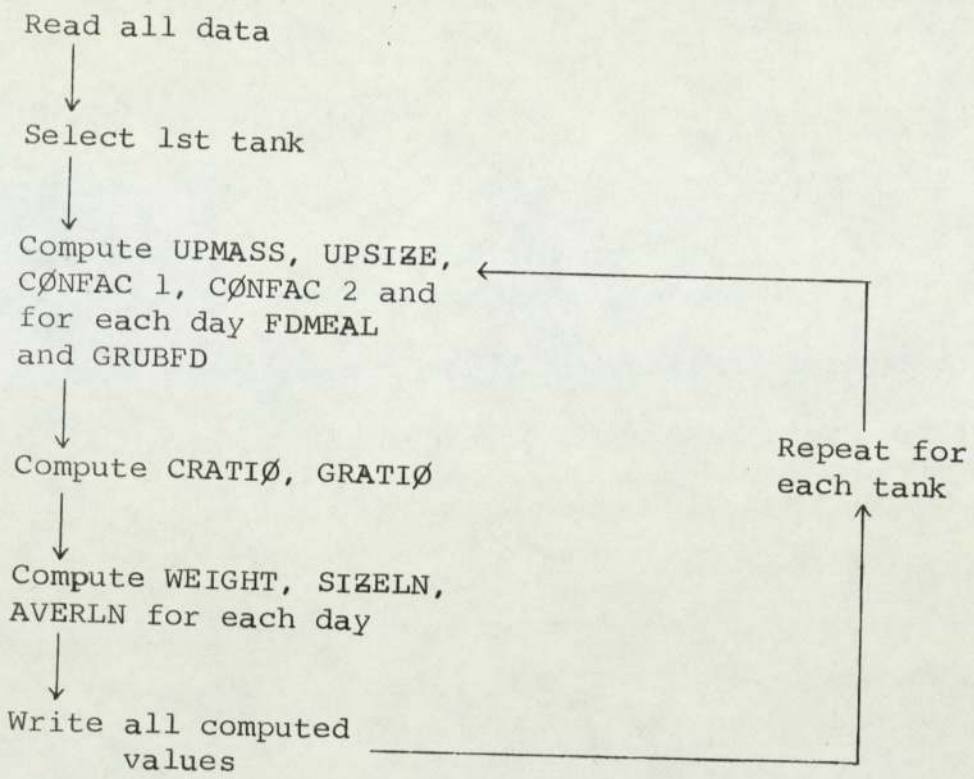


+	=	positive option	-	=	negative option
C	=	temperature	TØT	=	TOA
PKA	=	pK_a	PH	=	pH

Batch terminator dummy has -ve TØT

Data terminator dummy has -ve C

Figure 7.9 Basic pattern for GRØWCALC



UPMASS = $W_{14} - W_o$ (weight gain)

UPSIZE = cumulative-length gain

CØNFAC 1 = initial condition factor

CØNFAC 2 = final condition factor

CRATIØ = weight conversion ratio

GRATIØ = length conversion ratio

Figure 7.10 Flowchart for program GRØWCALC

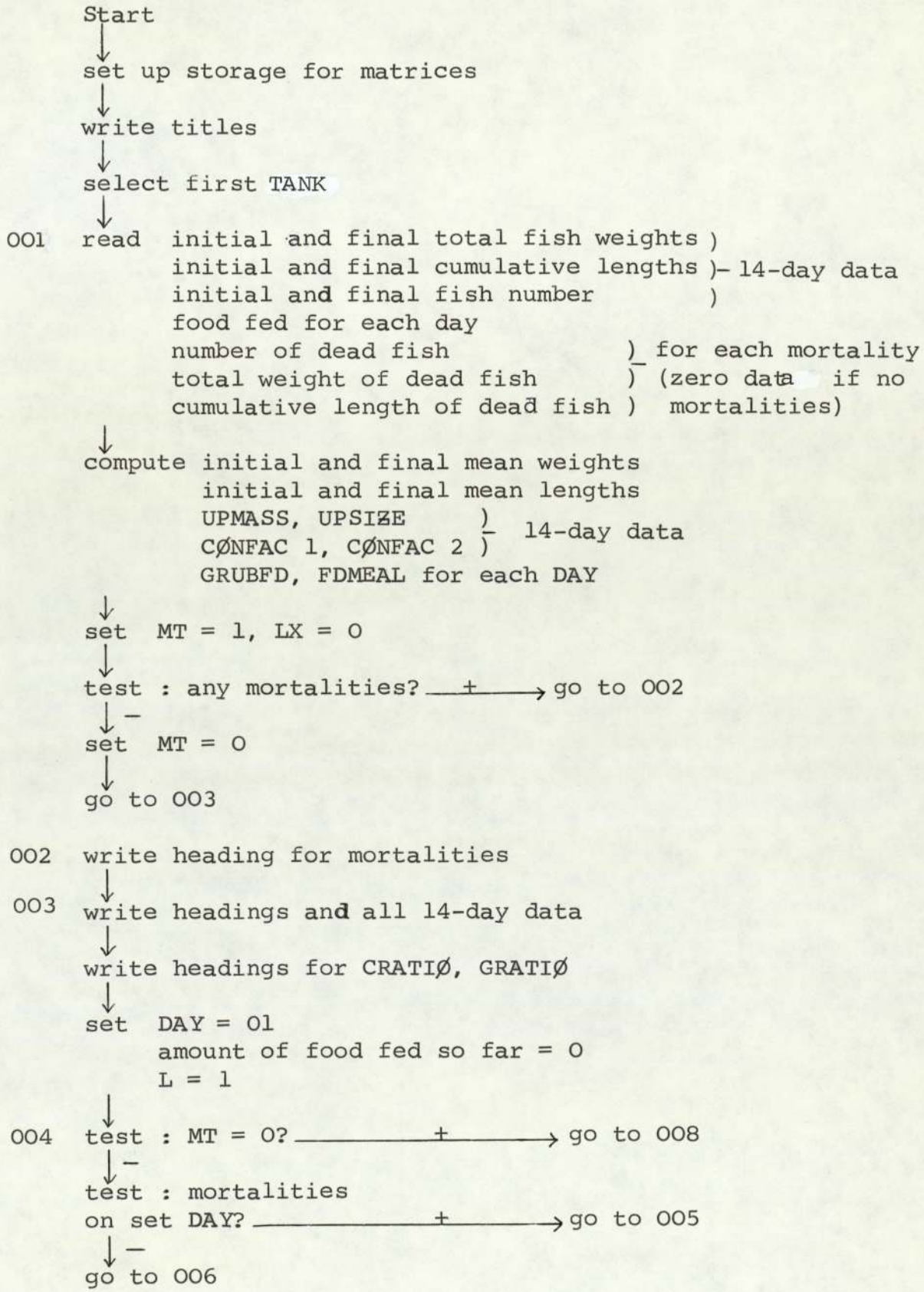


Fig. 7.10 continued

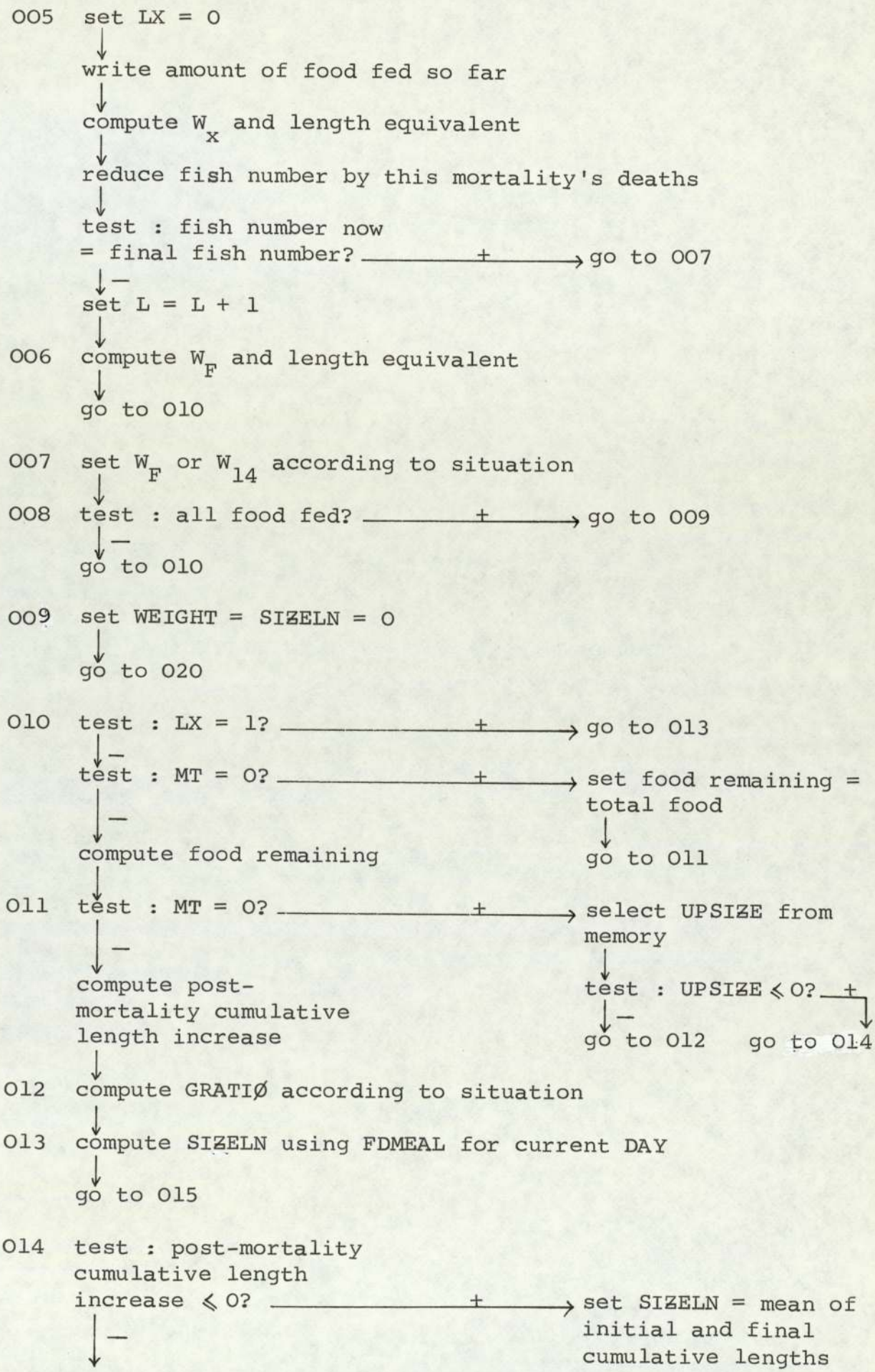


Fig. 7.10 continued

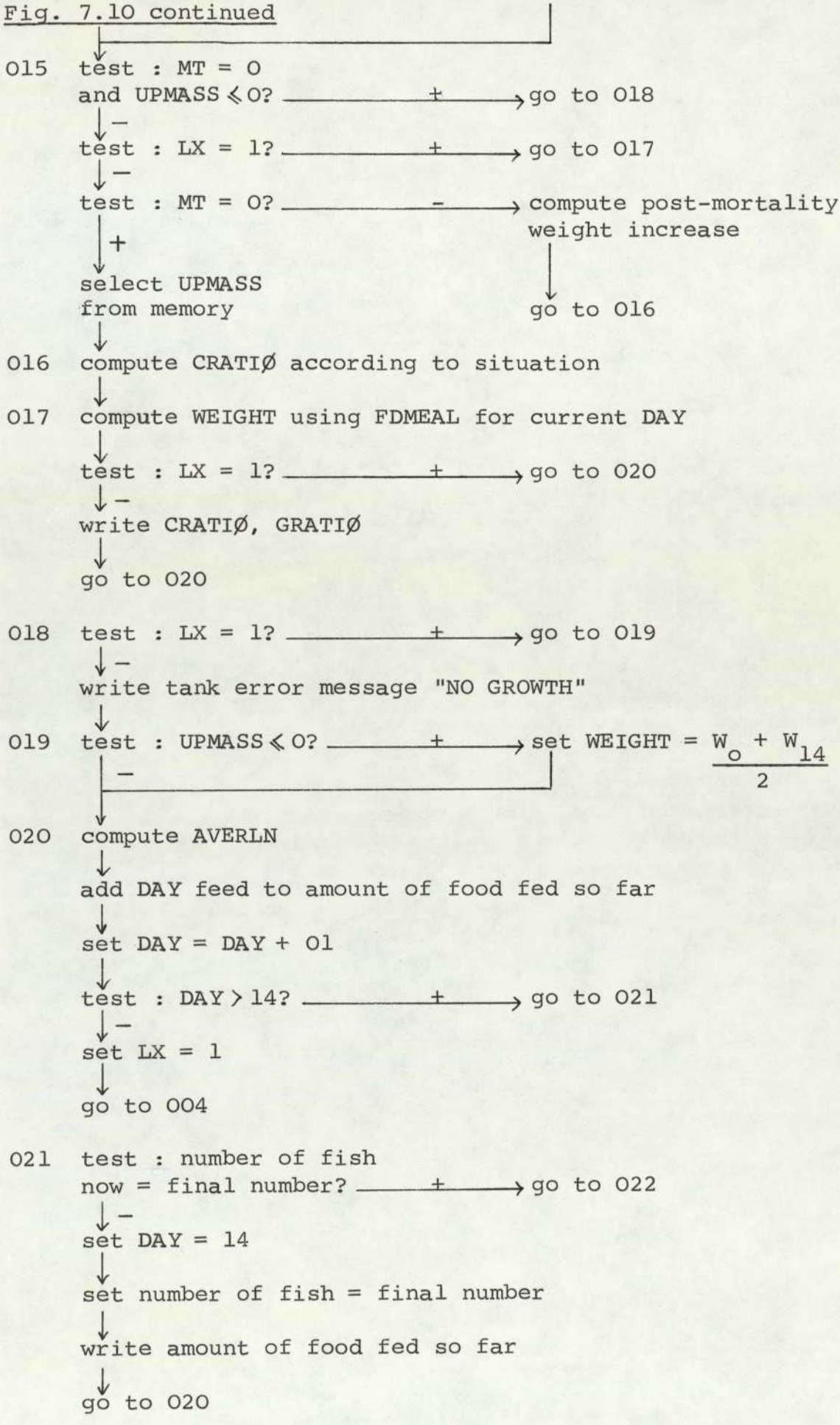


Fig. 7.10 continued

```
022  write headings for 1-day data
       |
       v
     write WEIGHT, SIZELN, AVERLN, GRUBFD, FDMEAL for each DAY
       |
       v
023  set TANK = TANK + 1
       |
       v
     test : TANK ≤ 8? ----------+----------> go to 001
       |  -
       v
     stop
```

Notes:-

a) UPMASS, UPSIZE, CONFAC 1 and 2, CRATIO and GRATIO are as defined in Fig. 7.9.

b) W_o, W_{14}, W_x and W_F are as shown in Fig.7.2 to 7.5 and described in para.7.46 to 7.48.

c) MT is mortality code. A distinction is drawn between a death (involving one fish) and a mortality (meaning an occurence of death involving one or more fish).

MT = 0 indicates no mortality:

MT = 1 indicates presence of one or more mortalities

d) LX is a repeater code for periods in between mortalities:

LX = 1 indicates that current CRATIO and GRATIO can be used for the next calculation of SIZELN and WEIGHT (skips irrelevant sections of program);

LX = 0 indicates that recalculation of CRATIO and GRATIO is required (at the beginning, or after a mortality) for the current tank.

e) L quantifies mortality; it is set to zero at the start, and is incremented by 1 for each mortality (not necessarily each death) for the current tank.

f) "Amount of food fed so far" refers to the total so far consumed at any particular point under consideration; at the end of consideration for one tank this quantity will have accumulated to the complete total fed (F_t).

g) As the program repeats itself for different DAYs and different tanks, quantities which have been calculated frequently need to be reset to zero or initial values, or updated to current values, to enable them to be re-used. For such operations the expression "set" is given in the flowchart, whereas straightforward calculations are expressed by "compute".

7.82 A sample of GRØWCALC output is shown in Fig.7.11. The growth indication data described in para.7.58 can be readily identified, and the required data for WEIGHT, SIZELN and AVERLN can be matched to any specified DAY.

Calculation of derived quantities

7.83 For the sake of pre-analysis information, a simple program entitled DERIVDATA was formulated to calculate values of SPEXRT, DNSTØK, DNLØAD and FACTØR from input values of 1-day data. The flowchart is given in Fig.7.12.

7.84 The programs GRØWCALC and DERIVDATA were later amalgamated into an overall program for pre-analysis treatment of E-series experiments, code-named ENVIRØDATA. Output from this program could be transferred to computer file storage when coded and stored in strings. The accumulated total storage of these coded data strings made up the content of the basic and calculated data matrices which form Appendix A3.

Multiple regression analysis

7.85 The data of Appendix A3 were stored, collected and edited on computer files to produce the single matrix of screened data which satisfied the criteria of para.7.58; this is given in Appendix A4 and was produced in correct format for the analysis requirements.

7.86 Multiple regression analysis, using the Appendix A4 data to supply quantities for independent variables with SPEXRT as the dependent variable, was carried out using the standard ICL Statistical Package (XDS3) software. Output includes the results of the analysis with multiple correlation coefficient, regression coefficients, statistical probability data, and limits and intercept of the regression equation. The same data could be reworked as many times as required, with specification of different independent variables.

7.87 Any individual multiple regression analysis carried out by the Statistical Package could be further scrutinised by means of a presentation program (see Appendix C).

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XX
XX

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XX

TANK	INITIAL WEIGHT (G)	FINAL WEIGHT (G)	UPMASS (G)	INITIAL SIZELN (CM)	FINAL SIZELN (CM)	UPSIZE (CM)	TOTAL FOOD FED (G)
5	530,0	628,0	98,0	346,5	354,5	8,0	95,0

XX
XX

INITIAL FISH NUMBER	FINAL FISH NUMBER	CONFAC1	CONFAC2	CRATIO	GRATIO
30,	30,	1,147	1,269	0,9694	11,8750

XX
XX

TANK	DAY	FDMEAL (G)	WEIGHT (G)	SIZELN (CM)	AVERLN (CM)	GRUBFD (G)
5	1	3,36	533,47	346,78	11,559	3,36
	2	7,72	541,43	347,43	11,581	2,68
	3	6,62	548,66	347,99	11,600	2,60
	4	7,34	555,83	348,61	11,620	3,44
	5	8,56	564,66	349,33	11,644	3,40
	6	8,02	572,93	350,00	11,667	2,92
	7	7,18	580,34	350,61	11,687	2,80
	8	5,66	586,18	351,09	11,703	1,46
	9	8,44	594,89	351,80	11,727	2,60
	10	7,06	602,17	352,39	11,746	3,16
	11	7,38	609,78	353,01	11,767	2,64
	12	6,56	616,55	353,57	11,786	2,60
	13	6,78	623,54	354,14	11,805	2,88
	14	4,32	628,00	354,50	11,817	0,00

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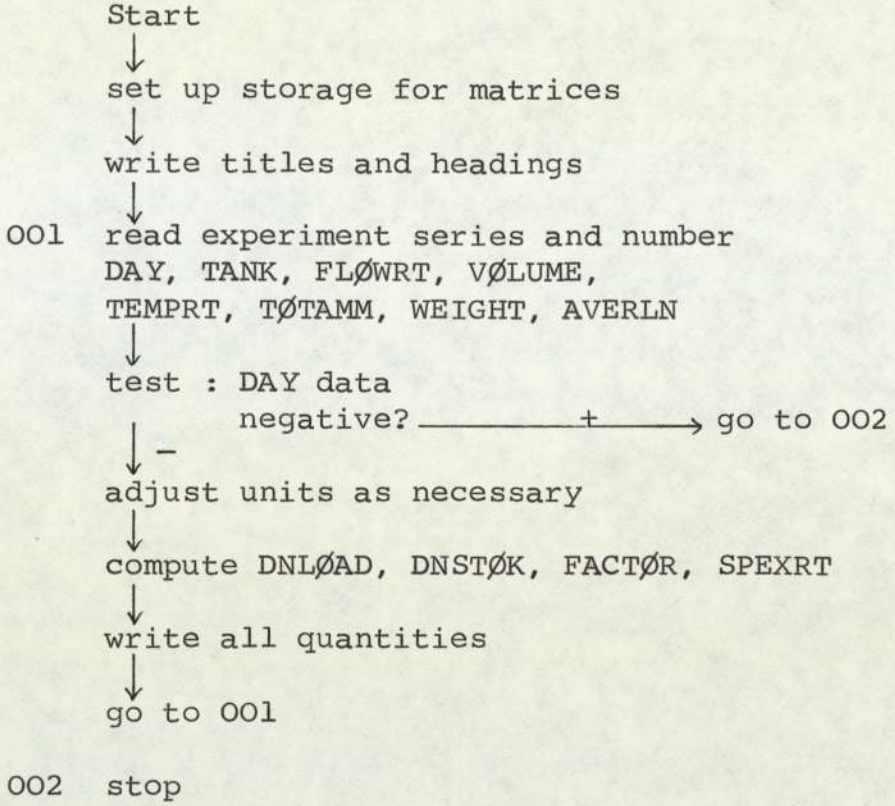
Figure 7.11 Sample of GRØWCALC output

Figure 7.12 Flowchart for program DERIVDATA



(Data terminator has negative value for DAY)

7.88 Basic and calculated data strings are given in total in Appendix A3. The screened data used for analysis are given in Appendix A4.

7.89 Table 7.3 lists the mean, minimum, maximum and standard deviation values of the quantities which formed the analysis observation matrix. Similar values are given in Table 7.4 for other quantities considered during analysis.

Individual correlations

7.90 During multiple regression analysis, the correlations between SPEXRT and the other individual quantities were produced as by-products. This enables one to gauge the efficiency of individual quantities as SPEXRT predictors under the conditions of these experiments (as well as enabling detection of high correlations between individual independent variables). A list of such correlation co-efficients is given in Table 7.5. It should be noted that with so many degrees of freedom, virtually any correlation coefficient is statistically significant.

7.91 Of the various quantities tested against SPEXRT, the best predictors are TEMPRT (temperature) in the positive sense, and DNFACR and DNLØAD (two different loading factors) in the negative sense. It is important to realise that this predictive ability lies only in that particular quantity taken alone; when groups of quantities are considered (as in multiple regression analysis) the situation is somewhat different. From the data of Table 7.5 it would appear that the best biophysical quantity on which to base simple "rule-of-thumb" prediction, for these experimental conditions is the loading factor which takes length into account by its relation with weight, DNFACR. It is interesting to note that the correlation between SPEXRT and FACTØR is relatively low, although FACTØR and DNFACR should be in theory different ways of calculating the same thing, provided the relationship between weight and length is constant. In the

Table 7.3 Summary data for observation matrix quantities

(n = 122)

QUANTITY	UNITS	MEAN	MINIMUM VALUE	MAXIMUM VALUE	STANDARD DEVIATION
SPEXRT	mg kg^{-1} h^{-1}	13.3751	3.829	34.845	7.05840
TEMPRT	^{o}C	9.97295	6.1	17.0	3.76612
ENUMBR	-	78.5164	19	350	87.9254
FLØWRT	ℓ min^{-1}	1.12267	0.228	3.000	0.671287
VØLUME	ℓ	34.5779	8.3	114.0	36.3247
AVERLN	cm	12.277	8.86	17.24	2.39057
DENSTY	ℓ^{-1}	2.71791	0.708	7.000	1.27894
DNLØAD	kg min ℓ^{-1}	1.52438	0.214	3.743	0.965343
DNSTØK	g ℓ^{-1}	57.4419	23.81	122.35	22.2050
GRUBFD	g	8.14139	1.46	30.12	7.21439
FDMEAL	g	21.3864	3.96	73.20	18.9725

Notes:-

a) Minimum and maximum values are given to the accuracy determined by readings.

b) Mean and standard deviation are given to the accuracy used by the computer during analysis (6 significant figures).

Table 7.4 Summary data for transformation quantities

(n = 122)

QUANTITY	UNITS	MEAN	MINIMUM VALUE	MAXIMUM VALUE	STANDARD DEVIATION
FACTØR	$kg\ min\ \ell^{-1}\ m^{-1}$	12.2161	1.98516	35.6781	7.41932
FREPAT	$ml^{1/3}$	0.613972	0.105258	1.21820	0.264166
EXRATE	min	27.2733	7.21717	74.4368	16.4905
AVEENU	m	9.67972	2.04820	33.1093	10.3046
DNFACR	$kg^{2/3}\ min\ \ell^{-1}$	1.28306	0.321354	2.25565	0.497329
STØLIN	$kg\ min\ \ell^{-2}$	0.0706914	0.0172581	0.170619	0.0448347
FDGØVN	$^{o}C\ cm^{-1}$	55.2109	17.0165	284.205	58.0796
STKFAC	$g\ \ell^{-1}\ cm^{-1}$	4.67125	2.20872	7.94283	1.47511
DNSTAC	$g^{2/3}\ \ell^{-1}$	5.50902	2.56906	10.2998	2.04448

Note:-
Values are given to the accuracy used by the computer during analysis (6 significant figures)

Table 7.5 Correlation of SPEXRT with other variables

VARIABLE	CORRELATION COEFFICIENT	
DNLØAD	- 0.600	(df = 120)
DNSTØK	- 0.473	
FACTØR	- 0.479	
DNFACR	- 0.635	
DENSTY	0.162	
FREPAT	0.377	
STKFAC	- 0.326	
DNSTAC	- 0.132	
STØLIN	- 0.278	
TEMPRT	0.778	
FDGØVN	0.102	
GRUBFD	- 0.274	
AVERLN	- 0.494	
EXRATE	- 0.425	
AVEENU	- 0.317	

conditions of these experiments the correlation between FACTØR and DNFACR was 0.818, showing variability in the length/weight relationship. A similar "equivalence" between STKFAC and DNSTAC showed a correlation of only 0.690.

7.92 The next-best biophysical predictor was DNLØAD, the simple loading factor (weight/flow), whereas the stocking factor DNSTOK (weight/volume) was relatively poor, and was exceeded in predictive capability by the simple length of the fish (AVERLN). All of the quantities so far mentioned had a negative correlation with SPEXRT, showing that as the loading of fish increased, ammonia excretion was depressed. However, temperature (which had the highest correlation of all) was positively correlated, indicating that ammonia excretion increased with increasing temperature.

Multiple regression analysis (OM 1)

7.93 Under the analytical procedure of OM 1 (see DATA ANALYSIS section), several different additive models were tried, using various combinations of the supposedly basic influences: temperature, some loading factor, stocking, the food and the squares and first-order interactions of these quantities. GRUBFD was chosen in preference to FDMEAL for consideration because it seemed likely that the excretory rate as sampled at 1330 would be most affected by the food fed at 1000 and 1200 on the same day, and much less by feeds on the previous day which fell within the same experimental DAY. (Substitution tests between these quantities showed no difference in the final analysis). Stocking and loading were both built into the models, but three different ways of expressing the loading were tested: (i) as DNLØAD + AVERLN, (ii) as DNFACR, (iii) as FACTØR. The basic pattern of the additive models was :-

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 \quad \ldots\ldots \text{(simple quantities)}$$
$$+ b_{1.2}x_1x_2 + b_{1.3}x_1x_3 \quad \ldots\ldots \text{(simple interactions)}$$
$$+ b_{1.1}x_1^2 + b_{2.2}x_2^2 + b_{3.3}x_3^2 \ldots\ldots \text{(squares)}$$

7.94 In addition, multiplicative models were tested, using log transformations of SPEXRT, TEMPRT, DNLØAD, DNFACR, FACTØR, AVERLN and GRUBFD. (In this case no interactions or squares were included owing to their difficulty of comprehension.) The multiplicative models had a basic pattern thus:-

$$\log y = \log a + b_1 \log x_1 + b_2 \log x_2 + b_3 \log x_3 \ldots.$$

or:-

$$y = a.\, x_1^{b_1}.\, x_2^{b_2}.\, x_3^{b_3} \ldots\ldots$$

Again the three possibilities of DNFACR, FACTØR and (DNLØAD, AVERLN) were tested as loading factors.

7.95 Results from the best additive and the best multiplicative models are shown in Table 7.6 and the regression coefficients of significant independent variables are given. It was found that in the full analysis the quantities DNFACR and FACTØR are interchangeable, with no effect on the resulting predictive equation, and that in both the additive and multiplicative types, the inclusion of both DNLØAD and AVERLN was preferable to the alternative expressions of loading, giving a higher overall multiple correlation coefficient. The equation suggested by the results for the best additive model (1) is:-

$$y = 48.622 - 2.620x_1 - 1.504x_2 - 0.324x_3 + 0.004x_3^2 - 0.018x_4^2 + 0.259x_1x_2 - 0.490x_1x_5 + 0.133x_1x_4 - 0.027x_2x_3 \ (\pm 6.376)$$

- where

x_1 = TEMPRT, x_2 = AVERLN, x_3 = DNSTØK,
x_4 = GRUBFD, x_5 = DNLØAD

(Cl_{95} given for one observation in parentheses.)

7.96 This model accounts for a high proportion of the variability in the data, having R = 0.899, and surpasses the corresponding multiplicative model (2) which has R = 0.808:-

$$\log y = 1.086 + 0.921 \log x_1 - 0.476 \log x_2 - 0.230 \log x_3 \ (\pm 0.268)$$

or:-

$$y = \frac{12.19x_1^{0.9}}{x_2^{0.5}\ x_3^{0.2}} \qquad (* 1.854)$$

Table 7.6 Multiple regression analysis OM 1 results

		X*	b
MODEL	(1) Additive	TEMPRT	- 2.6195272
		AVERLN	- 1.5037104
Y	SPEXRT	DNSTØK	- 0.3235561
R	0.899	$DNSTØK^2$	0.0042644
a	48.6218979	$GRUBFD^2$	- 0.0167329
ESS	1161.53	TEMPRT x AVERLN	0.2585517
SE_y	3.22037	TEMPRT x DNLØAD	- 0.4904210
Cl_{95}	6.376	TEMPRT x GRUBFD	0.1328849
		DNSTØK x AVERLN	- 0.0274240
MODEL	(2) Multiplicative	log TEMPRT	0.9213403
		log AVERLN	- 0.4757199
Y	log SPEXRT	log DNSTØK	- 0.2302780
R	0.808		
a	1.0859537		
ESS	2.16965		
SE_y	0.135598		
Cl_{95}	0.268		

Notes:-

a) X* = variables significant at level P = 0.05

b) b = regression coefficient

c) R = multiple correlation coefficient

d) a = intercept (log value for multiplicative model)

e) ESS = error sum of squares

f) SE_y = residual error = $\sqrt{\frac{ESS}{df}}$

g) CL_{95} = 95% confidence limits for observations = SE_y x 1.98 (since 1.98 is the t-statistic for P = 0.05 with df $\doteqdot$ 120)

h) Values are given as produced by computer, except for CL_{95} (which was calculated separately).

This approximates to:-

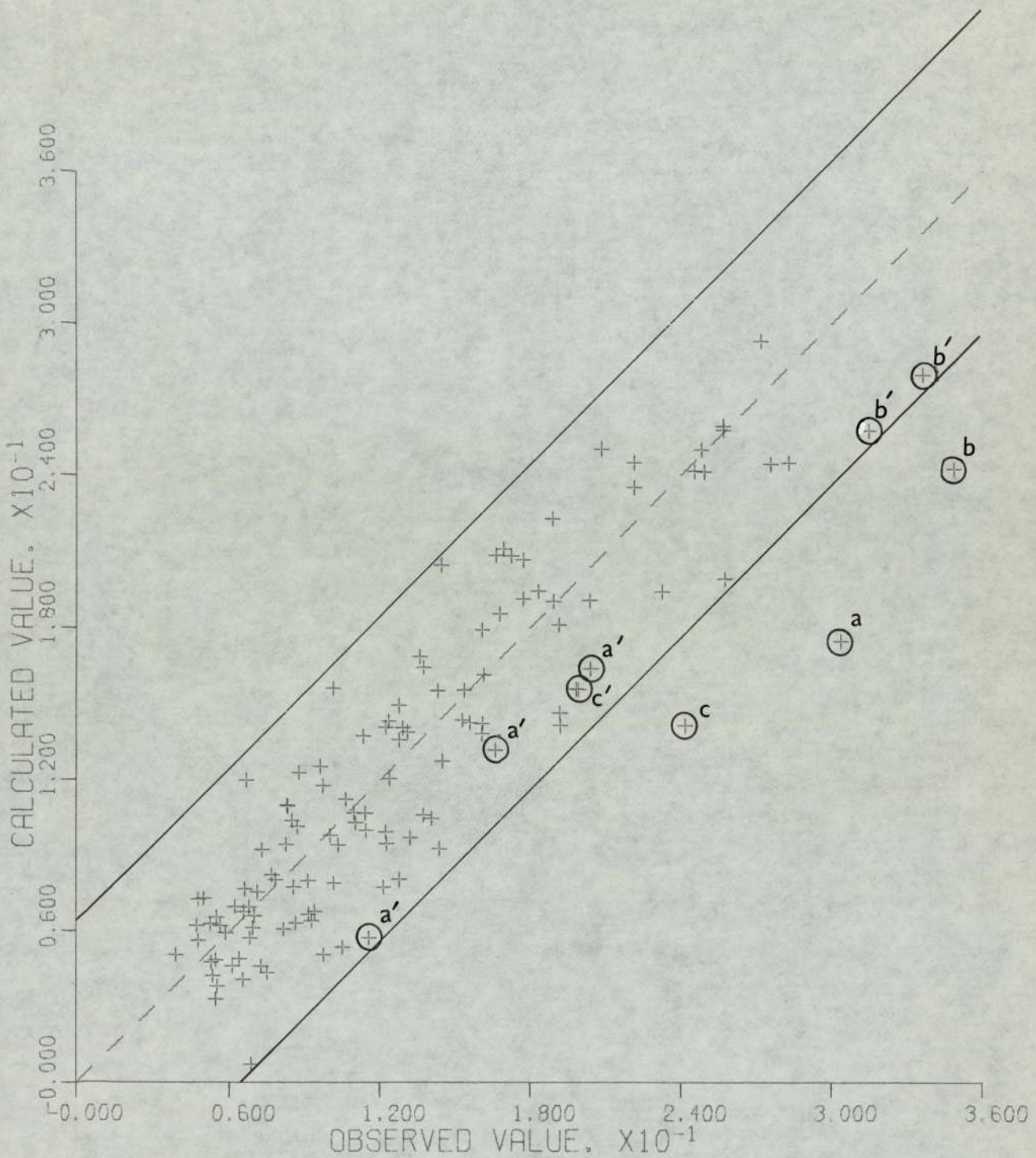
$$y = \frac{12x_1}{\sqrt{x_2} \cdot \sqrt[5]{x_3}}$$

7.97 Both of these models were subsequently submitted to the multiple regression presentation procedure, to produce graphical illustration of the models (Appendix C). Graph 7.2 (for model 1) shows values of SPEXRT calculated from the model ($\hat{y}$) plotted against observed values (y). The points are fairly well distributed about the mean line (dashes), with three particularly 'wild' points a, b and c (in descending order of deviation from the line), which fall outside the 95% confidence limits for y (solid lines - see Table 7.6).

7.98 Investigation of points a, b and c revealed special circumstances about their measurement. For points a and c an excessive amount of food had been fed to the fish in those particular tanks over the 24 h before TOA measurement (due to miscalibration of feeders by overshoot, see Chapter 7, para.7.27); this probably resulted in excessively high observed values. For point b the calibration values of TOA seemed to be unusually high compared with the values on other days; this could also account for an extra-high observed value. It was decided to remove a, b and c readings from the data matrix because of the doubts due to these circumstances. Following Wilson (1952), who maintained that:

"if a given circumstance is once used to justify discarding a discordant result, the occurrence of the same circumstance must cause rejection whenever it happens and whatever the result", - a thorough search was made for similar occurrences of these conditions. The case of b (high calibration) was true for the other readings of that day (b' in Graph 7.2), but was not otherwise found. The circumstances of a and c (sudden change in amount fed) were noted on these related occasions :- (see Graph 7.2)

a' : other tanks on the same day as for a also suffered 'overfeeding' to the extent of 56%, 52% and 50% increases over



Graph 7.2

OBSERVED V CALCULATED.

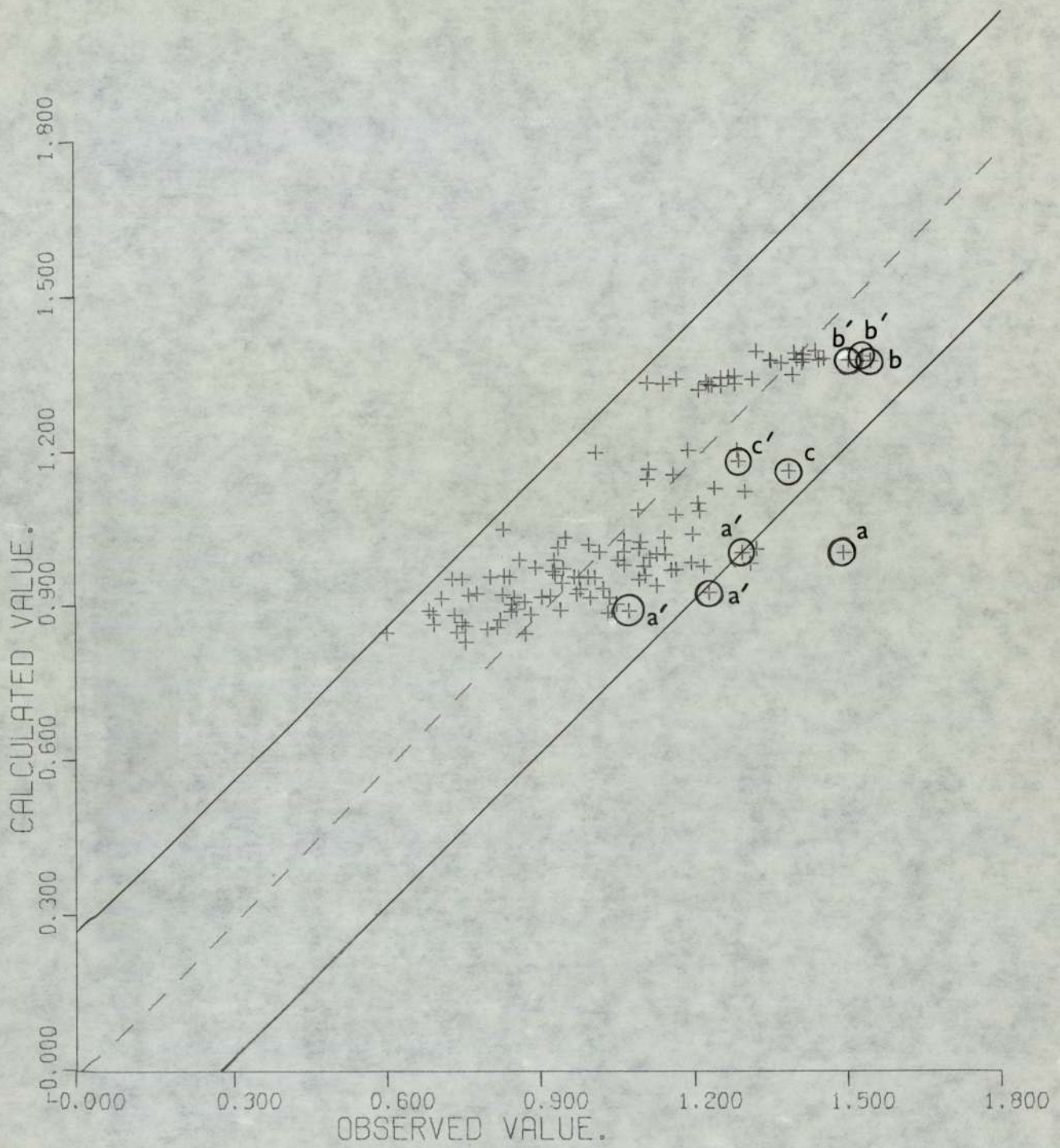
the previous DAY (a had a 50% increase);

c' : another tank suffered similarly on the same day as c, recording a 44% increase (c had 40% increase).

No other cases of exceptional 'overfeeding' occurred, and checks for 'underfeeding' by overshoot calibration of feeders revealed no instances where decrease was of a similar magnitude to the increases in a and c.

7.99 The corresponding graph for the multiplicative model (2) is shown in Graph 7.3, with points a, a', b, b', c and c' indicated. Some more 'wild' points are noted on this plot, but since this model is inferior to model 1, it was not further pursued: the data had already been checked for unusual circumstances. Comparison between the two graphs shows that (if points a, b and c are ignored), the points behave differently with respect to convergence: in Graph 7.2, there is neither a notable convergence nor a notable divergence of points as the values increase; however, in Graph 7.3, there is evidence of some convergence of points towards the higher values. It can be concluded that the standard deviation of Y is not dependent on the magnitude of the quantities; had that been so, there would have been appreciable divergence of scatter with increasing values in Graph 7.2, and the log transformation, which closes up higher values but opens out lower values, would have been more suitable as a model. In practice, the additive model (1) is superior on these grounds as well as its expression of a higher R value (Table 7.6)

7.100 For further analysis, points a, a', b, b', c and c' were eliminated from the observation matrix, and the regression analysis and presentation re-worked for the additive model. Corrected analysis results are given in the first part of Table 7.7, and it can be seen that an improved model (3) is generated, with R = 0.915 as opposed to R = 0.899 in model 1 (i.e. less of the variability in the data remains unaccounted for). Graph 7.4 shows the 'observed' against 'calculated' plot for model (3), and two points of interest arise: there are a few points just



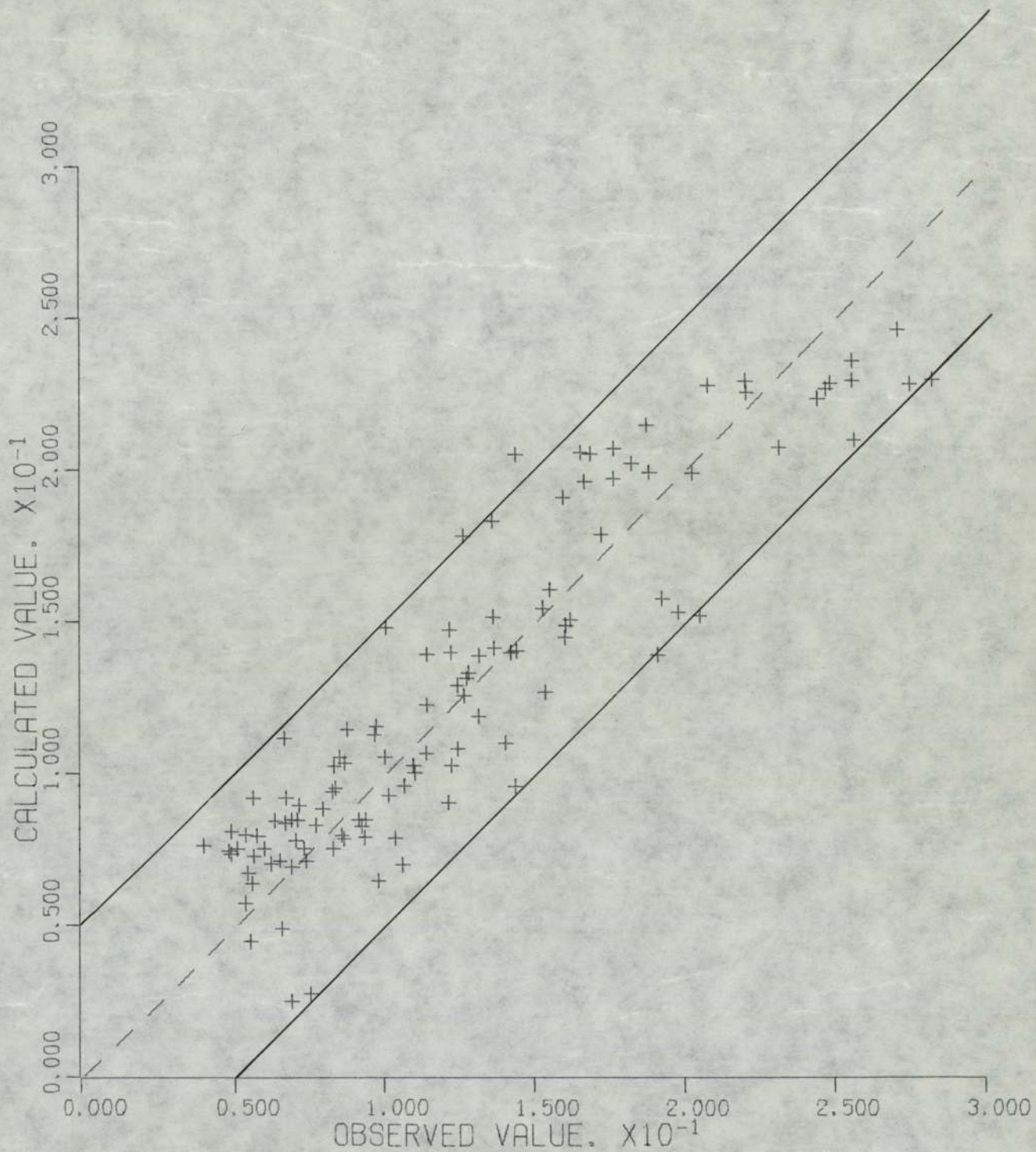
Graph 7.3

OBSERVED V CALCULATED.

Table 7.7 Multiple regression analysis OM 1 (corrected) and OM 2 results

		X*	b
RESULTS	OM 1 (corrected)	DNSTØK	- 0.3125522
MODEL	(3) Additive	$GRUBFD_2$	1.1121440
Y	SPEXRT	$TEMPRT^2$	0.0232537
R	0.915	$DNSTØK^2$	0.0018541
a	21.5339584	$GRUBFD^2$	- 0.0262277
ESS	691.956	DNLØAD x AVERLN	- 0.3275169
SE_Y	2.55497		
CL_{95}	5.059		
RESULTS	OM 2	$ENUMBR_2$	0.0427383
MODEL	(4) Additive	$TEMPRT^2$	0.0414127
Y	SPEXRT	$ENUMBR^2$	0.0001978
R	0.917	TEMPRT x FREPAT	0.4164954
a	5.7522365	ENUMBR x DNSTØK	- 0.0013503
ESS	676.424		
SE_Y	2.51430		
CL_{95}	4.9783		

(Notes apply as for Table 7.6)



Graph 7.4

OBSERVED V CALCULATED.

outside the Cl_{95} lines on this plot also, and it seems possible that at high observed values, the calculated values are tending to level off; the latter phenomenon may indicate the influence of some variable which has not been taken into account, especially since all observed values above about 23 mg kg^{-1} h^{-1} have low calculated values, causing points which fall well below the best-fit line.

Multiple regression analysis (OM 2)

7.101 Bearing in mind the additional factors envisaged under OM 2 (see DATA ANALYSIS section), and the possible influence of un-measured variables detected in OM 1 model (3), attention was focussed on the OM 2 analytical procedure, where the fundamental innovation was a separate consideration of the number of fish in the tank, especially as expressed in the factors ENUMBR, DENSTY and FREPAT. Once again, a battery of different models (all additive) were tried out in multiple regression analysis.

7.102 The introduction of ENUMBR and FREPAT for consideration seemed to remove the direct effects of food factors (GRUBFD or FDMEAL) from the list of significant quantities, and a simple model in terms of TEMPRT, ENUMBR, FREPAT, AVERLN and DNSTØK gave a promising result (R = 0.854) for unrevised data (i.e. including a, a', b, b', c, c'). When squares and first-order interactions were taken into account, R rose to 0.882.

7.103 Final modification was performed by using the revised observation matrix (without a, a', b, b', c and c') for the model described above. This final model (4) is detailed in the second part of Table 7.7, and results in a small improvement on model (3), having R = 0.917. This final model (4) has the advantage of an intuitively realistic intercept value ('a' in the Table) as compared with models (1) and (3); about 6 mg kg^{-1} h^{-1} seems a reasonable possibility for a basal excretion rate with most of the influencing factors at a minimum. Accordingly, a full presentation treatment was performed on model (4) (see Appendix C).

7.104 Graph 7.5, the 'observed' against 'calculated' plot, shows a fairly balanced distribution about the line although the extent of deviation of some points from it emphasises the limits of accuracy of the model.

7.105 By examination of Graph 7.5 and the other graphs also generated (see Appendix C), a number of other points became clear, and the overall results are detailed below.

Summary of results of multiple regression analysis

7.106 The best equation found for SPEXRT estimation was as follows (Cl_{95} for one observation given in parentheses):-

$$y = 5.7522 \times 0.0427x_1 + 0.0002x_1^2 + 0.0414x_2^2$$

$$+ 0.4165x_2x_3 - 0.0014x_1x_4 \quad (\pm 4.9783)$$

- where y = SPEXRT, x_1 = ENUMBR, x_2 = TEMPRT, x_3 = FREPAT, x_4 = DNSTØK

7.107 This model was selected by the best multiple regression analysis of the data available ($R = 0.917$) one limitation on its use is evident in the relatively wide and poorly-explained variability in SER in situations where <100 fish are present.

7.108 Since:

$$\text{FREPAT} = \frac{\sqrt[3]{\text{volume}}}{\text{number}} \quad \text{and DNSTØK} = \frac{\text{weight}}{\text{volume}},$$

then the model allows a fairly reliable estimate of SPEXRT in terms which involve mathematical manipulation of only four measured quantities; temperature, number of fish, volume of water and weight of fish.* Manipulated correctly, these variables can also account for the food fed, and the majority of variability found in measured SPEXRT (according to TOA concentrations), under the conditions of experiment.

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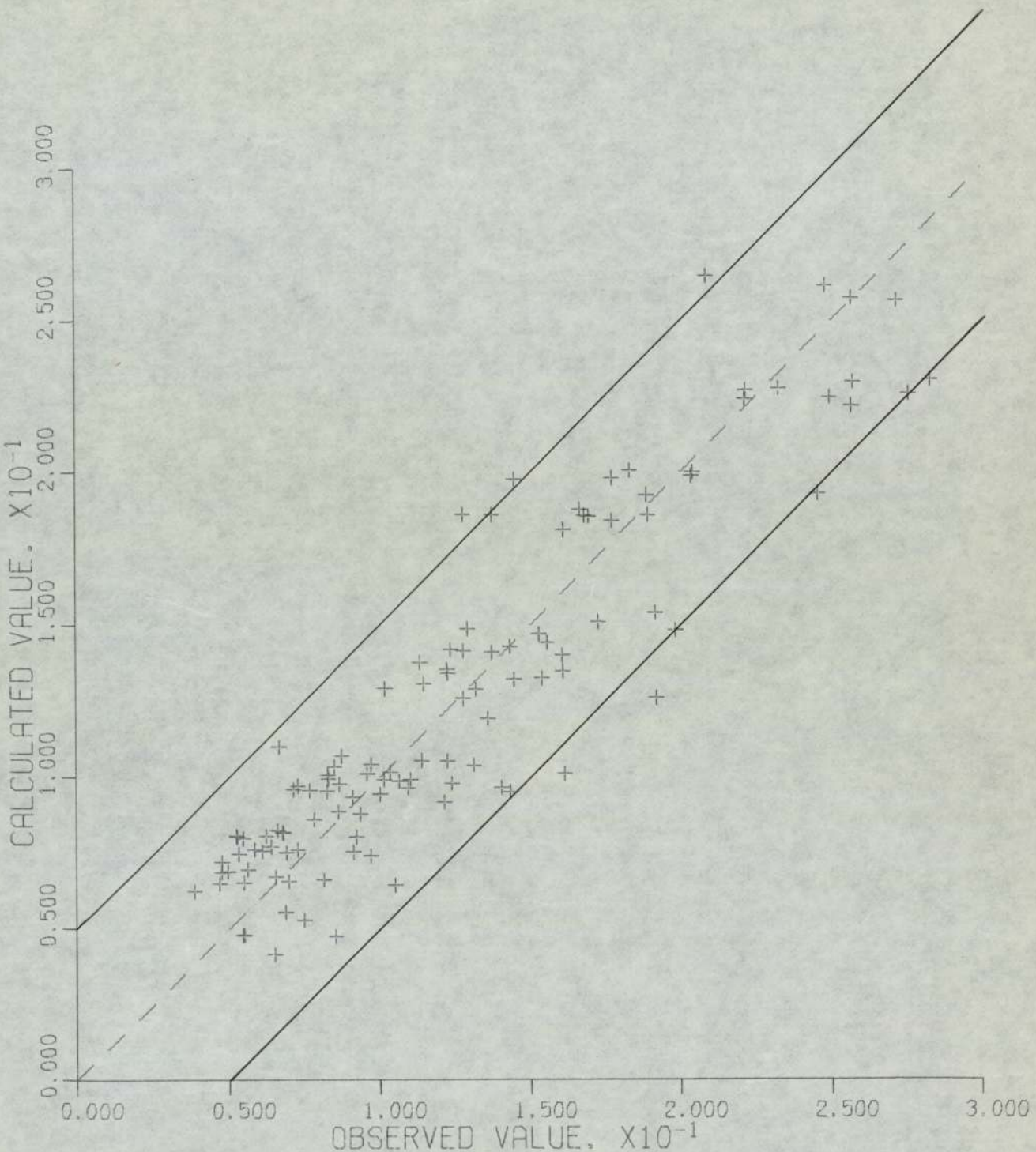
* See Appendix C for notes on independence of these quantities.



Graph 7.5

OBSERVED V CALCULATED.

7.109 The full E-series experiments were superior to the pilot experiments in several important respects:- (Ref. Chapter 6 DISCUSSION).

a) food consumption was maximised and, except for a few cases of accidental "overfeeding" (where food may not have been fully consumed) (see RESULTS section), the assumption of full consumption was justified by observation;

b) inauguration stresses (due to weighing out fish at the start of experiment) were minimised by improvement of technique and time was allowed for fish to settle down before readings were taken;

c) estimation of weight was improved to provide values for each experimental DAY;

d) a representative TOA reading was taken at the same point of the diurnal photoperiod cycle, in all cases (1330); if there is a diurnal excretory cycle this will have standardised its effect;

e) there was no sudden change in temperature or photoperiod during the changeover from holding to experimental conditions (due to HC2);

f) measurements were taken over a longer period;

g) tank size and configuration were improved, eliminating the extreme degree of 'crowding' mentioned in para.6.46.

7.110 With these improvements, a fairly reliable system was created for meaningful measurements of SER. The good hydraulic properties of the circular tank provided adequate mixing to ensure a fairly reliable sample from the effluent outflow (as contrasted with the raceway outflows used by Burrows: raceways have poor mixing qualities (Burrows & Chenoweth 1955)).

7.111 The final model for SER estimation, evolved from the results of E-series experiments, represents a surprisingly good explanation of the situation, bearing in mind that it is based only upon four measurable quantities. These quantities will affect the fish internally as suggested in Fig.5.6

(Chapter 5), and the external effect on TOA concentration will be by way of the changes in metabolism. However, it is almost certain that they will be unable to account thus for the complete total of variability in ammonia production.

7.112 This area of doubt is more closely examined in Appendix C. It seems probable that the best explanation of the lack of accuracy of the model is due to unmeasured quantities. Where unexpected deviation from the calculated (theoretical) SER values takes place, a hitherto intangible variable is suggested, which may be conveniently referred to as stress. In this particular instance, one possible type of stress might be quantitatively (and empirically) defined by the unexplained variability in excretion rate when the effects of normal biophysical parameters, and their measurement errors, have been taken into account. In the case of the final model for SER, this would be expressed in, and estimated by, the error limits of the equation (which also includes the measurement errors).

7.113 Summary of conclusions

1. The variability in SER is adequately explained ($R = 0.915$) by an additive model (3) which ignores the number of fish in the tank (OM 1), and hence allows SER description by quantities related to purely chemical or physical effects, i.e. treating the tank as a single ammonia-producing machine (see para.7.69).

2. The variability in SER is marginally better explained ($R = 0.917$) by an additive model (4) which takes the number of fish into account, together with three other basic quantities: temperature, fish mass and water volume.

3. The multivariate relationships which these models describe are sufficiently good ($R > 0.9$) to use as predictors of SER, within the limits of the experimental conditions, for future similar experiments.

4. Doubt exists as to the effect of number of fish below a value of about 100, but this does not seriously disturb the final model (4).

5. The error in observed SER values as compared to calculated (predicted) values may be interpreted as including a quantitative component attributable to stress, as well as measurement errors.

6. Future experiments are required to investigate more values of temperature, fish number and stocking, in order to verify the overall findings.

PART 4

TOLERANCE OF FISH

TO FISH EFFLUENT

8. INTRODUCTION TO AMMONIA TOLERANCE

LITERATURE REVIEW AND TOLERANCE THEORY

Toxicity and tolerance

8.1 Compounds which adversely affect the physiology of living organisms have long been termed toxic, principally when death results if they are allowed to act unchecked. Much literature has been devoted to identifying concentrations which produce measurable effects. This work is most advanced in medicine, but during this century attention has also focussed on other species. Fish have received much attention, especially in the particular context of pollution.

8.2 During this work, a variable terminology has grown up to describe toxicity; standard medical terminology has been involved, but recently some new definitions have been coined.

8.3 The effects associated with death are usually referred to as acute toxicity; and the quantity of compound causing death under stated conditions is the lethal concentration. Frequently the most important conditions involved are:

a) % of subjects dying (e.g. LC-50 indicates a lethal concentration at which 50% of subjects are killed);

b) time-span over which the effect occurs (thus 24 or 48 h LC-50);

c) temperature at which the effect occurs.

8.4 In many studies, however, one is not considering death; if suitable curative measures are to be taken, the identification of less severe criteria is clearly important. This kind of effect is described as sub-acute, sublethal or chronic toxicity, the latter emphasising that long periods of time may be involved. The looseness of the "medical" terminology (with its implication of time involved rather than effect caused) is unsatisfactory, especially since the criteria of effect may vary widely. For this reason, in common with several authors (e.g. Sprague 1971, Lloyd 1972, Webb & Brett 1972, 1973, Schulze-Wiehenbrauck 1974)

the term sublethal toxicity will be preferred in this account, and will be used to describe the whole range of adverse effects, short of death.

8.5 In contrast to the "negative" definition of chemical effects (emphasising adverse effects), it is possible to view the situation from the opposite end, and consider to what extent physiology is maintained as normal, despite the presence of a poison; or in what ways the physiology may be adjusted to cope with the substance. This is the phenomenon of tolerance, and it becomes increasingly important as the concentration involved decreases from lethal levels. For instance, when trying to establish "safe" levels of known pollutional chemicals to allow to be present in a waterway, either of two questions may be answered:-

a) At what level can no adverse effect whatsoever be detected?

b) What is the level up to which some physiological criterion of normality (e.g. breeding capability) is maintained?

The first question assumes that a level exists at which experimental and control tests will not differ, whatever the physiological criterion used; given the complexity of living organisms and their environmental interactions, this may often be an artificial assessment, since environmental variations are part of most organisms' "normal" conditions of life. At very "low" concentrations of the chemical, its effect may be lost in a complex pattern of interactions with other variables. The second question above assumes that a sufficiently sensitive criterion can be identified so that its maintenance will also guarantee preservation of more general qualities e.g. growth. This may lead to difficulties in assessing whether the criterion is of the correct sensitivity for the particular objectives envisaged.

8.6 In fish farming, there are probably two major types of objective borne in mind when assessing the fish environment. In one case the farmer may be most interested in the breeding

capability of the fish; but in the more generally important case, the farmer's objective is couched in growth terms. This may be expressed in different ways, according to circumstances; sheer speed of growth may be the objective in some cases, whereas in others the efficiency with which food is converted into fish flesh is more important. In the latter situation, the gross conversion ratio may well express the ratio between major outlay (food materials bought) and income (fish sold) assuming sales match production. Thus a suitable measure of tolerance may in many cases be the maintenance of growth rates or conversion ratios. Similarly, when considering recycling of water, there may be levels of dissolved excretory products which can be allowed to persist because they do not prevent the maintenance of economic growth rates or conversion ratios. If so, then a filtration unit need only remove excretory products down to such levels: complete removal may be unnecessary (if indeed practicable). The implications of this on filter unit cost may be important, particularly where filter efficiency is related to unit size as may be the case with biological filtration.

Ammonia as a toxicant

8.7 Given that ammonia is an acceptable index substance for dissolved excretory products, it is important to assess ammonia as a chemical toxic to fish, before considering it from the point of view of tolerance.

8.8 It has been recognised for about thirty years that the described toxic effects of ammonia on fish are due to the free radical NH_3^o (UIA) as opposed to the ion NH_4^+ (Wuhrmann & Woker 1948). Since that time much work has been done, especially on the lethal toxicity of UIA to fish, and the work is substantially reviewed in the FAO Report on ammonia and inland fisheries (EIFAC 1970), from which the expression below is taken:-

$$\% \text{ UIA in TOA} = \frac{100}{1 + \text{antilog } (pK_a - pH)}$$

where

pK_a = negative log of ionisation constant (dependent on temperature: determined by formula given in Robinson & Stokes 1969 [following Lloyd & Herbert 1960]).

8.9 The study of ammonia as a toxic substance can be split into two major areas: (a) studies in which fish are exposed to made-up solutions of ammonium compounds, and (b) studies in which adverse effects are described in conditions which imply that ammonia is the culprit. The literature of (a) is widespread and often scientifically rigorous, and has been extensively reviewed by EIFAC (1970); the major points of which are worth recapitulating. They include the early recognition of the importance of pH (Wuhrmann Zehender & Woker 1947, Wuhrmann & Woker 1948), contrasting with the approach of Grindley (1945), who calculated NH_3 as a molecular fraction of NH_4Cl or $(NH_4)_2SO_4$. The effects of oxygen (Downing & Merkens 1955, Merkens & Downing 1957, Lloyd 1961a), free CO_2 (Lloyd & Herbert 1960), temperature and other quantities (Lloyd 1961b, EIFAC 1970) on the lethal toxicity have been well described, so that given a chemical description of a water supply, the threshold lethal levels (in which prolonged exposure kills 50% of the fish) can be estimated (Lloyd 1961b). More recently Ball (1967) has compared the susceptibility of several species of fish to UIA and found little difference at around threshold values, with species differences more clear at higher levels (possibly indicating different modes of action in causing death).

8.10 Work has gradually extended into the sublethal region, following the spread of estimates for safe levels of ammonia (e.g. as reported in Ball 1967), couched in terms of LC-50. Recognition of the possibility of different physiological effects at different sublethal levels has prompted more recent workers to define sublethal criteria of toxicity independently of LC-50 (Reichenback-Klinke 1967, Fromm & Gillette 1968, Lloyd & Orr 1969, Schulze-Wiehenbrauck 1974).

8.11 Studies of adverse effects on fish in culture systems

have generally assumed that, because of the proven toxic effects of ammonia solutions in laboratory studies, ammonia is the culprit whenever high excretory levels would be expected and adverse effects occurred, with a strong tendency to indict ammonia especially for growth losses. Thus Brockway (1950) advises that 0.3 mg ℓ^{-1} "ammonia" affects blood oxygen content without specifying conditions or UIA content, and links this to probable effects caused by metabolic products in general. His quoted criterion of 0.1 mg ℓ^{-1} ammonia (TOA) as a "maximum that should be tolerated in waters used for fish culture" has often been quoted (e.g. Spotte [1970]), but has only recently been properly examined.

8.12 Early studies on metabolic product effects frequently involved simultaneously the problems of stocking (weight per unit volume), and subjective estimates of "stress" (Philips et al 1950, 1951); and growth-rate studies linked to measures of fish density or crowding cited ammonia as the agent of adverse effects (Kawamoto Inouye & Nakanishi 1957, Kawamoto 1958, Yashouv 1958), based on the reasonable assumption that denser collections of fish would give higher local ammonia levels, assuming that excretion rate was maintained. The point at issue, however, was whether ammonia, specifically, was the cause of growth losses under the particular conditions applying. From foregoing discussion, it is clear that

(a) pH and temperature, by their determining effect on UIA, could permit or prevent toxicity; and

(b) the knowledge that UIA can be toxic in controlled laboratory studies does not rule out other factors present in the fish culture situation as being involved in growth inhibition, either instead of, or as well as, UIA effect.

Such other factors could include:

a) direct action by other chemicals excreted,

b) feedback effect of stocking, loading or some measure of "crowding" (see Chapter 7), as a bio-physical effect,

c) direct or interactive effects of other variables allowed to operate on the system (e.g. oxygen content, temperature,

lighting regime, "disturbance stress", etc.). It is at this point that the more recent combination of laboratory experiments supplementing field observations has come into being.

8.13 Kawamoto (1961) followed the growth of carp (Cyprinus carpio) in an ammonia solution (held at roughly constant pH), compared to controls, and recorded a definite growth disturbance, although the data were variable and there was some good growth. He found that oxygen consumption of carp dosed with the growth disturbing ammonia solution displayed an altered pattern, being decreased at low temperatures but increased at high temperatures. His work, assuming that the considerations of (b) and (c) in para.8.12 can be ruled out, shows that, for carp, ammonia can have an adverse effect on growth. The question however arises of whether the effect is due to TOA or UIA. Kawamoto measured ammonia as TOA (this is assumed, since no mention is made of a distinction between TOA and UIA), and pH remained relatively low (6.25 - 6.72) so that dissociation would be low. Carp were exposed first to 0.3 mg ℓ^{-1} TOA (0.15 - 0.40 μg ℓ^{-1} UIA) and later to 1.2 mg ℓ^{-1} TOA (1.30 - 2.90 μg ℓ^{-1} UIA). There was most growth disturbance in the latter phase which coincided with the 4-fold rise in TOA (7- or 8-fold rise in UIA). Thus it seems that, at least for carp, either UIA or TOA could be causing growth inhibition at these levels.

8.14 For salmonids, more detailed consideration of the role of excretory products largely springs from the work of Burrows (1964), who looked at the effects of stated UIA levels on the gill histology and swimming stamina of chinook salmon (Oncorhynchus tshawytscha). Citing instances of growth rates being 10% lowered in "ammonia-dominated" raceways (see Chapter 5), Burrows goes on to nominate ammonia as a weakening agent which also predisposes fish to disease, notably bacterial gill disease (although Larmoyeux & Piper (1973) stress that oxygen depletion may also be involved). All of his work was conducted at pH 7.8, and three dosing solutions (0.3, 0.5 and 0.7 mg ℓ^{-1}

TOA) were used in tests of gill damage effect. Burrows estimated these at 6, 10 and 14 $\mu g\ \ell^{-1}$ UIA at 6°C; and 8, 12 and 18 $\mu g\ \ell^{-1}$ at 14°C. All produced gill hyperplasia, the severity of effect being correlated with concentration. Trussell (1972) has since pointed out that Burrows' UIA values are overestimates, and they should be approximately halved. The effect at "6 $\mu g\ \ell^{-1}$" has given rise to a common quoting of 5 $\mu g\ \ell^{-1}$ as the maximum acceptable UIA value for salmonid rearing (Liao & Mayo 1972, 1974). The order of magnitude of UIA involved is that which might have caused growth disturbance in Kawamoto's (1961) carp, and reinforces his findings as to the dangers of ammonia to sensitive species, among which chinook salmon can clearly be included.

8.15 More recently, the rainbow trout itself has been the subject of experiments, and at Bozeman Center studies on serial use of fish tanks generated interest in the combined effects of ammonia accumulation and oxygen depletion, (Larmoyeux & Piper 1973). Report on the effect of ammonia alone has been provided by Smith (1972) and Smith & Piper (1975). A preliminary six-week study of dosed NH_4OH at high oxygen levels indicated no loss of growth, at a concentration of 0.8 - 1.0 mg ℓ^{-1} TOA, (about 8 to 10 $\mu g\ \ell^{-1}$). These values of UIA are of the order of, or higher than, those causing growth disturbance and gill damage in chinook salmon (Burrows 1964); this indicates that rainbow trout may well be less susceptible to sublethal UIA toxicity.

8.16 Smith then performed a 12-month study at three TOA levels (0.6, 1.2 and 1.6 mg ℓ^{-1}) at 10°C and pH 7.75, using excreted ammonia, kept steady by adjusting the fish loading. Growth and stamina were measured, and internal organs histologically examined (see below), and in overall terms Smith could find no effects on growth except in his highest concentration (1.6 mg ℓ^{-1} TOA) and then only after 6 months' continuous exposure (Smith & Piper 1975). UIA values were about 6, 12 and 17 $\mu g\ \ell^{-1}$, in the various tanks. The "danger zone" for growth

disturbance is thus about 17 $\mu g\ l^{-1}$, corresponding to the 1.6 mg l^{-1} TOA tanks. The data support Smith's contention that Spotte's (1970) adherence to the 0.1 mg l^{-1} TOA limit is not applicable, and his further comment that it may be economically unfeasible to keep ammonia this low is particularly interesting, especially when the possible costs of biological filtration or other methods of achieving such a standard are taken into account.

Criteria of adverse effect of UIA

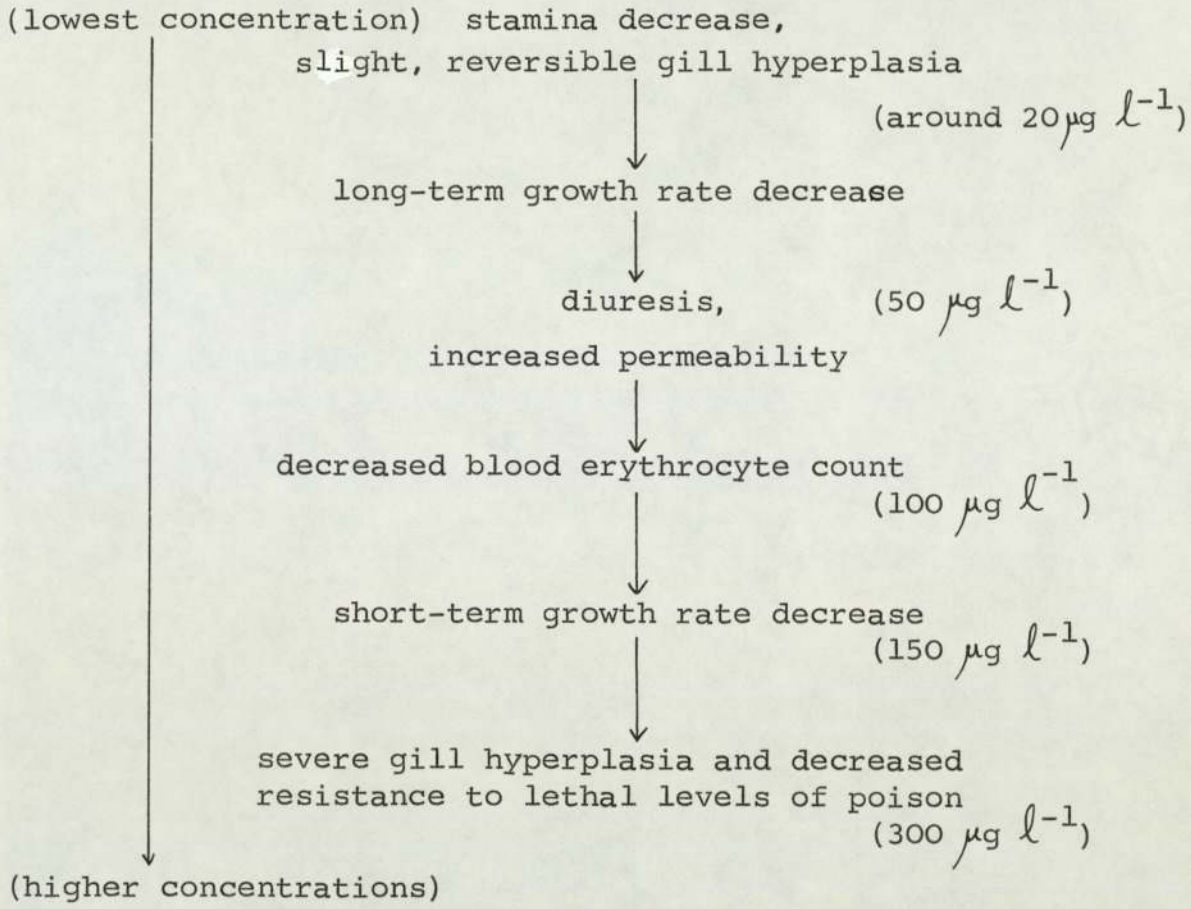
8.17 In recent studies of sublethal toxicity in dosage experiments, a variety of criteria of sublethal toxic effect have been used, and some of these are summarised in Table 8.1, for work directly using rainbow trout. It can be seen that

a) different effects can be associated with different levels, and

b) there can be controversy even over application of the same criterion.

Perhaps the difference between Smith (1972) and Schulze-Wiehenbrauck (1974) in their interpretation of growth rate changes lies either in the fact that Schulze-Wiehenbrauck's tests were limited to 7 weeks (Smith's ranged over 12 months) or that different sources of UIA were employed: Smith used excreted ammonia, whilst Schulze-Wiehenbrauck used a mixture of NH_4Cl and NaOH. It is probably that the 12-month study would reveal differences not found in 7 weeks, but with the extra passage of time, other variables may have had more opportunity to affect results, e.g. feedback effects of ammonia. Schulze-Wiehenbrauck maintains that the damage done to growth rates and food conversion which is observed at high fish densities is not due to ammonia, and that 100 $\mu g\ l^{-1}$ UIA can be regarded as harmless for young rainbow trout. This contrasts sharply with Smith's findings.

8.18 A possible interpretation of the results of Table 8.1 is that the various criteria represent different stages of toxicity in an ascending order such as:



At the top end of this range, lethal concentrations would be being approached (WPRL 1968, Lloyd & Orr 1969), and variations in conditions (including exposure time) might alter the exact UIA values involved at any stage, or indeed cause changes of position of effects in the scale.

8.19 In particular, Schulze-Wiehenbrauck's comment throws up doubts over the role of such factors as density of fish, stocking, etc. in either (a) modifying effective values, or (b) exerting effects of their own. Smith & Piper (1975) mention constant loading values used in their trials, but size and density certainly will have changed greatly over a growth period of 12 months. It is interesting that the one test which gave Schulze-Wiehenbrauck a definite difference in growth rate between controls and those treated with 170 $\mu g\ l^{-1}$ UIA, was that where highest stocking values were used (although still low, between about 10 and 20 g l^{-1}) in company with highest loading (1.1 to 2.0 kg min l^{-1}). This might lead to suspicion of a type (a) effect (above).

Table 8.1 Criteria of sublethal ammonia toxicity for rainbow trout

CRITERION OF TOXICITY	LOWEST EFFECTIVE CONC UIA ($\mu g\ l^{-1}$)	FOUND	NOTES
gill hyperplasia	> 300	Reichenbach-Klinke 1967	
gill hyperplasis and liver lesions	17	Smith & Piper 1975	
decreased blood erythrocyte count	100	Reichenbach-Klinke 1967	
diuresis (increased permeability)	90	Lloyd & Orr 1969	a)
decrease in stamina	17	Smith 1972, Smith & Piper 1975	b)
decrease in growth rate (weight)	170	Schulze-Wiehenbrauck 1974	c)
lowered food consumption			
decreased resistance	500	Schulze-Wiehenbrauck 1974	c)

Notes:-

a) Lloyd & Orr (1969) estimated no diuretic effect to occur at about 46 $\mu g\ l^{-1}$

b) Smith (1972) stressed that growth rate decrease was only found after 6 months continuous exposure

c) Schulze-Wiehenbrauck (1974) had doubts about his measured effects at 170 $\mu g\ l^{-1}$; he found an <u>increase</u> in resistance (to subsequent lethal UIA levels) at about 130 $\mu g\ l^{-1}$.

8.20 Sprague (1971) and Lloyd (1972) have reviewed sublethal effects of pollutants, and the problems of establishing "safe" water quality criteria. Sprague mentions that growth should always be monitored, but that it is not always a sensitive indicator. He also mentions disadvantages in relying on swimming speed/stamina trials or behavioural effects, encourages the possibility of measurement of scope for activity (see below), and cites reproduction and controlled ecological production experiments as particularly good assays. Sprague distinguishes between estimates of "safe" levels due to projections back from lethal concentrations (e.g. as reported in Ball [1967]), and those due to direct measurement of other sublethal criteria. Lloyd (1972) follows Sprague in advocating consideration of as many aspects as possible when setting water quality criteria, and warns of the danger of treating fluctuating concentrations of a toxicant (especially at sublethal levels) as the same as a steady dosage. He cites the combination of laboratory studies (Lloyd & Orr 1969) and field observations in arriving at a recommended standard for UIA in river water, as reported in EIFAC (1970). This standard is 25 $\mu g\, \ell^{-1}$ UIA, at which no diuretic effect should occur (Lloyd & Orr 1969), and which allows a safety margin for fluctuations.

8.21 It might be pointed out here that the work of Lloyd & Orr (1969) and Fromm & Gillette (1968), which described effects of ambient ammonia on excretion, was performed with fish which would almost certainly be losing weight due to lack of feeding before and during experiment. Also the fish were large and treated singly or in pairs. Although the relevance of these studies to setting of water quality standards for rivers may be great, it is important to contrast this type of work with that of Smith (1972) and Schulze-Wiehenbrauck (1974), who used populations of young fish which were feeding and actively growing. Clearly the latter type of experiment comes closer to the situation of the commercial fish farmer.

8.22 Nevertheless, the EIFAC (1970) standard of 25 $\mu g\ l^{-1}$, for no diuretic effect, seems a good basis on which to assume lack of short-term UIA effects in experiments, as in Chapters 6, 7 and 9. Although it is slightly above Smith's (1972) effective concentrations for stamina, histopathology and growth-rate decrease, these effects were found over a long period of time; and for the limited purposes referred to for this study, 25 $\mu g\ l^{-1}$ was accepted as the UIA concentration causing minimum effect on general physiology, including growth.

8.23 From this brief survey of the toxic effects attributed to ammonia, several points arise for consideration when the active growth situation of fish culture is envisaged:-

a) There is still a requirement to disentangle the effects of ammonia in general, and UIA in particular, from other factors, e.g. as described in para. 8.12.

b) There is a requirement for identification of the appropriate criteria of sublethal toxicity that should apply in a given situation.

c) There is, in particular, the necessity of assessing physiological responses of fish exposed to these culture environment effects.

Measurement of sublethal toxicity

8.24 Appendix D deals in detail with the rationale behind the use of the EC-50. This figure is equivalent to LC-50 in concept; EC-50 represents median effective concentration, the concentration at which 50% of subjects show a particular defined response under stated conditions. Whereas LC-50 implies the "lethal response" (death), an EC-50 can be defined for any desired quantity, e.g. loss of growth rate or loss of conversion rate. Since a level can be defined where 50% of subjects are affected, there can theoretically be an EC-0 where none are affected; in practice a true EC-0 is unobtainable. For this reason "no effect" values, or "safe" levels, such as the EIFAC UIA standard, are referred to in this study as IC_{max} (maximum ineffective concentration).

8.25 Use of the EC-50 does not imply necessarily the knowledge of the mode of action of the chemical involved; the concept is equally applicable to any desired criterion of sublethal toxicity, or tolerance.

Excretory materials

8.26 Since this appreciation of tolerance is independent of mode of toxic action, it can thus be independent of exact chemical identity of the toxicant. Provided that the toxicant supply is of relatively stable composition, any feature of it which is conveniently measurable as a concentration will suffice for describing an EC-50. Such an example is the excretory output of fish. This has been tacitly assumed to be ammonia; but as shown in Chapter 5, ammonia is not the only product; it is merely an indicator of nitrogenous excretory strength. Thus the question may be asked, is it possible and valid to determine the EC-50 for nitrogenous excretory materials taken together (using ammonia as an indicator), rather than to determine it for artificial ammonia solutions such as are used in most dosing experiments?

8.27 Herein lies a major difference between the fish-farming situation and the dosage experiments on ammonia toxicity: what is really important is not the effects of UIA, but the effects of dissolved nitrogenous excretory products in total. If Schulze-Wiehenbrauck (1974) is correct, and UIA is not responsible for growth losses when below 100 $\mu g\ l^{-1}$, then it is quite possible that some other excretory component is, quite aside from the effects of loading or stocking, etc. Putting this another way: what difference is there in growth effect between the dissolved nitrogenous excretory output of a fish tank and a made-up ammonia solution in dosage experiments?

8.28 It would seem that a useful approach to the consideration of the effect of recycled fish-farm water on the fish involved would be to investigate the tolerance of fish to their own dissolved nitrogenous excretory products (using ammonia as an index).

8.29 Finally, if as Schulze-Wiehenbrauck suggests, UIA is unimportant below about 100 $\mu g\ l^{-1}$, then within a reasonable pH range, TOA should be perfectly reliable as the ammonia index value, and TOA concentrations should suffice as indicators of dissolved nitrogenous excretory strength; TOA itself may be effective against growth rate, although the evidence to date (e.g. Burrows 1964) seems to suggest not.

INTRODUCTION TO T-SERIES EXPERIMENTS

Simulated recycled effluent

8.30 A scheme was prepared for investigating the growth-inhibition effects of the dissolved nitrogenous excretory products of rainbow trout in circular tanks in culture conditions.

8.31 Basic to this scheme was the provision of a source of such products. There were two possible sources:-

a) recirculation of the fish tank effluent back to the same tanks;

b) intallation of one series of tanks as an "effluent factory", with the effects of the effluent measured in a second series.

Because of the associated control problems of (a) (i.e. accumulation effects, deoxygenation of water problems, requirement for regulation of bleed-in water to a closed system), it was decided to use method (b) and set up an effluent "factory" of tanks serving a test series. The products of the "factory" required two major treatments before delivery to the experimental tanks: removal (screening) of solid wastes, and aeration to prevent interference by low oxygen effects.

8.32 Water produced by the "factory" tanks, and subsequently screened and aerated, is referred to as SREF (or simulated recycled effluent), and the design of T-series experiments is fundamentally a preliminary stage in the exploration of the growth-tolerance of rainbow trout to SREF.

Aims of study

8.33 Originally, the intention was to explore tolerance to SREF in terms of EC-50, as described in the preceding section. In

practice, shortage of time allowed for just two preliminary growth studies which can be viewed as pointing the way to further experiments. Fish from holding tank environments (and thus acclimated to low ambient inflowing TOA) were exposed to SREF in experimental tanks, whilst similar populations of fish under otherwise similar conditions were treated with control (inflow) water. SREF was quantified in terms of TOA measured, and information was sought on a series of effects:-

a) growth rate)
b) appetite) — over a 6-week growth trial
c) conversion ratio)

As only two experiments were possible, only two levels of SREF could be investigated.

8.34 Originally the intention was to chemically alter the SREF to a pH at which ammonia-dissociation would be higher. However, preliminary investigation with "natural" methods of raising pH, such as crushed calcareous shell (recommended in Burrows & Combs (1968) and Spotte (1970) for maintenance of pH in closed-system biological filter beds), proved ineffective. Clearly their effects in a closed system depend on accumulation as the water is recycled. Direct chemical dosing with alkali was then considered, using NaOH or a combination of NaOH and Na_2HPO_4. However, the quantities of chemical needed to meet the theoretical requirements of the fairly swift water flow proved excessively high. At very high alkali concentrations, the rate of drip-feed addition to the factory effluent would have been so slow as to require special equipment to maintain a correct feed (e.g. see Schulze-Wiehenbrauck 1974).

8.35 In practice it was decided to leave the pH of the SREF unaltered, thereby avoiding problems of chemical alteration. Whilst this limits the validy of the present study to soft water of relatively low pH (see Chapter 1), this is not necessarily too important. Modern fish-farming ventures, such as those of Shearwater Fish Farming, may well make use of soft water of low pH. The major difference is that almost all the

ammonia present is ionic, with negligible UIA: if Schulze-Wiehenbrauck is correct in his assessment of UIA growth-effects, this will not matter since the other factors will be causing any growth effects found. Also the subsidiary question arises of growth effects due to NH_4^+ itself: assuming negligible UIA, how concentrated can NH_4^+ become before growth is affected? This is, however, a separate question from the effects of SREF.

8.36 In addition to the growth parameters listed in para.8.44, several other criteria of effect were considered for investigation:-

a) haematocrit (as a measure of general health of the blood);
b) oxygen consumption capacity (as a measure of overall metabolic rates, or (in extreme) of damage to gills (frequently reported for UIA toxicity);
c) histological examination of selected organs, especially gills, liver and kidney (based on the known effects of UIA, as in Flis (1968), Smith & Piper [1975]).

These measures are all clearly measures of toxicity rather than of tolerance; they were considered from the point of view of additional evidence to support or contradict tolerance measures; in the case of contradiction, the way would be open for more rigorous toxicological assessment.

8.37 With regard to these additional parameters, Sprague (1971) remarks that haematocrit is useful only in so far as one can define "normal" or healthy values: in the present case no attempt is made to compare haematocrits on an absolute scale, only between control and experimental tanks. On oxygen consumption, Sprague laments the lack of experiments evaluating toxicants in terms of scope for activity (see Appendix D). It is important to emphasise that in the current study no full attempt to describe a stress in terms of oxygen consumption was made, although this is highly desirable once EC-50 levels have been roughly assessed. Instead, a few sample experimental and control fish were taken at the end of one of the T-series experiments, and allowed to settle in identical conditions to some-

where near a base level of activity (i.e. approximately the physiological 'standard metabolism', although the precise level is unimportant provided it was the same for all fish). Then their oxygen consumption rates were measured over a short period. This process demanded only a crude form of respirometer, but since the results were intended to be comparative (between experimental and control fish) and not definitive, this was acceptable for the purposes in hand. The only results envisaged as being important would be if there was a great difference between experimental fish and control fish (e.g. an order of magnitude), which might indicate a need for further assessment either by respirometry or toxicological methods.

8.38 In view of these considerations, it was decided not to proceed with histological assessment unless other measurements indicated a requirement to do so. (However, tissue samples were taken, fixed and wax-embedded in case of need.)

8.39 These two growth trials were envisaged as preliminary explorations only, and the conclusions drawn from them are consequently only pointers towards further examination at some future period.

9. TOLERANCE EXPERIMENTS

PREPARATION

Introduction

9.1 Basic and structural facilities were as described in Chapter 3, with some differences to meet the requirements of T-series experiments.

9.2 Facilities were required for:-

a) production of a fish effluent preferably containing high TOA,
b) screening out of effluent solid waste,
c) distribution of the screened effluent to experimental tanks,
d) a duplicate system for control tanks.

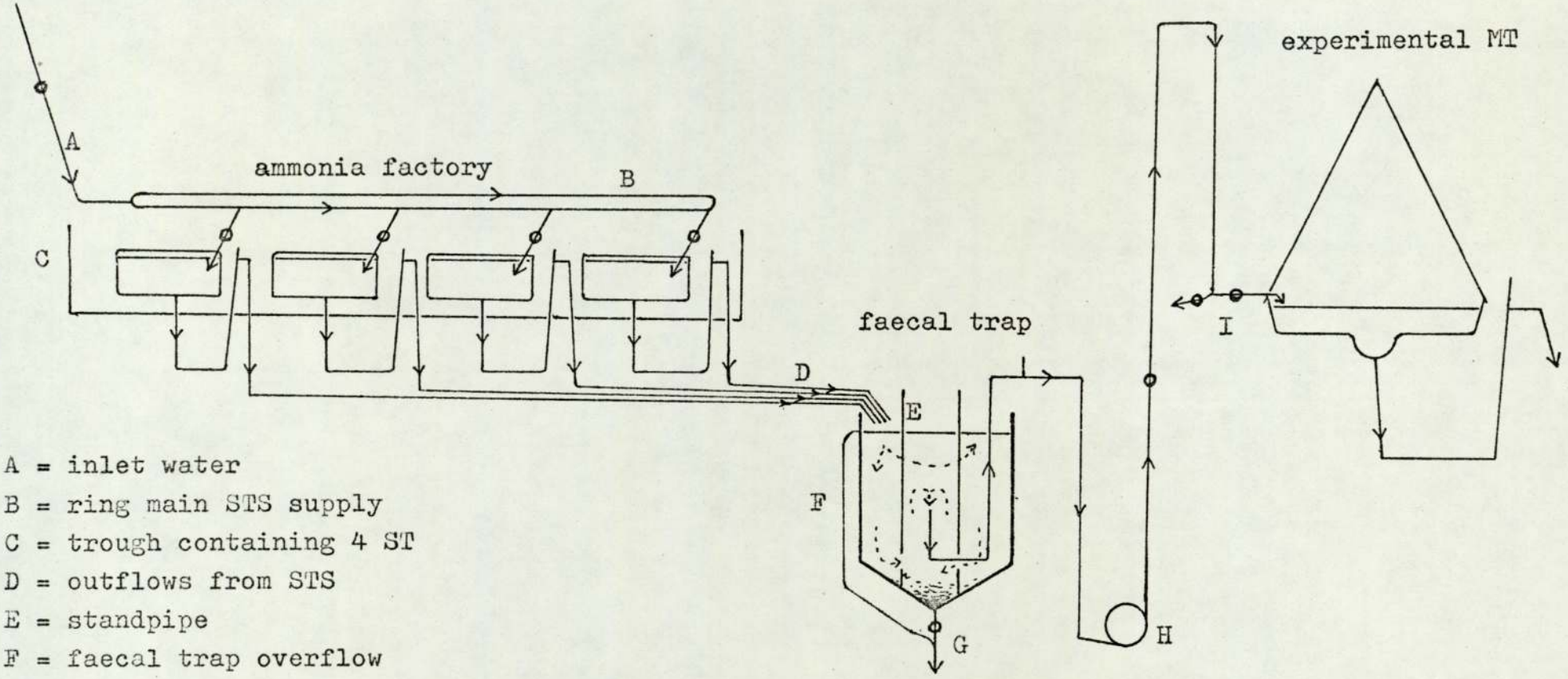
9.3 Figure 9.1 shows in diagrammatic form the system devised. Effluent water from four heavily-loaded tanks ("ammonia factory") was conducted to a faecal trap for solid-settling, and then pumped to experimental tanks. The system was duplicated from the faecal trap stage onwards for control water taken direct from inlet supply.

STS arrangement

9.4 Four ST type 2 were employed, clamped to the floor of the trough, with external overflow levelling, overhead lighting, blackout curtains and U-tubes.

9.5 It was necessary to have ready access to the fish in the tanks, and so the shroud used in other systems was replaced by the original lid across the tank top. Two holes pierced each lid, one to provide entrance for an air-line and diffuser stone, and a second for food. The latter had a tight-fitting plastic funnel-shaped cup inserted into the hole.

9.6 Fig.9.2 shows the major modifications in the STS when used for T-series experiments. The trough was maintained empty of water, acting as a safety overflow reservoir and helping to screen tanks. Above each tank twin feeders dispensed food; they were controlled by the automatic system and mounted on support arms attached to the STS framework. Food was dispensed through the tank lid receiving cup.



A = inlet water
B = ring main STS supply
C = trough containing 4 ST
D = outflows from STS
E = standpipe
F = faecal trap overflow
G = debris drainage
H = pump
I = inlet to experimental tanks (only one shown)
ø = control clip

Figure 9.1 T - series system : basic plan

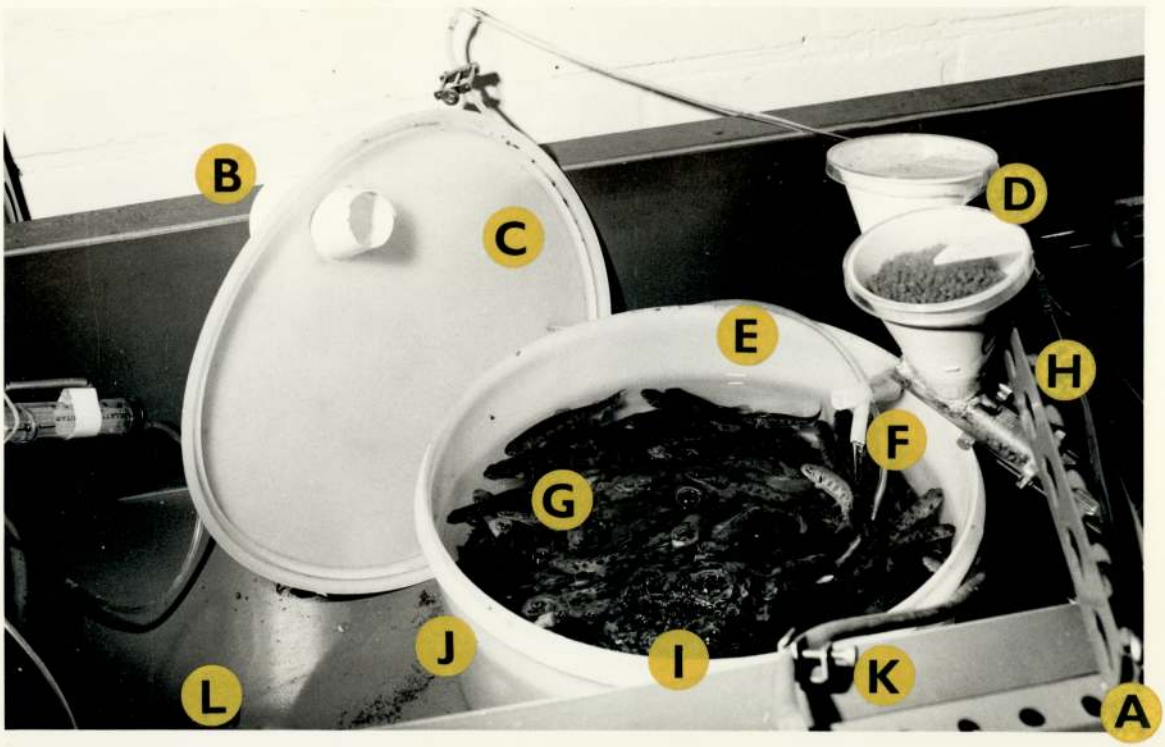


Figure 9.2 STS in T-series configuration

A STS framework
B Food receiving cup
C Tank lid (removed)
D Twin feeders for the tank
E Air line supplying tank through lid
F Densely-packed small fish
G Tank water inlet
H Feeders support arm
I Bubbles from diffuser stone
J ST with high water level
K Water inlet control at front
L Trough (empty of water)

9.7 U-tube arrangement was as for the MTS, the plastic tap of pilot experiments being unnecessary. At the overflow level controls, the outflow from each tree was continued directly into an individual effluent line (13 mm plastic tubing), which conducted effluent direct into the faecal trap. (The STS gutter was only used during cleaning operations and for trough overflow). Each effluent line was interrupted at convenient points by short rubber tubing sections at which the line could be opened, and a cleaning brush could be inserted, in between experiments or as required (the clear plastic allowed debris build-up to be observed).

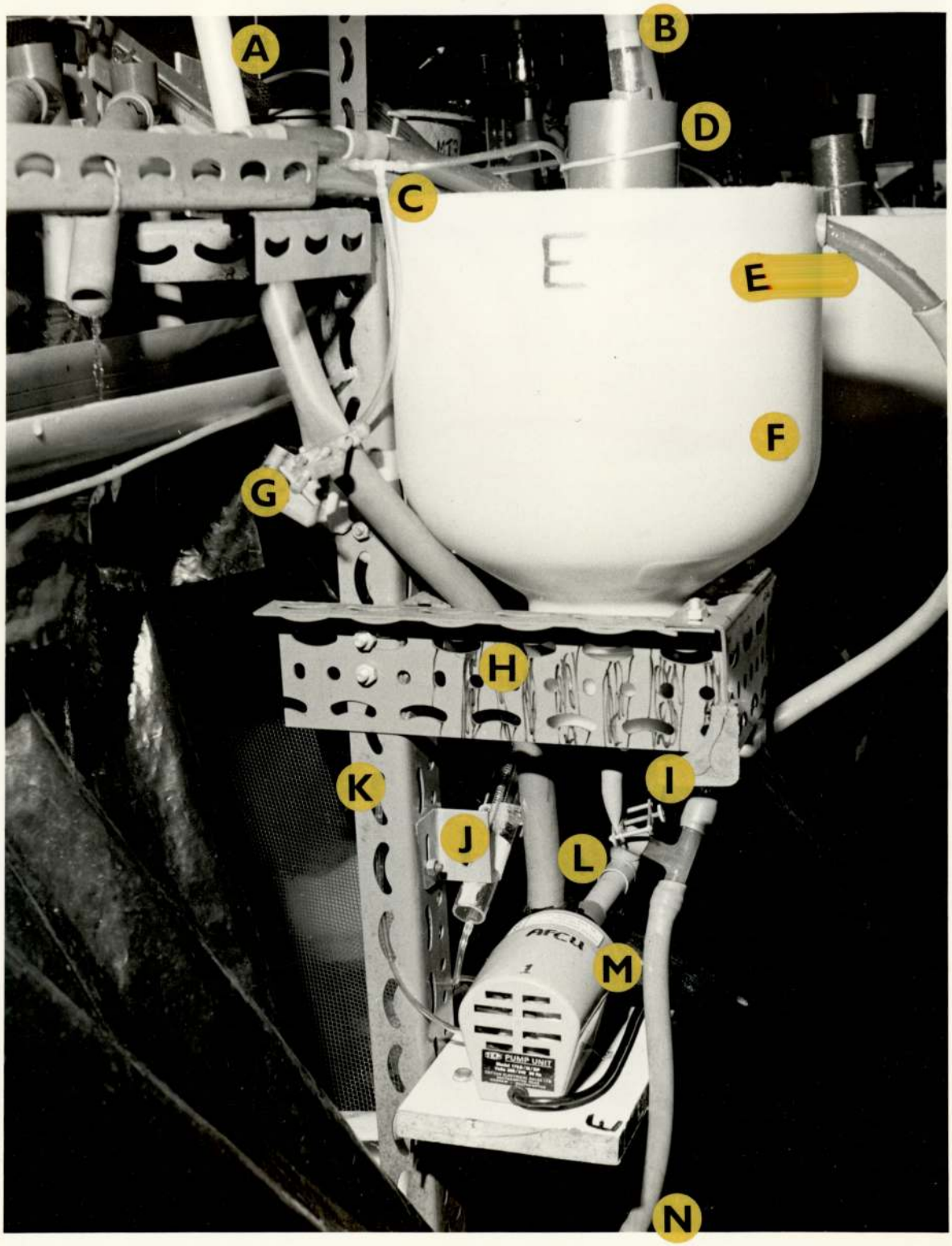
9.8 Individual ST effluent lines were used in preference to a larger common line in case of temporary loss of flow due to debris, which would thus only affect one tank at a time, instead of cutting off the complete flow. This factor was important for the continuity of pumping (see below).

Faecal trap and MTS arrangement

9.9 Four MT from the system were turned over from E-series experiments: the remaining four were maintained in use for E-series experiments, in company with some of the LTS.

9.10 Figures 9.3 and 9.4 show the equipment for screening and distributing STS effluent. Effluent entered the faecal trap from the individual lines at an angle, and so caused circular flow round the trap, during which faeces and any uneaten food wastes sedimented to the bottom. Here they could be removed by using a drain clip (Fig.9.3). The trap water level was controlled by overflow from a point 3 cm from the top, since inflow to the trap always exceeded the pumped outflow.

9.11 The trap was of high-density polyethylene, cylindrical over most of its height (25 cm) but tapering to an outflow point. The top diameter was 27 cm, and the volume approximately 11.4 ℓ. Overflow and drain lines were of 13 mm tubing. The trap was supported by a square section of framework around the bottom, bolted onto the major arch-leg of the main framework.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9.12 Inside the trap a vertical PVC standpipe (6 cm diameter, 36.5 cm height) was placed, with a ring of holes around the lower end allowing water entry (see Fig.9.1). Inside the standpipe a 2 cm diameter PVC exit pipe was positioned, with mouth opening vertically upwards, 9 cm from the bottom of the standpipe. This pipe passed through a slot in the side of the standpipe and conducted SREF upwards and out over the rim of the trap (see Fig.9.1) and then down to the pump below the trap (Fig.9.3) At the highest point (next to the trap rim), a "visitube" branch led vertically upwards for 2 cm and was securely bunged. This access branch could be used for filling the system and pump-priming; being of transparent plastic it also afforded a monitoring point for checking that the system was full of water (without air locks) during operation. Small bubbles could ascend to this point without blocking the exit tube; since the trap was directly aerated by two diffuser stones this was important.

9.13 SREF was pumped by a Totton Model 175B/M/DP electric pump into an ascending water pipe of 2 cm rubber tubing. The pump was screwed to a piece of wooden board securely bolted onto the main framework 20 cm below the trap. With the pump at the bottom of the system, there was a danger of spillage affecting it, but the danger of loss of pump water supply was minimised. In practice, relatively little splash-water found its way to the pump, and a tissue matting between pump and board absorbed it.

9.14 Control of the pumped SREF was exercised by a large Hoffman clip on the ascending water pipe. With a combination of control at this point and an extended head through which to push water the pump was effectively "throttled down" to give the required delivery.

9.15 The ascending SREF pipe was continued upwards by a PVC pipe (Fig.9.4) to a head of 120 cm above the pump. At this point it was conducted over the MTS superstructure girder and continued as a descending PVC pipe ending in a glass T-piece 5 cm above the rims of MT1 and 2. The cross-arms of the

Figure 9.4 MTS in T-series configuration

A Descending PVC SREF pipe

B Ascending PVC SREF pipe (cross-pipe above)

C MT1 (experimental tank)

D MTS superstructure framework: beyond, MT3 and 4 (control tanks)

E Normal MT2 inlet (closed off) behind shroud

F T-series inlet to MT2 passing through shroud; control clip beside T-piece

G T-series inlet to MT1 passing through shroud (control clip not shown)

H Tubing supplying volume indicator (see Chapter 3)

I MTS drainage gutter

J Faecal trap aeration line

K Four individual effluent lines from STS discharging into faecal trap

L Volume indicator tube and scale (see Chapter 3)

M MTS trestle framework

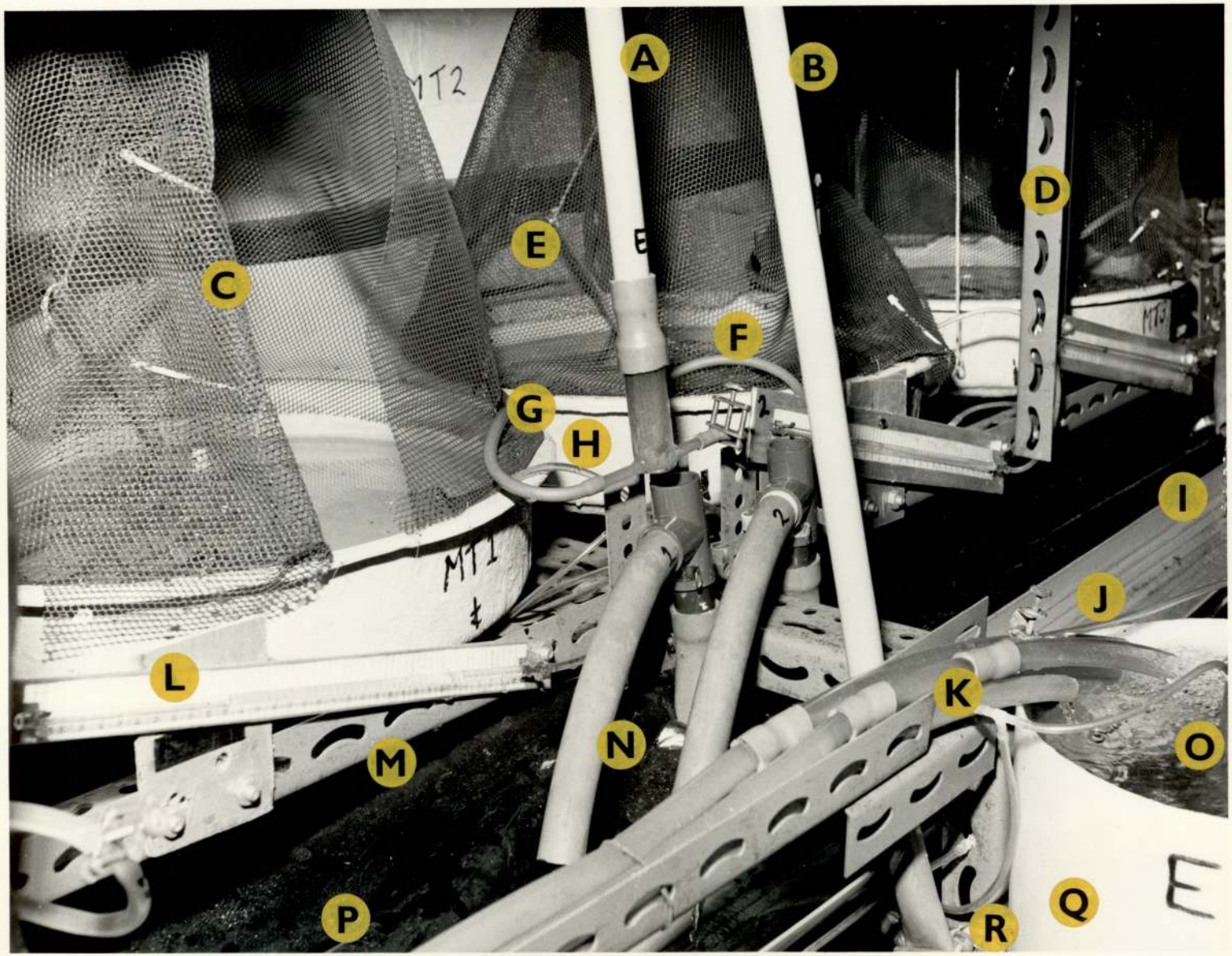
N MT1 overflow discharge pipe to gutter

O Faecal trap standpipe

P Blackout separator between MTS and LTS

Q Faecal trap

R Main control clip on ascending rubber pipe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T-piece were of 6 mm diameter, and from the arms short sections of rubber tubing (with control clips) conducted SREF to the experimental tanks, passing through the shrouds just above the tank rims, and supplying water at an angle for peripheral flow. (The normal MT inlets were closed off).

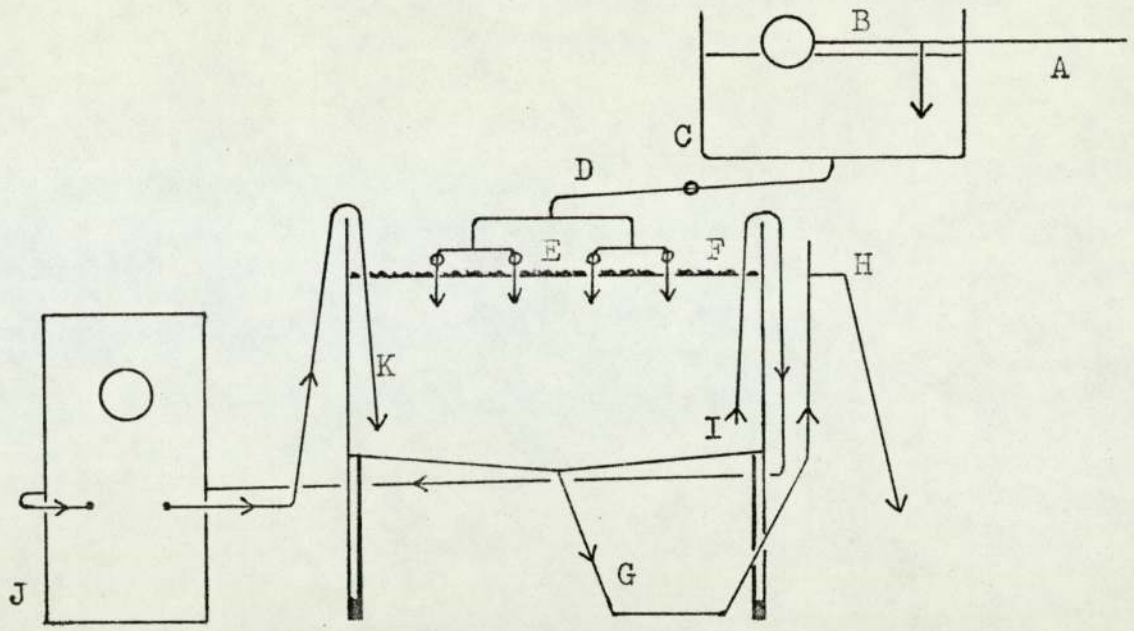
9.16 The system as described served two experimental tanks, MT1 and 2. Two further tanks, MT 3 and 4, were the controls. Their normal water supplies were also shut off, and water from a specially-inserted branch line off the MTS supply was used. This supply was controlled by a Hoffman clip, and conducted by 13 mm plastic tubing along the front of the MTS to discharge into a duplicate faecal trap, pump, and delivery system (mounted in a similar way to the first). The single water line to the control trap could handle enough water to match the experimental system's flow in four separate lines. In both systems the trap supply was greater than the pump output, the excess flowing to waste via the overflow tube.

9.17 The feeders and feeding attachments of MT 1, 2, 3 and 4 were removed, since feeding was by hand.

LT 1 adaptation

9.18 At the end of each T-series experiment, a large-capacity water-bath was required, for oxygen consumption tests, equipped with a supplementary controlled water supply. This was achieved by conversion of LT 1 as in Fig.9.5. The feeder and shroud were removed and normal inlet supply closed off. A 2 ℓ-capacity cistern was installed on a supporting framework approx. 30 cm above the tank. From this cistern one supply line (6 mm tubing) led downwards and branched by means of T-pieces into four equal supplies, each controlled by a clip. These supplies could discharge into the LT or into any apparatus placed in it, when a water supply was connected to the cistern ballcock valve. The flow to the tank escaped via the normal tank outlet/overflow system.

9.19 As a separate circuit, two 13 mm plastic tubes connected



A = inlet water supply
B = ballcock valve
C = cistern header tank
D = main supplementary supply
E = 4 branch supplementary supplies
F = expanded polystyrene pieces
G = LT outlet
H = overflow level control
I = outflow to chiller
J = chiller/thermocirculator
K = inflow from chiller
ø = control clip

Figure 9.5 Plan of arrangement of LT1 adapted as water-bath

the LT water-bath with the chiller/thermocirculator used in pilot experiments. This circuit controlled the water-bath temperature, aided by a layer of expanded polystyrene pieces spread across the LT water-surface. These pieces provided thermal insulation between the water and the air above, and the water-bath could easily maintain a desired low temperature. Black-out curtains were retained for screening-off LT 1.

Equipment sources

9.20 Manufacturers of important items are listed below:

Water pumps	- Totton Electrical Sales Ltd. Southampton.
Faecal traps (purchased as container bottles; bottoms removed)	- Jubb Containers
Glassware	- Glassblowers, Department of Physics, University of Aston
Oxygen measurement aquaria (see METHODS section) (to specification)	- Workshop, Dept. Biological Sciences, University of Aston

METHODS AND MATERIALS

Fish

9.21 In each experiment, two fish batches were used, all from Lots 03 and 04 pooled. The smallest fish (FCAT 1, 2 and 3) were used in STS, since smaller fish metabolise (and hence excrete ammonia) at a higher rate, weight for weight, than larger fish.

9.22 Fish in the MTS were all of FCAT 3. All fish selected had a healthy appearance and good maintenance record; all had been held at Aston for at least three months before use.

Feeding

9.23 During experiment, all MTS fish were fed Trout No.4 (floating) pellets. STS fish were fed a mixture of Salmon No.2 and No.3 (sinking) diets.

9.24 The STS feeding regime was as determined by the automatic procedure used for E-series experiments (see Chapter 7), with

five automatic feeds per day, dispensed from the twin feeders mounted over each ST. Amounts were calculated according to temperature from Table 1.3 (Chapter 1) and the twin feeders calibrated accordingly.

9.25 For the MTS fish, a measure of appetite was required (see Chapter 8) and so fish were fed by hand "ad-lib", through the tank shroud, until satisfied, twice during each day. Food in measured amounts was weighed out daily into small plastic containers, one per tank, and at each feed several pellets were sprinkled into the water flow in the tank, for each tank in turn. All these pellets were usually eaten at once, and then a few more were dropped in, from the appropriate container, again for each tank in turn. This process was repeated as necessary for each tank until at least four pellets were left uneaten between one visit and the next. This was taken as a signal of satiation. Experience with this method of feeding has confirmed that even excess pellets are usually consumed within the next 15 min or so; and the error due to loss of pellets rarely exceeds about 0.5 g if the feeding is carefully carried out (P.Smith, personal communication). After feeding, the containers were re-weighed and the food consumed found by difference; records were kept for each tank. In all cases consumption was excellent.

Tank fish densities

9.26 For the STS, the objective was to produce an effluent containing as much TOA as possible. This was done by crowding the tanks as full as seemed reasonable with small fish; although loading was not extremely excessive due to the low fish weight, stocking and fish density were high. In experiment TO1, the STS population density was 150 fish per tank, or about 15 fish ℓ^{-1}, and in TO2 this was increased to 200 fish per tank (20 fish ℓ^{-1}). Since the STS water flow averaged 0.5 ℓ min^{-1} per tank, the loading and stocking values would be in the region of 1.0 kg min ℓ^{-1} and 50 g ℓ^{-1} respectively, although they would fluctuate as fish grew. As this was a highly stressful environment,

and the fish employed were growing slowly, there was a steady mortality of one or two fish per tank per day. This was made up from the Lot 03/04 reserve stocks. It should be stressed that the sole function of the STS was as a fish effluent "factory" and the physiology of the fish was not studied in any other respect.

9.27 In the MTS, a minimal stress was required. Numbers were restricted to 20 in each tank, and so with water level maintained almost full and a flow of about 0.75 ℓ min $^{-1}$ through each tank, the following figures would roughly apply at the start of each experiment:

density : 2.0 fish ℓ^{-1}
stocking: 20 g ℓ^{-1}
loading : 0.3 kg min ℓ^{-1}

(The latter two values increased as fish grew.)

Sorting/weighing

9.28 Weighing took place fortnightly over the 6-week period of each experiment. Procedures were broadly as described in Chapter 7, but with the major difference that all fish were individually weighed to the nearest 0.1 g. Individual lengths were also measured in TO2. For individual weighing, anaesthetised fish were carefully "blotted" on damp absorbent paper to remove excess water, then placed on a dampened pad (tared) on the balance. The procedure could be speedily combined with length measurement, and the time that each fish was exposed to sorting stress (exclusive of anaesthetising)would not exceed 1 minute. In all cases fish recovered swiftly from anaesthetic and exhibited no side-effects, eating quite normally on the following day.

Procedure

9.29 After initial sorting (during an afternoon) each experiment lasted for 42 days. During this time, the fish were weighed again on day 14, day 28 and finally on day 42. Fish were not re-sorted on these occasions, but maintained as the same tank population throughout. During TO1, fish were weighed with empty guts (not fed on the morning previous to weighing) in the

interests of optimium comparability between weights. This had the disadvantage of interrupting the feeding schedule and thus the growth pattern. For TO2, the feeding schedule was maintained throughout, so that fish were weighed with guts in various stages of digestion. It was felt that the error thus introduced would average out over a tank population, and still allow comparability between experimental and control tanks. Initial sorting was on 19/11/74 for TO1 (final weighing 31/12/74), and on 13/1/75 for TO2 (final weighing 24/2/75).

9.30 Water samples for TOA measurement were taken as for E-series experiments. Volume and flow through tanks were also monitored daily and adjusted when necessary to preserve environmental conditions; fluctuation in flow occurred due to build-up of debris in the effluent lines, faecal trap and pumping circuit, hence daily monitoring and debris-removal was necessary. Pump operation, aeration, temperature, lighting, STS condition and MTS condition were also monitored daily. pH was monitored on every occasion of TOA measurement.

Haematocrit measurement

9.31 At the end of each experiment, two further tests were carried out on selected experimental and control fish; haematocrit readings and crude measurement of oxygen consumption capability. For haematocrit, a majority of fish from each tank (12) were removed to an aerated holding bucket one or two days after final sorting; in the meantime they had been maintained under experimental conditions. Each fish was anaethetised lightly in a bucket of MS.222 solution at 80 mg l^{-1}, killed by severing the backbone just behind the cranium, and the tail was cut off at a point just behind the vent. The cut surface was swiftly "blotted" with absorbent tissue (since dilution with water causes haemolysis), and blood collected from the caudal artery in a heparinised micro-haematocrit tube; this was allowed to fill to about two-thirds of its length when held horizontally. In this way sufficient blood could be obtained for measurement. (For TO2, duplicate tubes were taken for each fish). Micro-

haematocrit tubes were sealed at the clean end by careful rotation in a micro-burner flame (without affecting the blood), allowed to cool, placed in a haematocrit centrifuge, and spun at a frequency of 20 Hz for 7 min. They were then placed on a micro-haematocrit reader apparatus and the % volume of red cells read off on the scale and recorded. (This method follows the recommendations of Snieszko [1960].)

Oxygen uptake measurement

9.32 The apparatus used consisted of four modified small rectangular aquaria, each of volume about 7 ℓ and equipped with a sealed lid with inlet and outlet ports (Fig.9.6). For use, the aquaria were placed in the LT water-bath previously described (para. 9.18) with four supplementary water supplies connected to the inlets and under flow. The surrounding tank water level was below the central port in the lid. Two fish were selected from each MT (experimental and control) on the day of final weighing, care being taken to make sure that they were among neither the largest nor the smallest, but otherwise selected randomly. Each pair was placed in an aquarium through the central port, and the bung was inserted, thus sealing the aquarium and freeing it from air-bubbles. The LT water level was then raised (by adjusting the overflow) to cover the aquaria, which were weighted on top to ensure that they remained submerged.

9.33 The aquaria were screened from one another with sheets of opaque plastic, and the LT water-bath screened from surroundings by blackout curtain. The fish were left in darkness without food at a constant low temperature (about 6^{o}C) for 24 h, served by a flow of clean water from the cistern. After 24 h, a 50 ml syringe with flexible tubing attached was inserted into each aquarium outlet port, and a water sample removed for dissolved oxygen analysis. This was done in darkness, with as little fish disturbance as possible. The water supplies were then closed off and the outlet ports bunged, and the system left for 2 h exactly. Then a second water sample was taken from each aquarium and the fish were then removed from the aquaria and weighed individually.

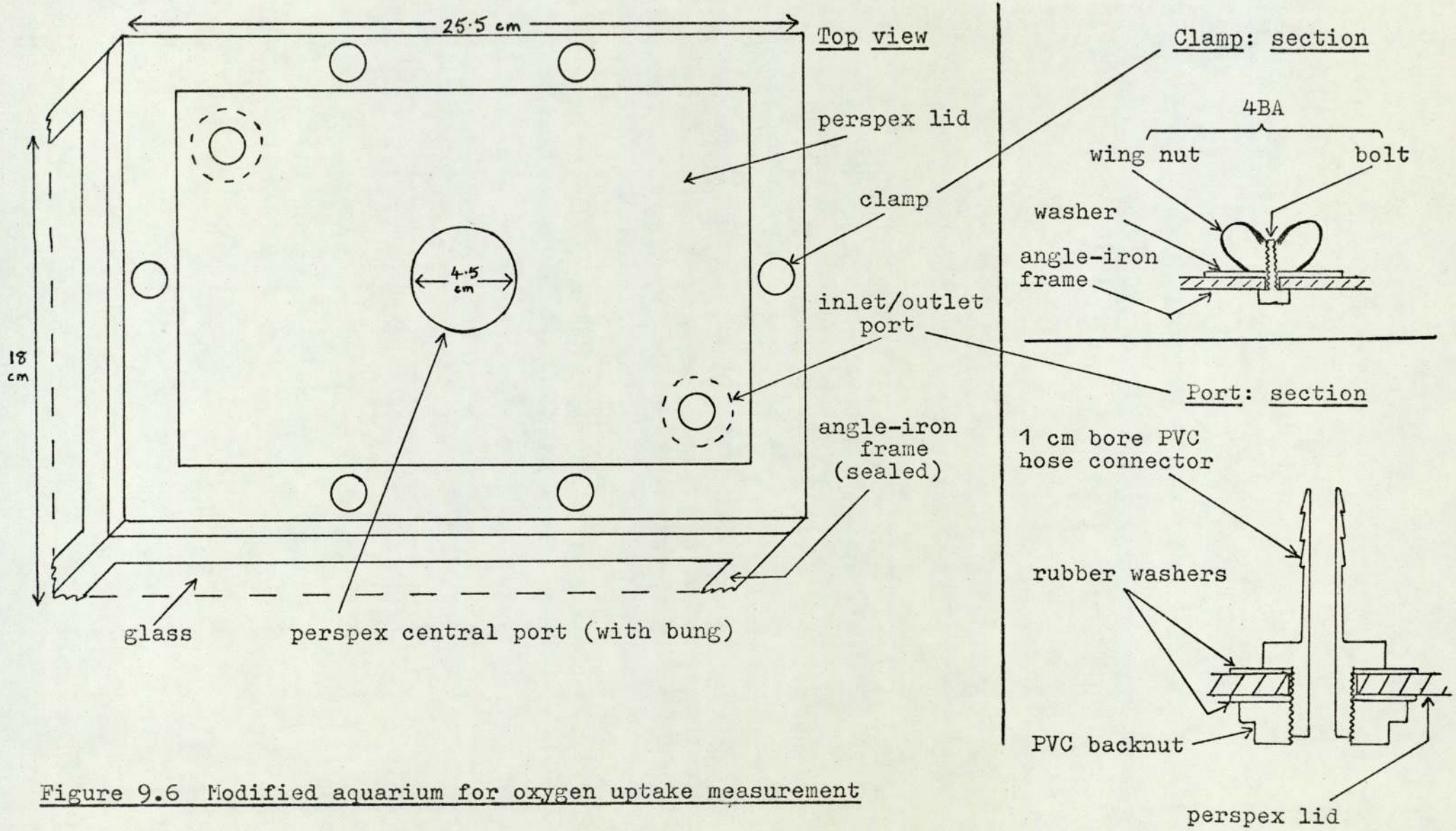


Figure 9.6 Modified aquarium for oxygen uptake measurement

Subsequently the full volume of each aquarium was determined accurately. From these measurements the fish oxygen uptake during the 2 h was estimated.

RESULTS

TOl conditions

9.34 Several quantities in the experimental conditions were variable, and required assessment in case of differential effects on experimental and control tanks. The major factors were temperature, flow, volume, stocking, loading (which were similar in all tanks), and inflow excretory product (which was intentionally different between experimentals and controls).

9.35 Temperature, volume and flow were measured at intervals, as was TOA in the water inflow to the tanks. Intermediate weight estimations were made, to correspond with these values, using the process described in Chapter 7. Thus appropriate values of stocking and loading could be calculated. All conditions are summarised, and their statistical analyses described, in Table 9.1. A multiple range test (Duncan 1955) was used to partition significant results and identify significant differences between tanks. Volume and stocking were found to differ significantly between tanks, but the test showed that the differences were due to individual tank fluctuations and did not represent a difference between experimental tanks on the one hand and control tanks on the other. Thus for volume:

$$\text{MT } \underline{1 < 4 < 3} \ll 2$$

- where

underlining indicates no significant differences, and a double magnitude sign represents a significant difference ($P = 0.05$). Similarly, for stocking:

$$\text{MT } \underline{3 < 2 < 4} \ll 1$$

(Although not analysed, it is clear that for TOA:

$$\text{MT } \underline{3 \doteq 4} \ll \underline{1 \doteq 2})$$

9.36 This type of analysis assumes that results from different days are completely equivalent, and that time (stage of experi-

Table 9.1 Environmental conditions in TO1

Quantity	Units	MT	MEAN	SEM	MINIMUM	MAXIMUM	ANALYSIS
Temperature	(^{o}C)	1	7.606	0.092	7.1	8.5	F = 2.2490
		2	7.611	0.091	7.1	8.5	P > 0.05
		3	7.361	0.087	6.9	8.0	NS
		4	7.378	0.084	6.9	8.0	
Volume	(ℓ)	1	9.46	0.21	8.4	11.1	F = 4.8917
		2	10.22	0.17	9.5	12.2	0.001 < P<0.01
		3	9.71	0.05	9.3	10.2	**
		4	9.67	0.10	9.3	10.9	
Flow	(ℓ min^{-1})	1	0.565	0.040	0.221	0.916	F = 1.4003
		2	0.571	0.033	0.229	0.851	P>0.05
		3	0.651	0.028	0.444	0.945	NS
		4	0.594	0.031	0.425	1.034	
Stocking	(g ℓ^{-1})	1	25.69	8.64	20.8	32.9	F = 7.5183
		2	21.96	6.30	19.0	27.5	P<0.001
		3	21.86	6.77	17.5	26.7	***
		4	22.00	5.21	18.5	25.7	
Loading	(kg min ℓ^{-1})	1	0.4924	0.0608	0.211	1.328	F = 2.4451
		2	0.4365	0.0505	0.216	1.190	P>0.05
		3	0.3402	0.0221	0.189	0.518	NS
		4	0.3773	0.0240	0.174	0.562	
TOA	(μg ℓ^{-1})	1, 2	359.7	13.3	268	445	(not analysed)
		3, 4	(<50)	-	(<50)	(<50)	

Table 9.1 continued

Notes:-

a) n = 18

b) MT1 and 2 are experimental, MT 3 and 4 control.

c) TOA was measured for each inlet supply (experimental and control), and not for all four tanks; all control values were always measured as below 50 $\mu g\ l^{-1}$ and so their accuracy is highly dubious and the values probably negligible; since the difference between these values and those of the experimental tanks is so large (by design), no analysis was performed.

d) The analysis of variance tested for significant differences between the four tanks for each environmental condition; probabilities are expressed against a null hypothesis; for the F values: df = 3,68.

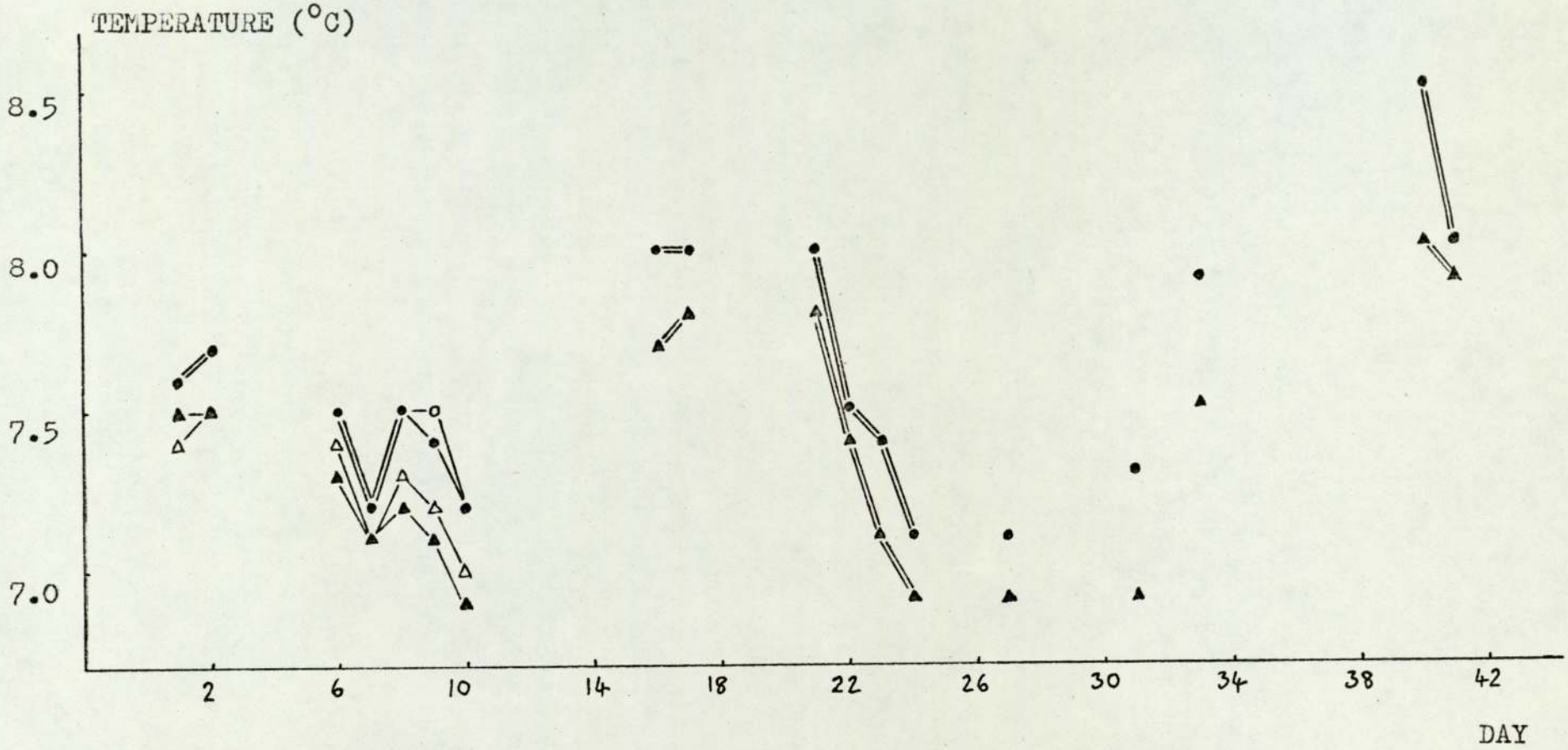
ment) had no effect; so that mean values for environmental conditions are compared in the context of their variances according to tank only. However, it must be recognised that experimental and control tanks might have differed if time were taken in account. Accordingly, this was checked and only temperature (Graph 9.1) shows a clear and consistent difference between experimental and control tanks. This, although small in relation to the total variability of the quantity in each tank, must be considered in any further analysis. (see DISCUSSION section).

9.37 pH was monitored and was consistently lower in the experimental tanks (mean pH 6.60, minimum 6.25, maximum 6.80), as would be expected from the pilot excretion experiment results. Control values were: mean pH 7.02, minimum 6.80, maximum 7.30. The effect of this pH difference in altering UIA would be insignificant; TOA in control tanks was too low for there to be appreciable UIA, whilst higher TOA in the experimental tanks, at lower pH, would still cause negligibly small UIA; i.e. orders of magnitude below the diuresis IC_{max}.

TO1 measured effects

9.38 For experiment TO1, weight was the only growth criterion measured directly, and fish weight results are summarised in Table 9.2. No mortalities occurred. Within each tank, the passage of time and growth opened out the range of weights about the mean for each weighing, as shown in Graph 9.2 for MT 1. Mean and SEM values are shown for all tanks and phases in Graph 9.3; it is clear that there is some divergence of the lines, and this was subsequently statistically tested.

9.39 Feeding was measured in terms of the food weight consumed, and daily records were kept for each tank; Table 9.3 gives a summary of food consumption data. As there were a few unavoidable occasions when feeding was missed, the trends are better appreciated by "ironing out" the effects of these occasions and the subsequent "compensation" feeding on the days following.



Graph 9.1 TO1 temperature results

Table 9.2 Weight results for TO1

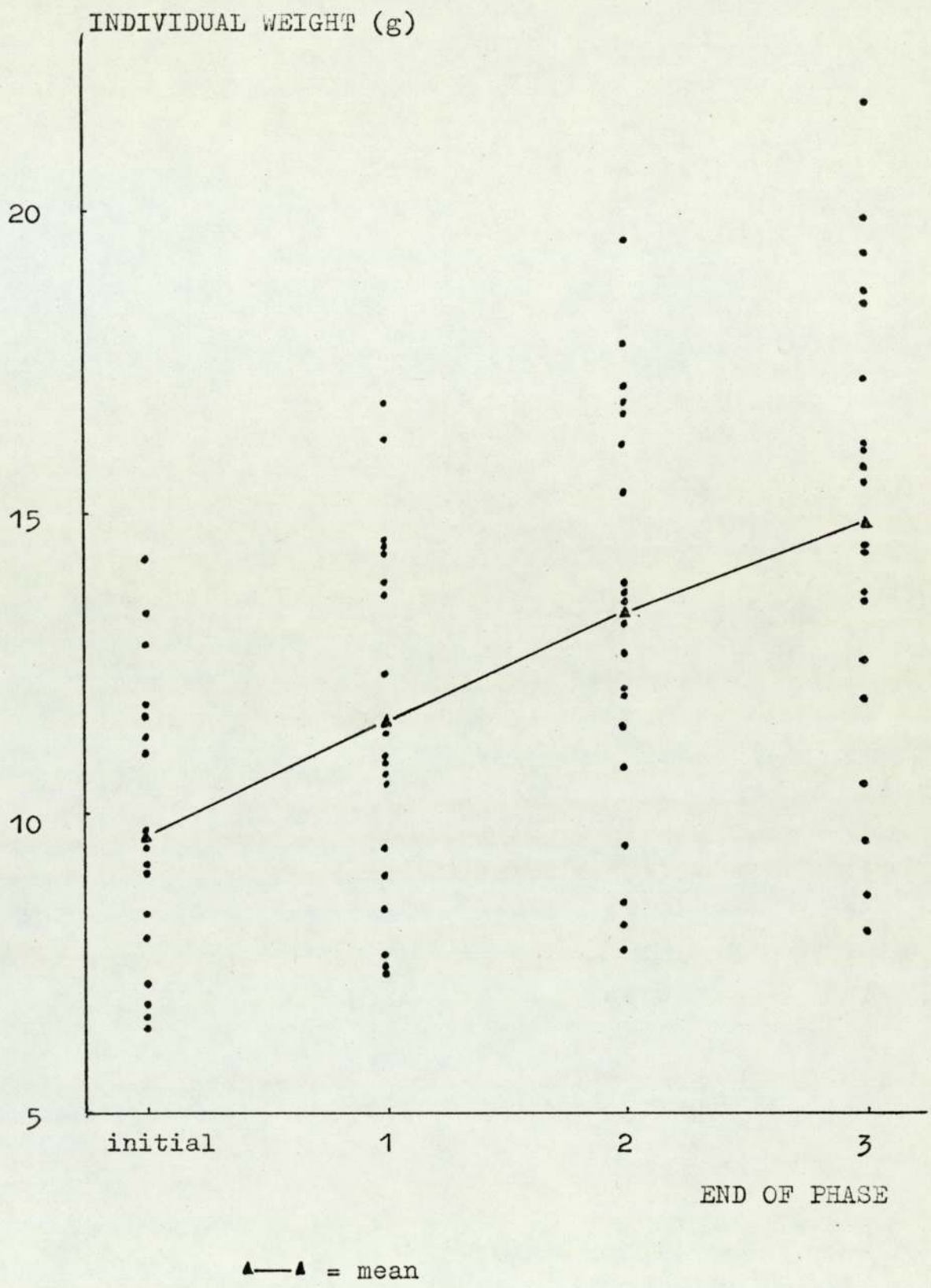
MT	INITIAL	END OF PHASE 1	END OF PHASE 2	END OF PHASE 3/FINAL
1	9.600	11.515	13.285	14.775
(EXPTL)	0.525	0.647	0.760	0.863
	6.4	7.3	7.7	8.0
	14.2	16.8	19.5	21.8
2	8.960	10.715	12.175	13.740
(EXPTL)	0.495	0.629	0.780	0.924
	6.0	7.5	8.1	8.3
	13.4	17.1	19.7	22.4
3	8.670	10.130	11.410	12.835
(CONTL)	0.452	0.535	0.740	0.890
	6.0	6.9	6.8	7.2
	12.8	15.9	18.5	20.6
4	8.905	10.070	11.510	12.830
(CONTL)	0.422	0.590	0.650	0.805
	6.5	6.6	7.6	7.7
	13.4	15.4	18.5	21.4

Notes:-

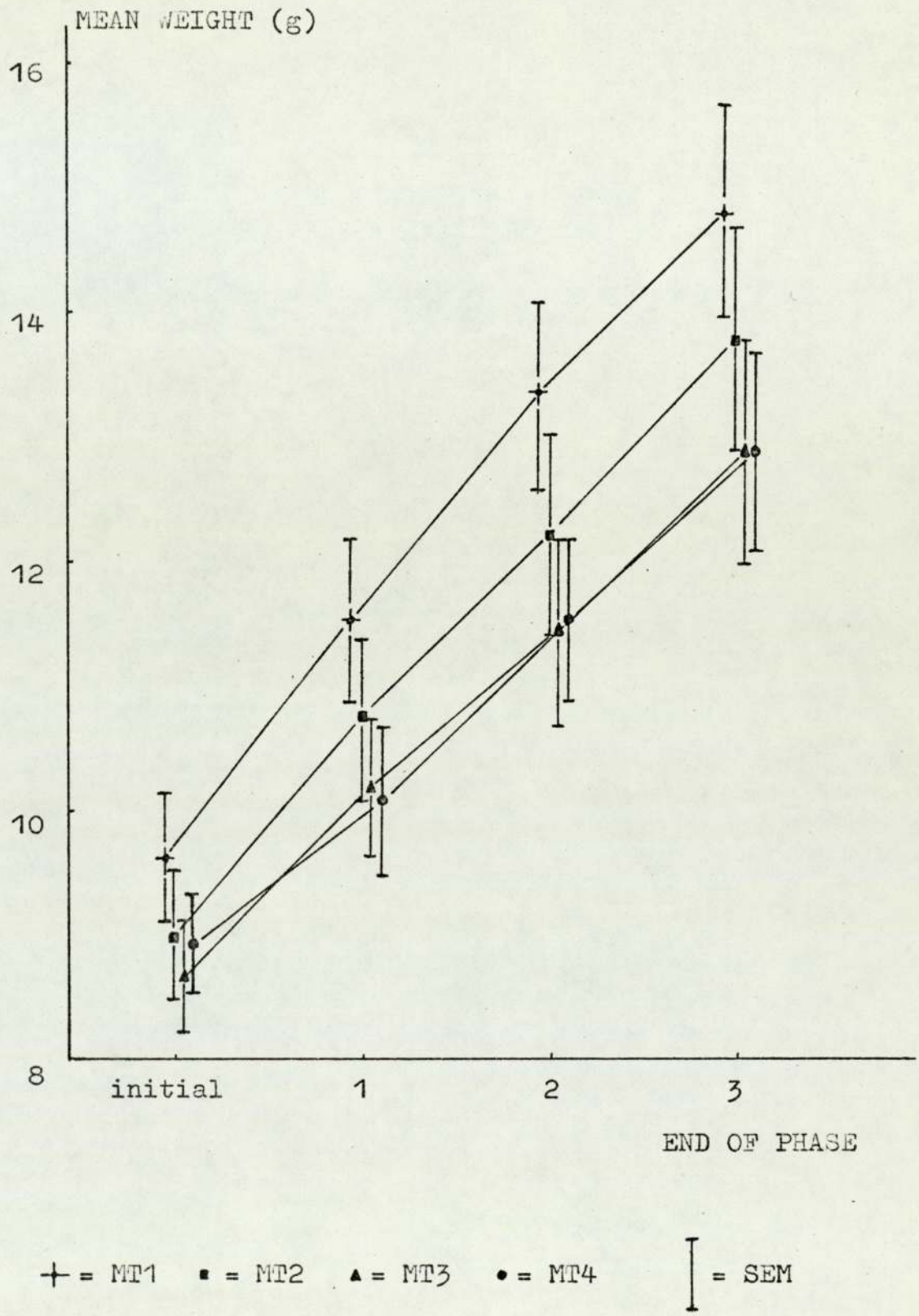
a) All values are in g; EXPTL = experimental; CONTL = control

b) Each cell contains values for mean, SEM, minimum and maximum, respectively. (n = 20)

c) Analysis of variance on the initial data for the four tanks indicates that there was no significant difference in the spread of mean weights between tanks at the start of the experiment. ($F = 1.4518$; $df = 3,76$; $P > 0.05$)



Graph 9.2 T01 individual weights of fish



Graph 9.3 T01 mean weights of fish

Table 9.3 Food consumption in TO1

PHASE	MT 1	2	3	4
1	44.6	40.5	38.1	37.8
2	46.2	40.6	39.8	39.0
3	45.4	43.2	40.4	42.3
OVERALL TOTAL	136.2	124.3	118.3	119.1
OVERALL DAILY MEAN	3.243	2.960	2.817	2.836

All values in g

Table 9.4a Gross conversion ratios in TO1

PHASE	MT 1	2	3	4
1	1.164	1.154	1.305	1.622
2	1.305	1.390	1.555	1.326
3	1.523	1.380	1.418	1.602
OVERALL	1.316	1.300	1.420	1.517

Analysis of variance : phases - $F = 1.3301$; $df = 2,6$; $P > 0.05$ NS
tanks - $F = 1.2718$; $df = 3,6$; $P > 0.05$ NS

Table 9.4b % weight increase in TO1

PHASE	MT 1	2	3	4
1	19.95	19.59	16.84	13.08
2	15.37	13.63	12.64	14.30
3	11.22	12.85	12.49	11.47
OVERALL	53.91	53.35	48.04	44.08

Analysis of variance: phases - $F = 7.7155$; $df = 2,6$; $0.01 < P < 0.05$*
tanks - $F = 1.1601$; $df = 3,6$; $P > 0.05$ NS

This was done by taking a moving average set of values (span 3 days) and such a set is plotted both for MT1 (experimental) and MT3 (control) in Graph 9.4. (The duplicate experimental and control tanks' values were extremely similar to those illustrated.) Feeding data were further analysed as indicators of appetite as described in the DATA ANALYSIS section.

9.40 Gross conversion ratios were calculated for each tank for each period, and are shown in Table 9.4a. A two-way analysis of variance indicated that there was no significant difference due to tanks, nor due to phase of the experiment. Percent weight increase was calculated as

$$\left(\frac{\bar{x}_1 - \bar{x}_o}{\bar{x}_o} \right) \times 100$$

- where $\bar{x}_o$ = initial) mean weight for one phase
$\bar{x}_1$ = final)

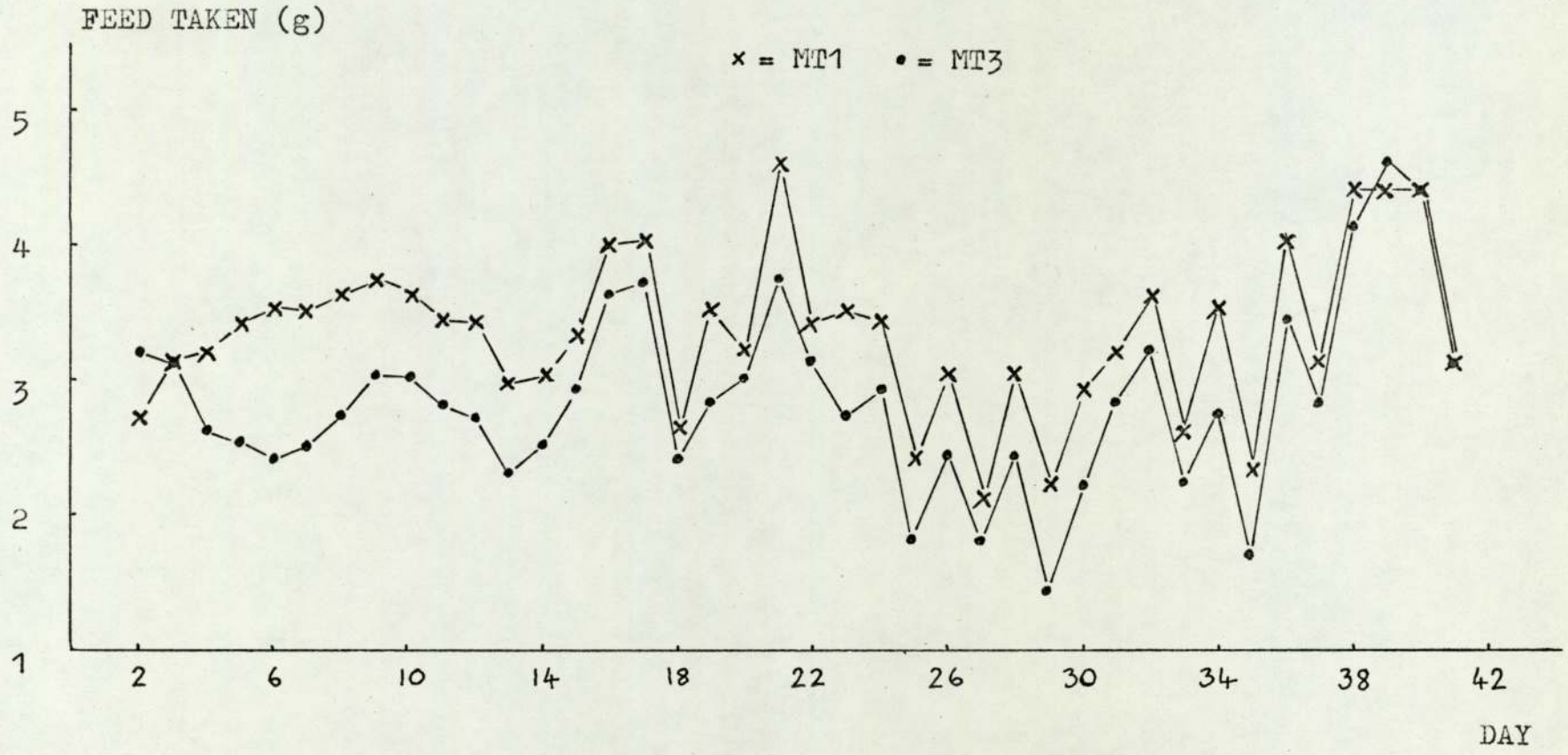
This showed no significant difference between tanks, but there was a difference according to phase of the experiment.

9.41 During haematocrit measurement, some tubes were lost due to breakage in the centrifuge, but the results from the remainder are summarised in Table 9.5a, followed by a comparison of the means for the tanks, using t-tests (Table 9.5b). MT1 was found to differ significantly from the other three tanks, but no other significant results were found.

TO2 conditions

9.42 As with TO1, experimental conditions were statistically tested for differences between experimental and control tanks. As length of fish was measured (as well as weight), the intermediate weight and length estimations could be used to determine the length-related loading factor (FACTØR in Chapter 7), and so this also was tested. Table 9.6 gives a summary of conditions and their analyses. Values for MT3 (control) are missing, since a structural failure on the 19th day caused the tank to be closed down.

9.43 The Duncan test was again applied to the significant



Graph 9.4 T01 food consumption: 3-day means

(plotted on middle day)

Table 9.5a Haematocrit from fish in TO1

QUANTITY	MT 1	2	3	4
MEAN	49.80	43.46	42.57	42.00
SEM	1.50	2.31	2.18	1.36
MINIMUM	40	36	33	38
MAXIMUM	57	64	50	53
n	10	11	7	11

Values are expressed as % red blood cells in the volume of blood.

Table 9.5b Comparison of TO1 haematocrit values

MT	1	2	3
4	$0.001 < P < 0.01$ **	$P > 0.05$ NS	$P > 0.05$ NS
3	$0.01 < P < 0.05$ *	$P > 0.05$ NS	
2	$0.01 < P < 0.05$ *		

$(df = n_1 + n_2 - 2)$

Values are given as probability results from t-tests between values from the tanks indicated.

Table 9.6 Environmental conditions in TO2

QUANTITY	UNITS	MT	MEAN	SEM	MINIMUM	MAXIMUM	ANALYSIS
Temperature	(^{o}C)	1	6.815	0.171	6.1	8.4	F = 1.3057
		2	6.792	0.170	6.1	8.4	P>0.05
		4	6.485	0.142	5.9	7.7	NS
Volume	(ℓ)	1	9.90	0.13	9.1	11.1	F = 5.9386
		2	10.37	0.10	9.7	11.0	0.001<P<0.01
		4	10.11	0.04	10.0	10.4	**
Flow	(ℓ min^{-1})	1	0.510	0.039	0.199	0.795	F = 1.7706
		2	0.534	0.041	0.209	0.755	P>0.05
		4	0.598	0.017	0.464	0.667	NS
Stocking	(g ℓ^{-1})	1	27.79	0.67	24.7	31.4	F = 0.1938
		2	28.09	1.04	21.4	33.2	P>0.05
		4	27.33	0.85	21.8	31.4	NS
Loading	(kg min ℓ^{-1})	1	0.5849	0.0531	0.317	1.128	F = 2.4978
		2	0.5972	0.0579	0.333	1.084	P>0.05
		4	0.4650	0.0154	0.349	0.521	NS
Loading factor	(kg min ℓ^{-1} m^{-1})	1	5.883	0.568	3.27	11.92	F = 2.0503
		2	5.893	0.590	3.48	11.41	P>0.05
		4	4.700	0.134	3.71	5.27	NS
TOA	(μg ℓ^{-1})	1					
		2	698.2	60.5	370	1180	(not analysed)
		4	(<50)	-	(<50)	(<50)	

Notes:- (a) n = 13;(b) Notes (b) and (c) for Table 9.1 also apply here;(c) For analysis F values, df = 2,36

quantity, volume, and indicated:

$$MT1 < \underline{4} < 2 \qquad (1 \ll 2)$$

Thus the significant difference lay between experimental tanks 1 and 2, and not between experimental and control.

9.44 Temperature, volume and flow were checked against time, and once again only temperature shows a consistent difference between environmental and control tanks.

9.45 pH monitoring gave the following values for TO2:-

MT1/2 : mean 6.59, minimum 6.25, maximum 6.85

MT4 : mean 7.35, minimum 7.00, maximum 7.75

UIA values were negligible.

TO2 measured effects

9.46 Weight and length results are shown in Table 9.7. No mortalities occurred. Mean weights are plotted against time in Graph 9.5 and against mean lengths in Graph 9.6, where the lines for all three tanks appear to be in good agreement, the slight divergence of MT4 in phase 1 being removed by the end of the experiment. This suggests that all fish in TO2 can be considered as a homogeneous population.

9.47 Food consumption results were treated similarly to those of TO1; they are summarised in Table 9.8. Gross conversion ratios are shown in Table 9.9a and % weight increase values in Table 9.9b. Two-way analysis of variance indicated that neither quantity was significantly different, according to tanks or phases.

9.48 Haematocrit results are summarised in Table 9.10a followed by a comparison of means by t-test in Table 9.10b. No significant differences were found.

9.49 Results from the oxygen consumption test are shown in Table 9.11. Although only single values were obtained, and hence the tests have no statistical validity, the similarity in the values does not suggest any difference between experimental and control tanks. (For comparison, Davis (1956) quotes a range of 84 to 727 mg kg^{-1} h^{-1} oxygen consumption in salmonids under hatchery conditions.)

Table 9.7 Weight and length results for TO2

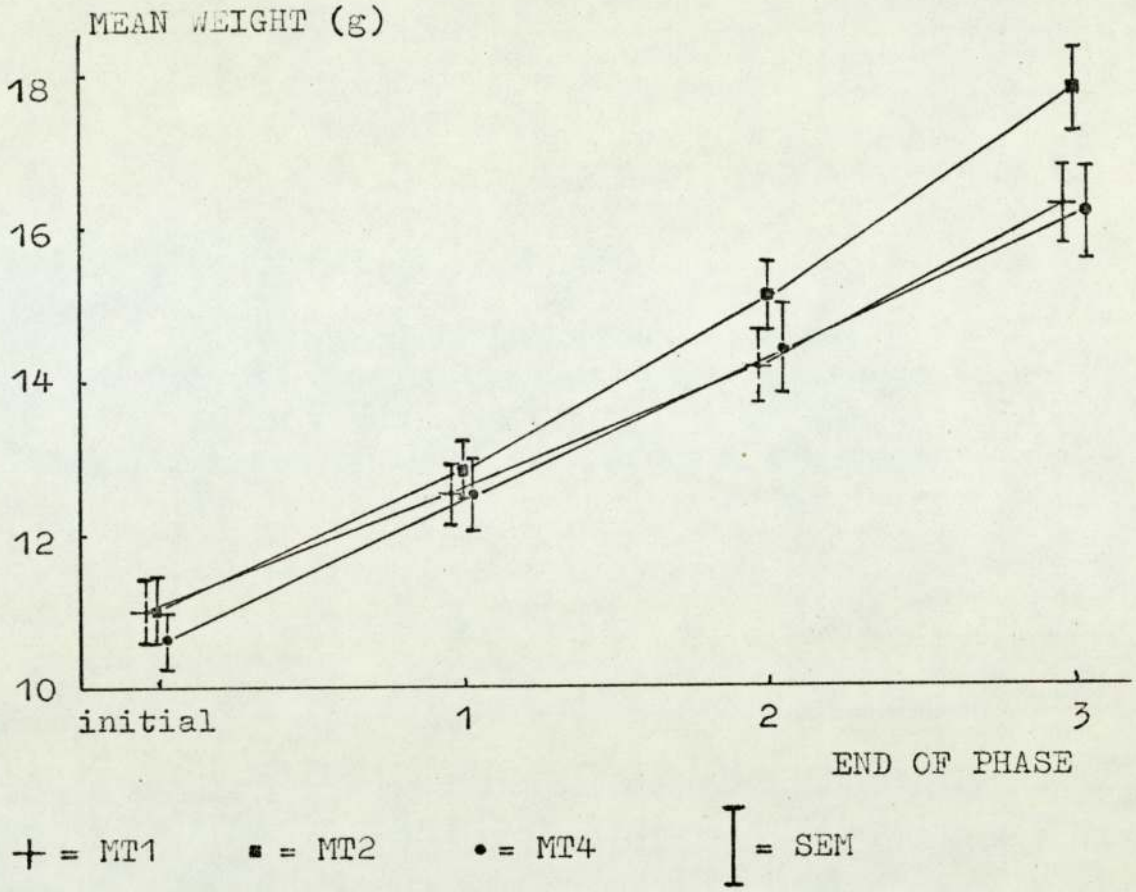
MT	WEIGHT (g)				LENGTH (cm)			
	INITIAL	END PHASE 1	END PHASE 2	END PHASE 3/FINAL	INITIAL	END PHASE 1	END PHASE 2	END PHASE 3/FINAL
1	10.970	12.460	14.165	16.330	9.425	9.675	10.050	10.525
	0.386	0.421	0.457	0.539	-	-	-	-
	7.1	8.5	9.8	10.8	8.5	8.5	9.0	9.0
	14.3	15.6	17.1	20.3	10.0	10.5	11.0	11.5
2	11.020	12.760	15.120	17.820	9.450	9.750	10.250	10.800
	0.365	0.405	0.449	0.566	-	-	-	-
	7.8	8.5	10.2	11.9	8.5	8.5	9.0	9.5
	14.5	16.0	18.7	22.9	10.0	10.5	11.0	11.5
4	10.565	12.470	14.430	16.245	9.300	9.525	10.000	10.450
	0.360	0.451	0.541	0.630	-	-	-	-
	7.2	8.7	9.3	10.4	8.5	8.5	9.0	9.0
	13.6	15.8	19.2	22.0	10.0	10.5	10.5	11.5

Notes:-

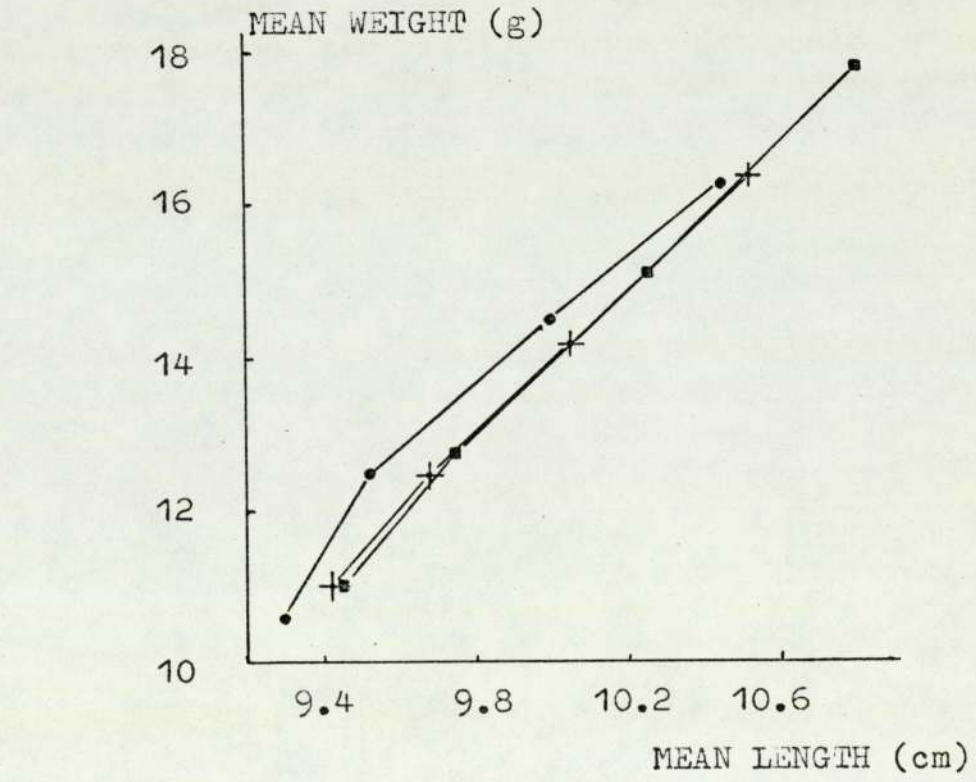
a) Each cell contains values for mean, SEM, minimum and maximum, respectively (n = 20).

b) SEM values for length are omitted, as length was measured in 0.5 cm classes.

c) Analysis of variance on initial weight data indicates no significant difference between tanks ($F = 2.2036$; $df = 2,57$; $P > 0.05$).



Graph 9.5 T02 mean weights of fish



Graph 9.6 Length and weight of fish in T02

Table 9.8 Food consumption in TO2

PHASE	MT 1	MT 2	MT 4
1	37.8	44.4	44.4
2	45.2	54.2	53.0
3	56.4	63.1	58.7
OVERALL TOTAL	139.4	161.7	156.1
OVERALL DAILY MEAN	3.319	3.850	3.717

All values in g

Table 9.9a Gross conversion ratios in TO2

PHASE	MT 1	MT 2	MT 4
1	1.268	1.276	1.165
2	1.326	1.148	1.352
3	1.303	1.169	1.617
OVERALL	1.300	1.189	1.374

Analysis of variance: phases - $F = 0.5672$; $df = 2,4$; $P > 0.05$ NS
tanks - $F = 1.1016$; $df = 2,4$; $P > 0.05$ NS

Table 9.9b % weight increase in TO2

PHASE	MT 1	MT 2	MT 4
1	13.58	15.79	18.03
2	13.68	18.50	15.72
3	15.28	17.86	12.58
OVERALL	48.86	61.71	53.76

Analysis of variance: phases - $F = 0.0872$; $df = 2,4$; $P > 0.05$ NS
tanks - $F = 1.5676$; $df = 2,4$; $P > 0.05$ NS

Table 9.10a Haematocrit from fish in TO2

QUANTITY	MT 1	2	4
MEAN	38.33	39.92	36.64
SEM	1.10	1.70	1.54
MINIMUM	34	32	27
MAXIMUM	45	53	45
n	12	12	11

Values are expressed as % red blood cells in the blood volume.

Table 9.10b Comparison of TO2 haematocrit values

MT	1	2
4	$P>0.05$ NS	$P>0.05$ NS
2	$P>0.05$ NS	$(df = n_1+n_2-2)$

Values are given as probability results from t-tests between values from the tanks indicated.

Table 9.11 TO2 oxygen-consumption test results

MT	MEAN TITRE (ml)			DO FALL (mg ℓ^{-1})	BOX VOLUME (ℓ)	FISH WEIGHT (g)	OXYGEN CONSUMPTION (mg kg^{-1} h^{-1})
	INITIAL	FINAL	CHANGE				
1	2.86	2.47	-0.39	1.505	7.05	32.6	171.4
2	2.85	2.48	-0.37	1.504	7.00	27.5	191.3
4	2.84	2.36	-0.48	1.951	7.08	36.7	188.1

Notes:-

a) Temperature = 6.0°C.

b) Titre is in ml of N/80 thiosulphate solution required during Winkler estimation; value given is mean of two estimations; INITIAL indicates before 2 h of test run (see METHODS section), and FINAL indicates immediately afterwards.

c) DO (dissolved oxygen) FALL is given by:- $\frac{\text{titre difference} \times 101.6}{\text{volume of sample titrated}}$

d) FISH WEIGHT is the sum of the two individual weights.

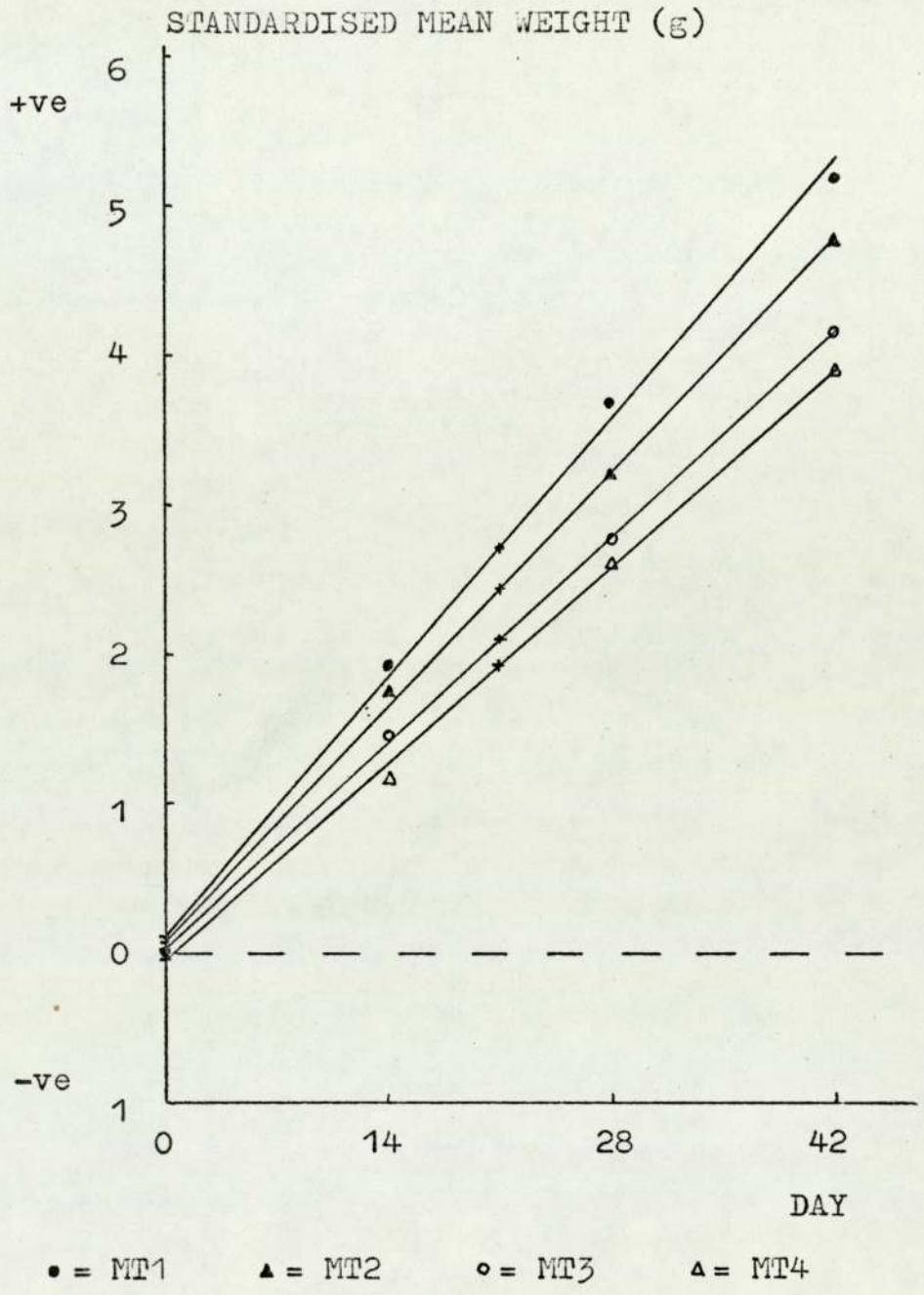
DATA ANALYSIS

9.50 All calculations were performed on an Olivetti Programma P101 desk computer unless otherwise stated.

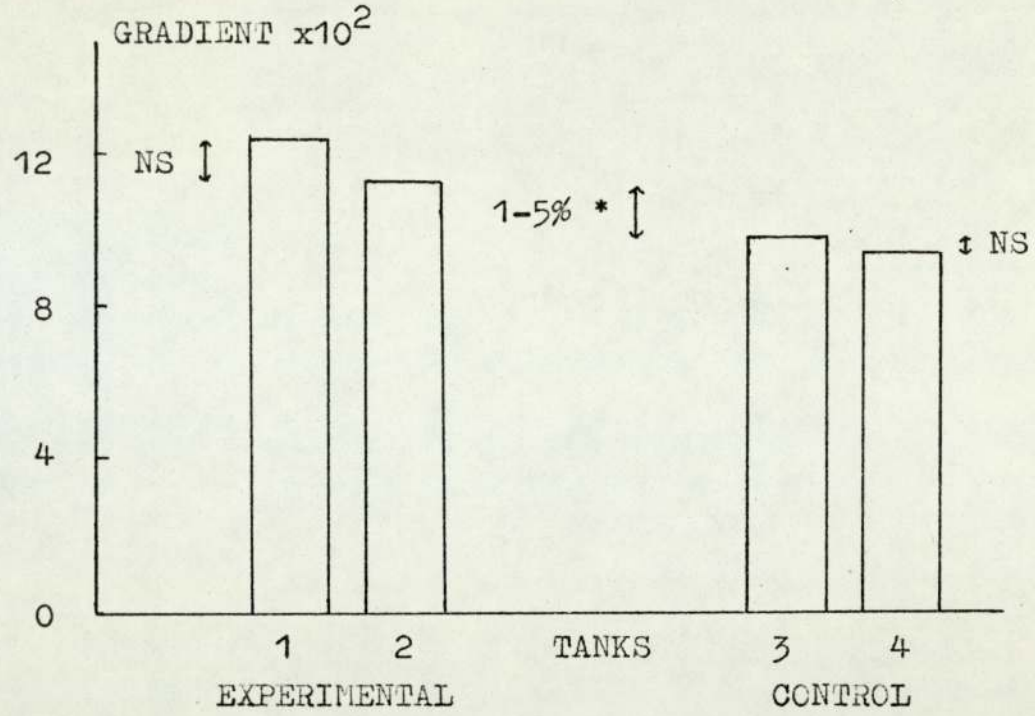
Analysis of growth in T01

9.51 Graph 9.3 showed some growth line divergence in T01, and in order to assess the importance of this, the slope differences between the lines were statistically tested. This demanded two steps; (a) regression of the points to define an equation which would reveal the overall slope, and (b) statistical test of the slope differences. For (a), the simplest method was to perform a regression analysis on the untransformed mean weight data. However, when Haskell (1948) compared growth curves for salmonids, including using simple weight data, log transformation, and cube root transformation, his recommendation was to transform mean weight values to their cube roots to give the best straight-line. In the present case, all three methods were used, and the best straight line selected by comparison of correlation coefficients. Slopes for the four tanks were then compared statistically according to a standard process (Cole 1975).

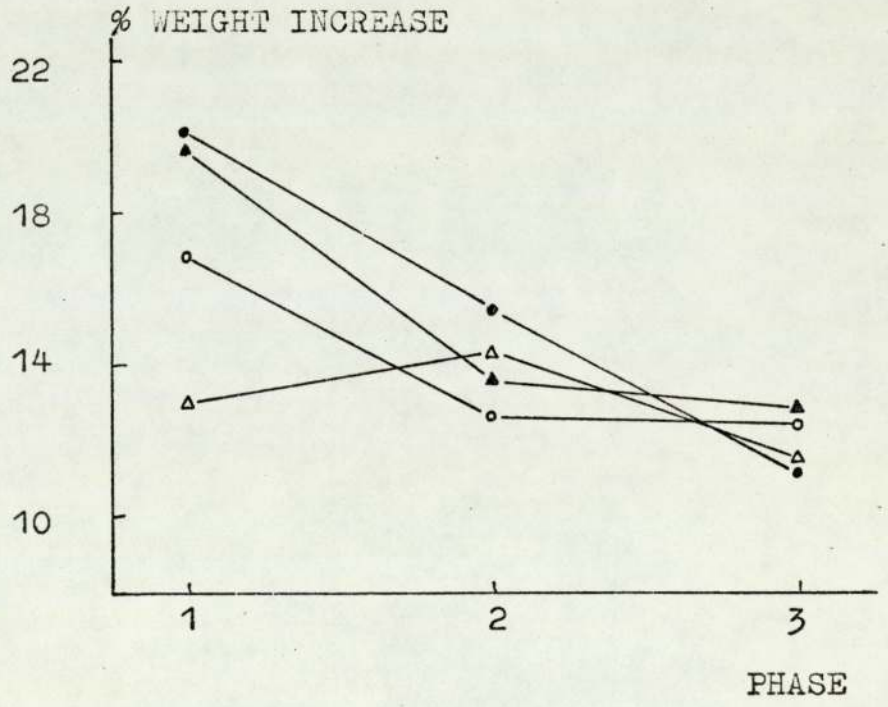
9.52 Table 9.12a shows the correlation coefficients, and the one selected is that with the highest average value over the four tanks; in this case simple mean weight. Values for this quantity are plotted against time, with regression lines, in Graph 9.7, having first been standardised to make the graph clearer. (The standardisation is equivalent to assuming that fish in all tanks started off at the same mean weight, artifically set to zero.) Limits are not given for the regression lines or predicted Y values, since the lines are only for comparison and not for prediction. Table 9.12b gives details of the regression lines and the slope analysis; and the significant difference between the experimental and control groups is illustrated in Graph 9.8. Similar analyses performed on log and cube root transformations show no significant differences.



Graph 9.7 Regression of standardised mean weight with time for T01



Graph 9.8 Gradient differences for T01 regression lines



Graph 9.9 T01 % weight increase by phase

9.53 This analysis was carried out on the ICL 1905 computer using program GRADIENTS (flowchart in Fig.9.7). The following calculations were performed, from sets of data input for time, mean weight, log mean weight, and cube root of mean weight; for each tank:-

(x = time and y = weight data)

a) $\Sigma x,\ \bar{x},\ \Sigma x^2,\ \frac{(\Sigma x)^2}{n}$

b) SS_x (sum of squares) $= \Sigma x^2 - \frac{(\Sigma x)^2}{n}$

s_x^2 (variance) $= \frac{SS_x}{n-1}$

c) $\Sigma y,\ \bar{y},\ \Sigma y^2,\ \frac{(\Sigma y)^2}{n}$

d) $SS_y = \Sigma y^2 - \frac{(\Sigma y)^2}{n}$

$s_y^2 = \frac{SS_y}{n-1}$

e) $\Sigma xy,\ \frac{(\Sigma xy)^2}{\Sigma x},\ \frac{\Sigma x.\Sigma y}{n}$

f) $SS_{xy} = \Sigma xy - \frac{\Sigma x.\Sigma y}{n}$

g) s_{xy}^2 (covariance) $= \frac{SS_{xy}}{n-1}$

h) $R = \frac{s_{xy}^2}{\sqrt{s_x^2 . s_y^2}}$

$b = \frac{s_{xy}^2}{s_x^2}$

$a = \bar{y} - b\bar{x}$

Then for each pair of tanks (denoted as 1 & 2):-

i) $s^2_{y_1.x} = \frac{n_1 - 1}{n_1 - 2}(s_{y_1}^2 - b_1^2 s_x^2)$

$s^2_{y_2.x} = \frac{n_2 - 1}{n_2 - 2}(s_{y_2}^2 - b_2^2 s_x^2)$

} population variances

j) $F_{12} = \dfrac{s^2_{y_1.x}}{s^2_{y_2.x}}$ (tested for significance, df = n_1-1, n_2-1)

Assuming F is not significant, and thus population variances are $\pm$ equal, then:

k) $$s^2_{y.x.p} = \frac{(n_1 - 2)s^2_{y_1.x} + (n_2-2)s^2_{y_2.x}}{n_1 + n_2 - 4}$$

(pooled variance)

l) $$s^2_{(b_1-b_2)} = s^2_{y.x.p.}\left(\frac{1}{[n_1 - 1]s_x^2} + \frac{1}{[n_2-1]s_x^2}\right)$$

(variance of b_1-b_2)

and, finally:

m) $$t = \frac{b_1 - b_2}{\sqrt{s^2_{(b_1 - b_2)}}}$$

(df = $n_1 + n_2 - 4$)

9.54 In all cases, for both TO1 and TO2, the F-test at (j) proved non-significant, and the t statistic was thus valid, and its probability could be checked against the usual t distribution.

9.55 Percent weight increase is illustrated in Graph 9.9. Overall % weight increase is lower in control tanks, and % weight increases for different phases. The trend is clearly towards a lower value in all tanks as expected; fish once having passed their earliest growth phases tend to decelerate in growth rate. There is no clear difference btweeen experimentals and controls. Further analysis of % weight increase can be achieved by plotting it against the mid-phase mean weight (i.e. $\frac{\bar{x}_o + \bar{x}_1}{2}$

where $\bar{x}_o$ = initial) mean weight for one phase).
$\bar{x}_1$ = final)

Such a plot (Graph 9.10) for TO1 shows a suggestion of a difference between the estimated average for experimental tanks and the average for control tanks. This suggests a possibility of

Figure 9.7 Flowchart for program GRADIENTS

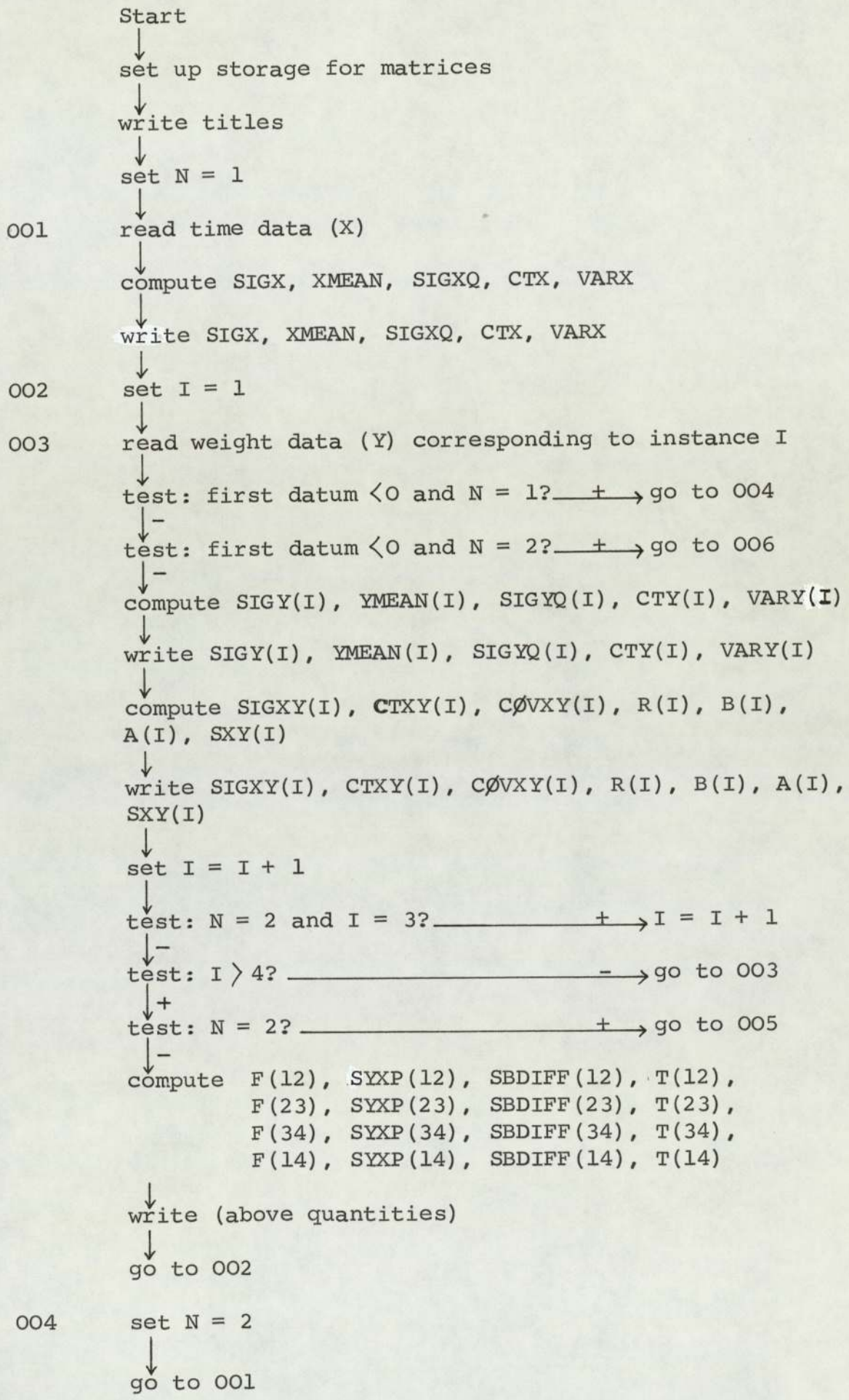


Fig. 9.7 continued

```
005     compute  F(24), SYXP(24), SBDIFF(24), T(24),
                 F(41), SYXP(41), SBDIFF(41), T(41),
                 F(21), SYXP(21), SBDIFF(21), T(21)

        go to 002

006     stop
```

Notes:-

a) Codes refer to the quantities described in para. 9.53 as follows:-

X :	x	Y :	y
SIGX :	$\lesssim x$	SIGY :	$\lesssim y$
XMEAN :	$\bar{x}$	YMEAN :	$\bar{y}$
SIGXQ :	$\lesssim x^2$	SIGYQ :	$\lesssim y^2$
CTX :	$(\lesssim x)^2/n$	CTY :	$(\lesssim y)^2/n$
VARX :	s_x^2	VARY :	s_y^2

SIGXY : $\lesssim xy$

CTXY : $(\lesssim x . \lesssim y)/n$

CØVXY : s_{xy}

R : R

B : b

A : a

SXY : $s^2_{y.x}$

F(12) : F_{12}

SYXP(12) : $s^2_{y.x.p}$

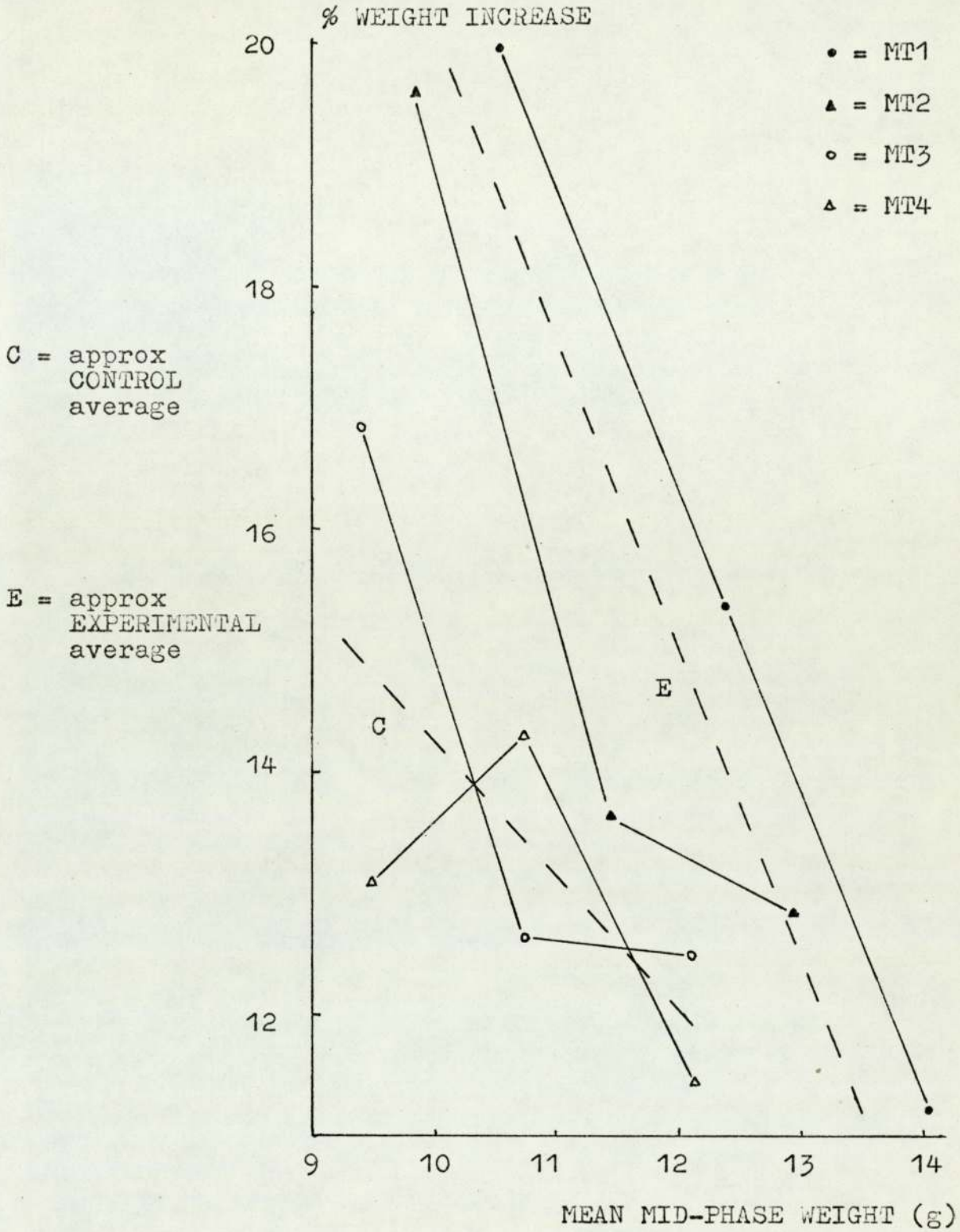
SBDIFF(12) : $s^2_{(b_1-b_2)}$

T(12) : t

} etc., where numbers refer to tanks under comparison

b) I and N are repeater codes for program loops

c) "set" and "compute" used as in Chapter 7, Fig.7.9, note(g)



Averages show increased growth rate in experimentals compared to controls; agrees with Webb & Brett (1973) observations for levels below EC-50

Graph 9.10 T01 % weight increase by size

an increased growth rate (as measured by phase % weight increase) at a given individual fish weight in experimental tanks, although the average lines look as though they are converging as fish become heavier. Since the data are too scanty for statistical analysis, however, this can only remain a suggestion.

Analysis of appetite in TO1

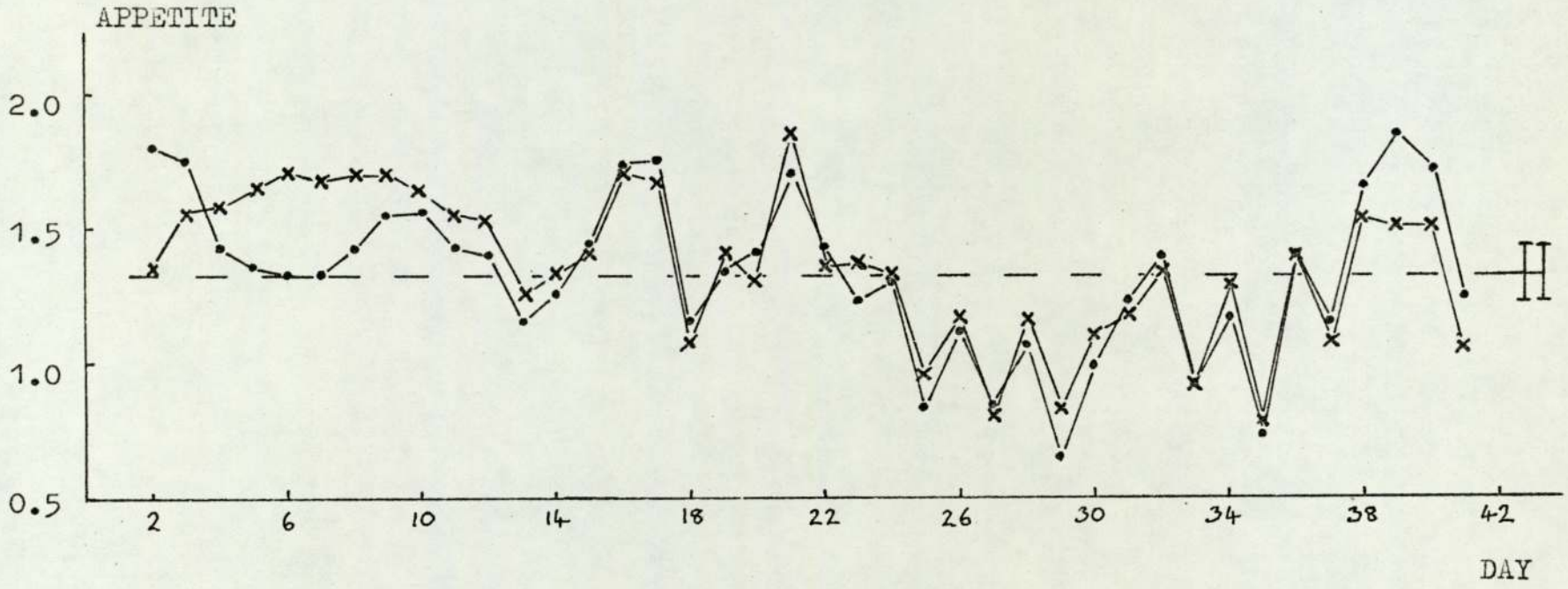
9.56 Food consumption was measured as weight fed per tank per day. In order to compare tanks containing different fish weights, the quantity used for comparison was appetite, arbitrarily defined as:

$$\left(\frac{\text{weight of food fed}}{\text{total weight of fish}}\right) \times 100$$

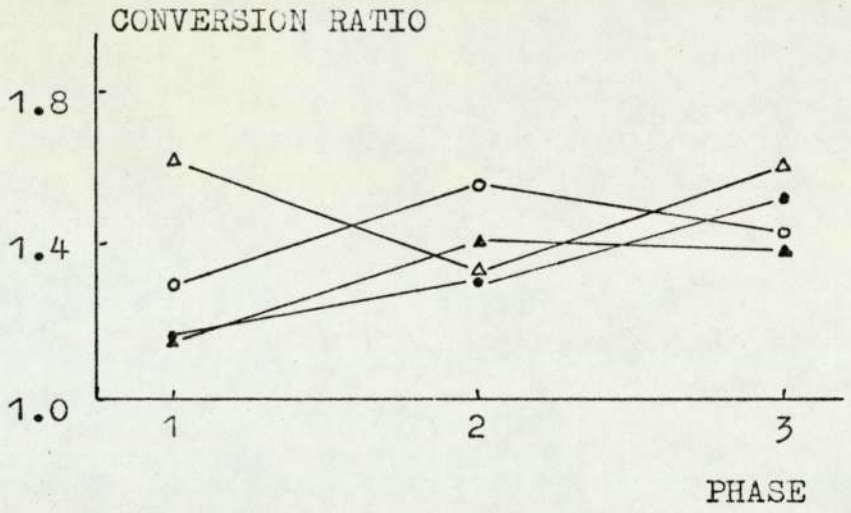
(or food consumed as % of body weight)

This involved intermediate weight estimation as in Chapter 7. As there were no mortalities, the accuracy of this process was at maximum. Due to the fluctuations mentioned in para.9.39, a moving average was used (span 3 days) to provide a plot on which trends could be assessed. Graph 9.11 shows such a plot for one experimental and one control tank. The patterns and values are very similar; the overall trend is downwards, with a rise in the last few days. Differences between the extreme mean values (Table 9.13, MT1 & 2) were found to be statistically insignificant.

9.57 Gross conversion ratio is analysed in Graph 9.12 and Graph 9.13 (plotted against mid-phase mean weight). Overall values are slightly higher (worse) in control tanks; and the tanks display a slight upward trend in phase values (not statistically significant). Graph 9.13 agrees with the impression of Graph 9.10 for % weight increase: the approximate average for experimental tanks appears to be generally superior to that for controls, suggesting that the reason for the suspected superior growth rate in experimentals is that the fish would be converting food at a better rate (appetite is approximately the same in all tanks).

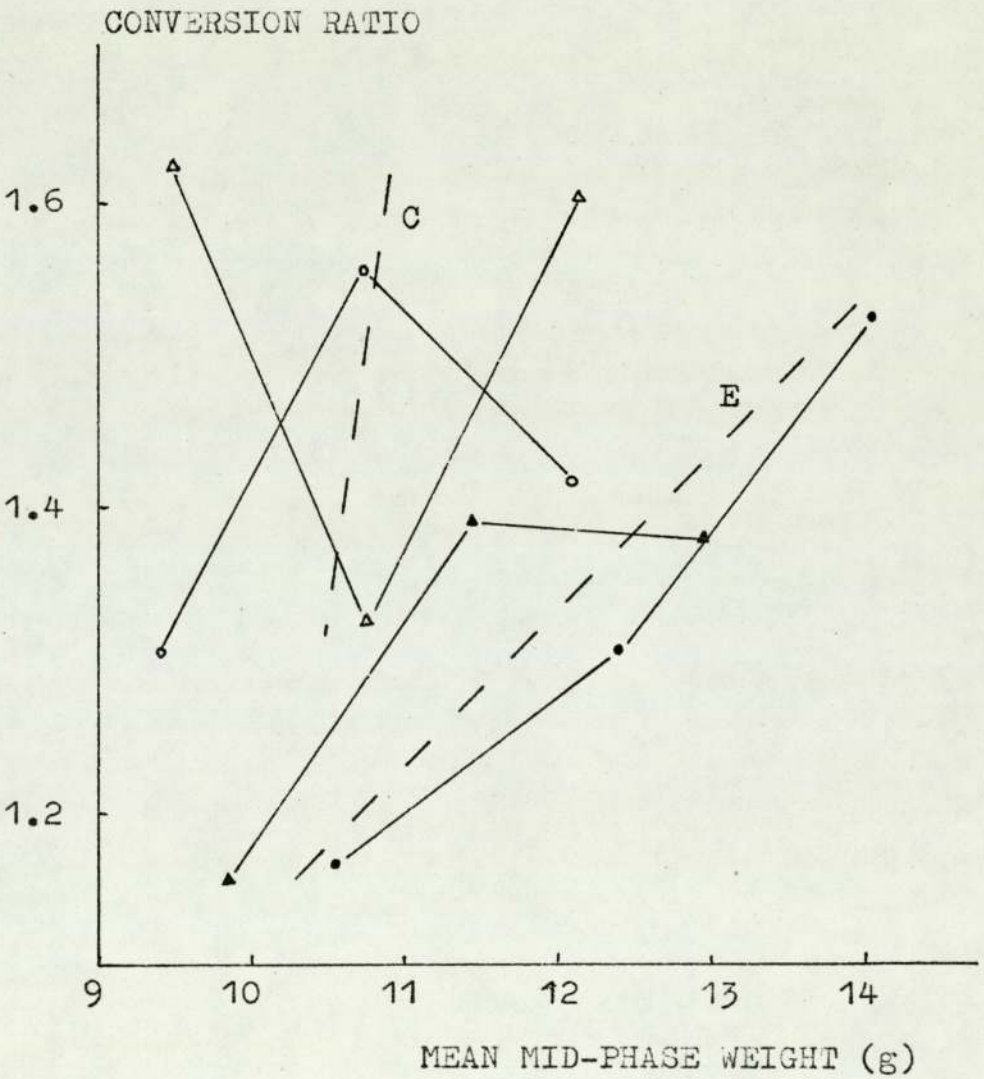


Graph 9.11 T01 appetite: 3-day means (plotted on middle day)



• = MT1 ▲ = MT2 ○ = MT3 △ = MT4

Graph 9.12 T01 conversion ratio by phase



• = MT1 ▲ = MT2 ○ = MT3 △ = MT4; C,E as in Graph 9.10

Graph 9.13 T01 conversion ratio by size

Analysis of growth in TO2

9.58 TO2 growth data were analysed similarly to those of TO1; correlation coefficients for the three weight data presentations are shown in Table 9.14a, and regression data in Table 9.14b. In this case, the log transformation gave the best average fit, and the regression graph of log mean weight against time is plotted in Graph 9.14, the data having been standardised as in Graph 9.7. The only significant slope difference found was between the two experimental tanks. Regression and slope analysis on the mean and cube root weight data showed: no significant differences, and a lower level of significance, respectively, between MT1 and 2. MT4 mean weights showed a correlation coefficient of 0.9999 (± unity) indicating perfect straight line growth (whilst MT1 and 2 displayed accelerating growth).

9.59 Percent weight increase is illustrated in Graph 9.15. Overall values show the control tank between the two experimentals, and the phase values show an interesting difference in pattern. MT4 follows the trend of TO1 results in clearly declining with time, but the MT1 and 2 pattern is one of stability or increase, indicating a possible rise in growth rate over the experimental period. When these results are plotted against phase mean weight, this difference is clearly repeated (Graph 9.16), reinforcing the suggestion of increasing growth rates in experimental tanks.

Analysis of appetite in TO2

9.60 Food consumption data were transformed to appetite. A plot of 3-day span moving average for appetite (Graph 9.17) shows an overall slight increase in all tanks, and a significance test between the mean values showed a significant difference between MT1 and MT4, but not between MT2 and MT4 (Table 9.15). Thus it was not through lack of appetite that MT4 showed poorer growth patterns than the experimental tanks.

9.61 Gross conversion ratio was higher (worse) overall in MT4, and the phase values (Graph 9.18) show this tank's conversion

Table 9.14a Correlation coefficients for TO2 weight and time data

	WEIGHT DATA TRANSFORMATION		
MT	MEAN	log MEAN	$\sqrt[3]{\text{MEAN}}$
1	0.9963	0.9996	0.9989
2	0.9955	0.9995	0.9987
4	0.9999	0.9973	0.9986
MEAN	0.99723	0.99880	0.99873

All values are highly significant ($P<0.001$, df = 3).

Table 9.14b Regression data, TO2: log mean weight and time

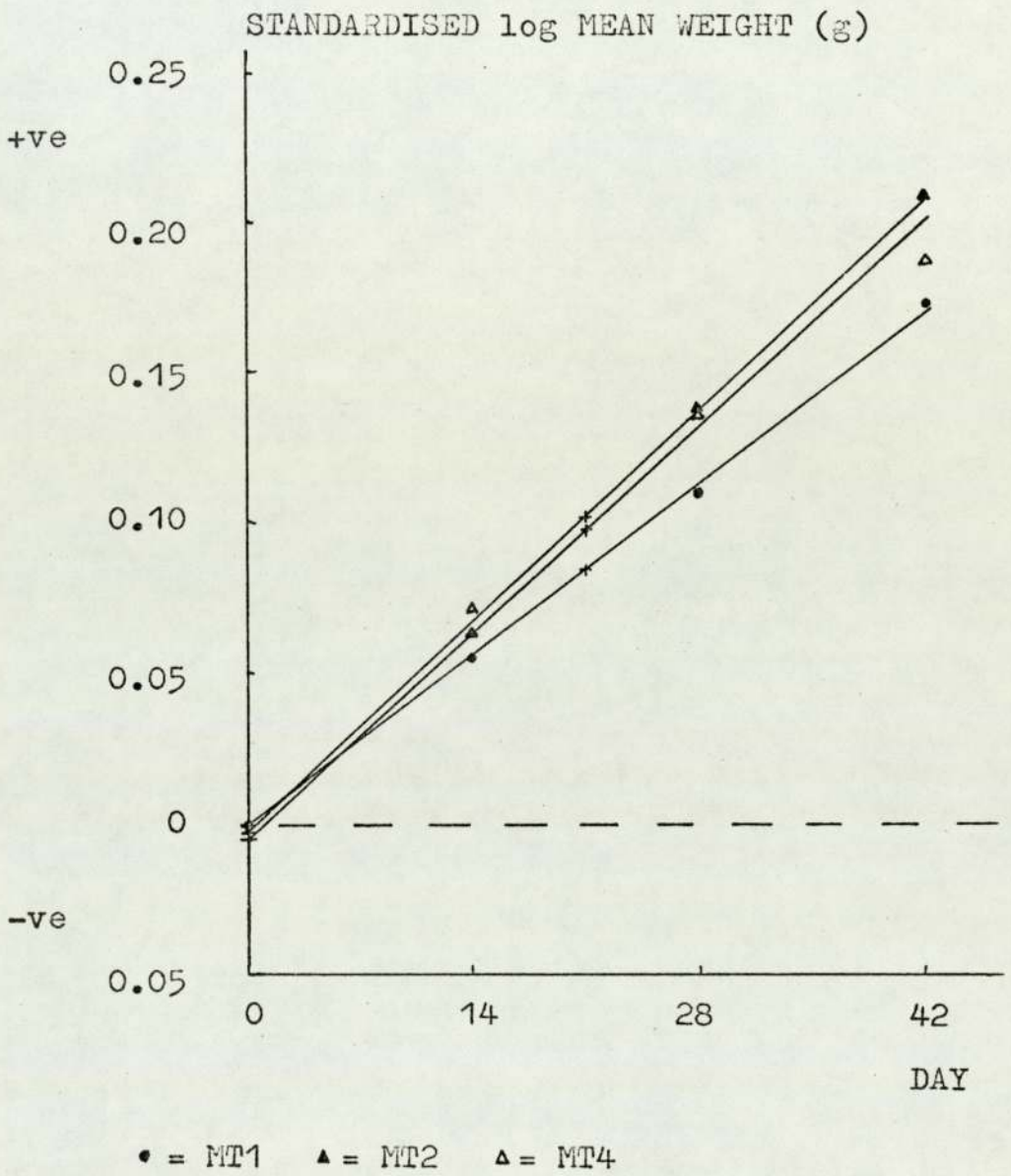
	MT		
QUANTITY	1	2	4
R	0.9996	0.9995	0.9973
a	1.0389	1.0397	1.0288
b	0.0041	0.0050	0.0045
t	6.7316	2.1225	
P	$0.001 \longrightarrow 0.01$ **	>0.05 NS	
	$t = 1.4570$; $P>0.05$ NS		

Notes:-
a) Notes (a) and (b) from Table 9.12b also apply here.
b) Overall finding:- MT1 < 4 < 2 (1 ≪ 2)

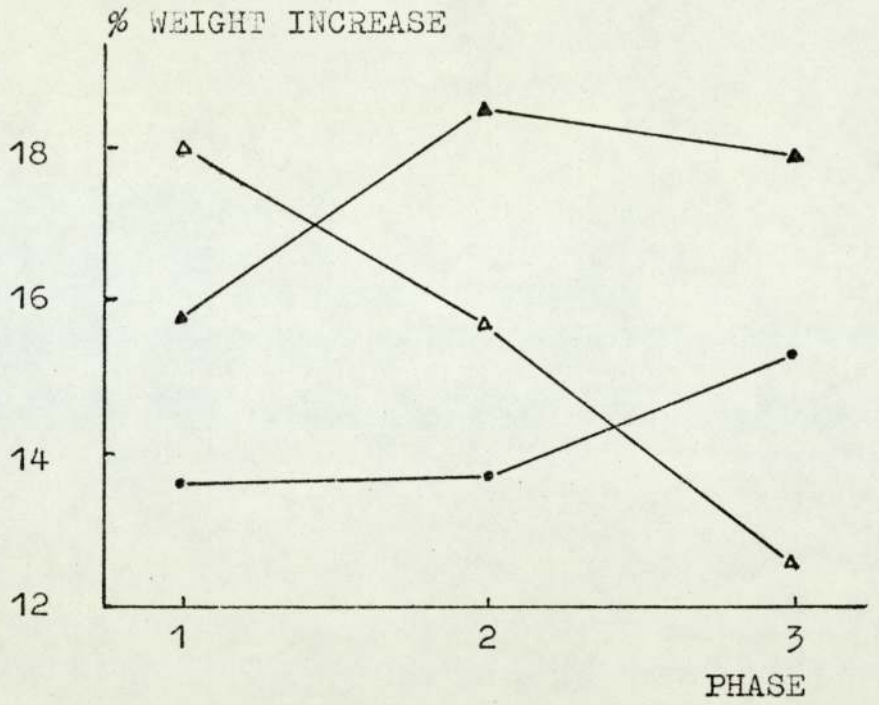
Table 9.15 Appetite in TO2

	MT		
QUANTITY	1	2	4
MEAN	1.2241	1.3581	1.3785
SEM	0.0599	0.0553	0.0472
t	1.6429	0.2808	
P	>0.05 NS	>0.05 NS	
	$t = 2.0235$; $0.01<P<0.05$ *		

(df = $n_1+n_2-2 = 82$)
Notes:-
a) Values in % of body weight food consumed per day.
b) t and P as in Table 9.14b (df = 82)

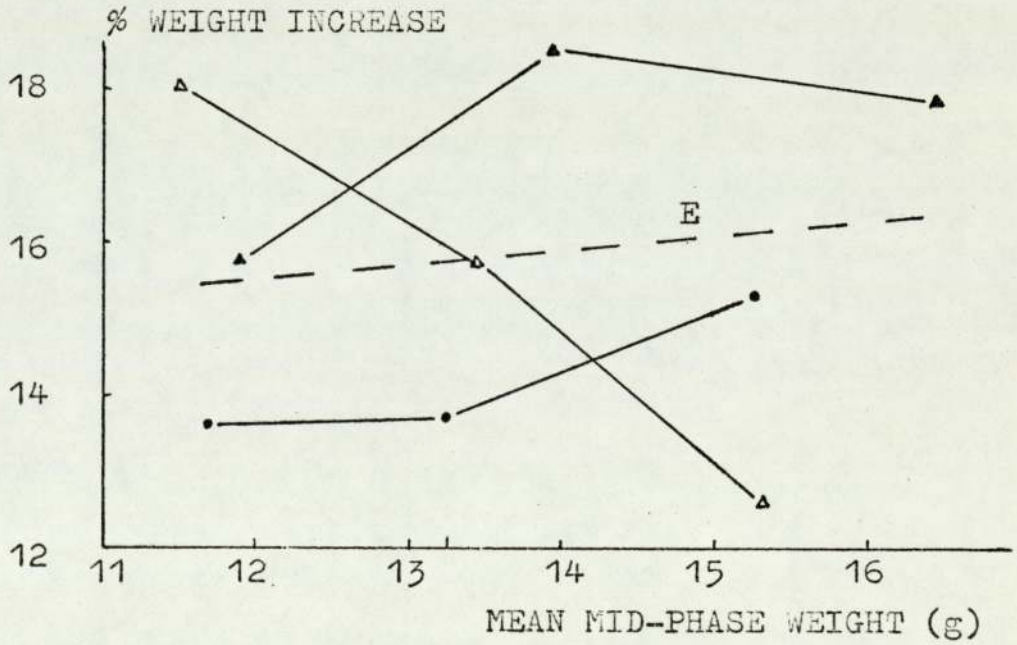


Graph 9.14 Regression of standardised log mean weight with time for T02



Graph 9.15 TO2 % weight increases by phase

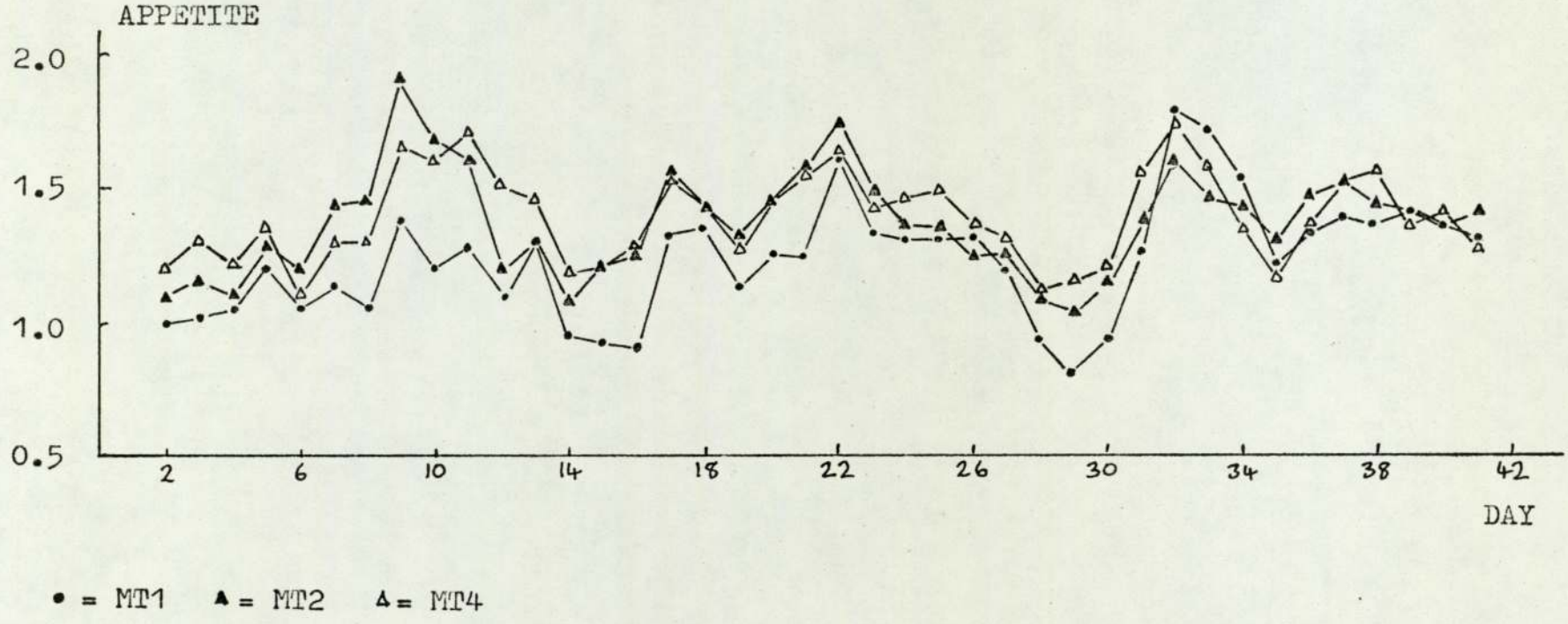
● = MT1 ▲ = MT2 △ = MT4



Average shows increasing growth rate in experimentals compared to fall in control; in general agreement with Graph 9.10

E as in Graph 9.10

Graph 9.16 TO2 % weight increase by size



Graph 9.17 T02 appetite: 3-day means (plotted on middle day)

steadily worsening with time while those of experimental tanks were maintained. This pattern agrees with that of Graph 9.15, and shows that, although having a good appetite, fish in MT4 had a poorer conversion of food. This is emphasised by the plot of conversion against mid-phase mean weight (Graph 9.19); weight for weight, experimental tank fish converted better than controls, in the last two phases of the experiment. In all these graphs, the data for phase 1 show MT4 as superior to the experimentals, but the situation is fully reversed by the end of the experiment.

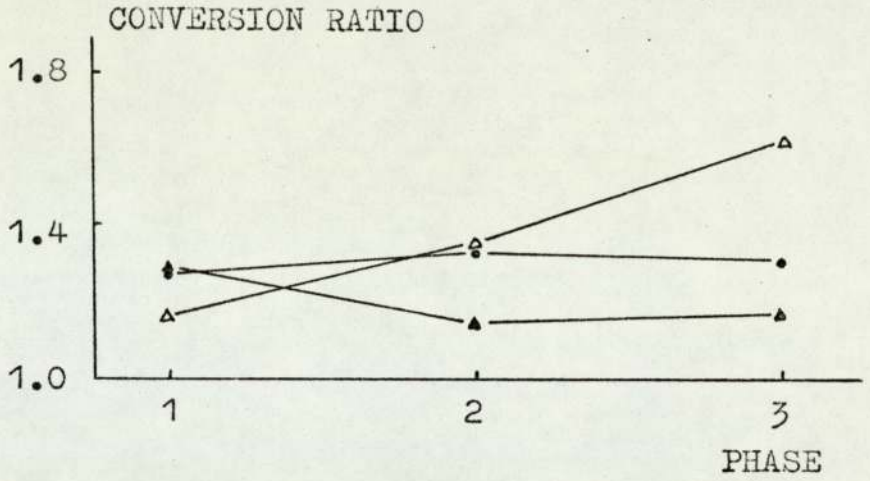
9.62 Table 9.16 gives a full summary of all T-series conditions and measured effects results.

DISCUSSION

9.63 TO1 and TO2 results give rise to several points for comment, of which the most important must be the equivocal results in growth rate differences; it is noteworthy that the log and cube root transformations of TO1 data did not show significant differences. In general growth effects have not been conclusively shown to be different between controls and fish exposed to SREF. In particular, no negative effect has been shown; and a positive effect is possible.

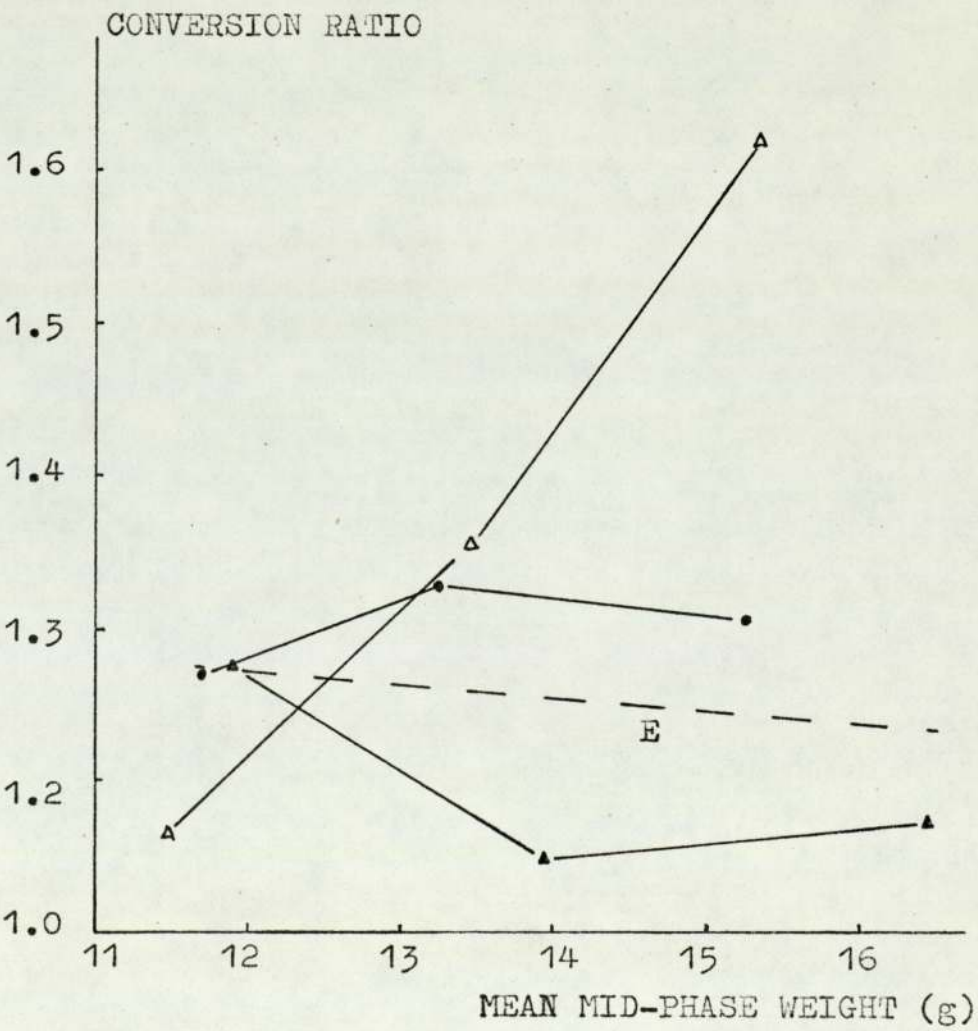
9.64 Differences between conditions in the experiments were mostly insignificant, or did not follow an "experimental v. control" pattern. Temperature must be regarded with some suspicion, as it was demonstrably, though slightly, different between experimentals and controls, and any future work would be better accomplished at a definite fixed temperature. Significant growth differences in TO2 agree with the pattern for volume, and this may explain why there is a significant difference between the two experimental tanks.

9.65 There remain two aspects of SREF which might be responsible for detected differences between experimentals and controls: carbon dioxide and pH changes due to excretion. The latter



Graph 9.18 TO2 conversion ratio by phase

• = MT1 ▲ = MT2 △ = MT4



E as in Graph 9.10

Graph 9.19 TO2 conversion ratio by size

Table 9.16 Summary of TO1 and TO2 results

QUANTITY		TO1	TO2	
Measured effects	Slope of growth regression	4<3≪2<1 *	1<4<2	(1≪2 **)
	% weight increase	4<3<2<1	1<4<2	
	Conversion	2<1<3<4	2<1<4	
	Appetite	2<4<3<1	1<2<4	(1≪4 *)
	Haematocrit	4<3<2≪1 *	4<1<2	
	Oxygen uptake	-	N 1<4<2	
Conditions	Temperature	3<4<1<2	4<2<1	
	Volume	1<4<3≪2 **	1<4<2	(1≪2 **)
	Flow	1<2<4<3	1<2<4	
	Stocking	3<2<4≪1 ***	4<1<2	
	Loading	3<4<2<1	4<1<2	
	Loading factor	-	4<1<2	

Notes:-

a) 1, 2 (experimental) and 3, 4 (control) refer to tanks.

b) Magnitude signs show relationships between tanks for the quantity referred to.

c) Double magnitude sign shows statistically significant difference, followed by rating; underlining indicates no significant difference between the tanks underlined.

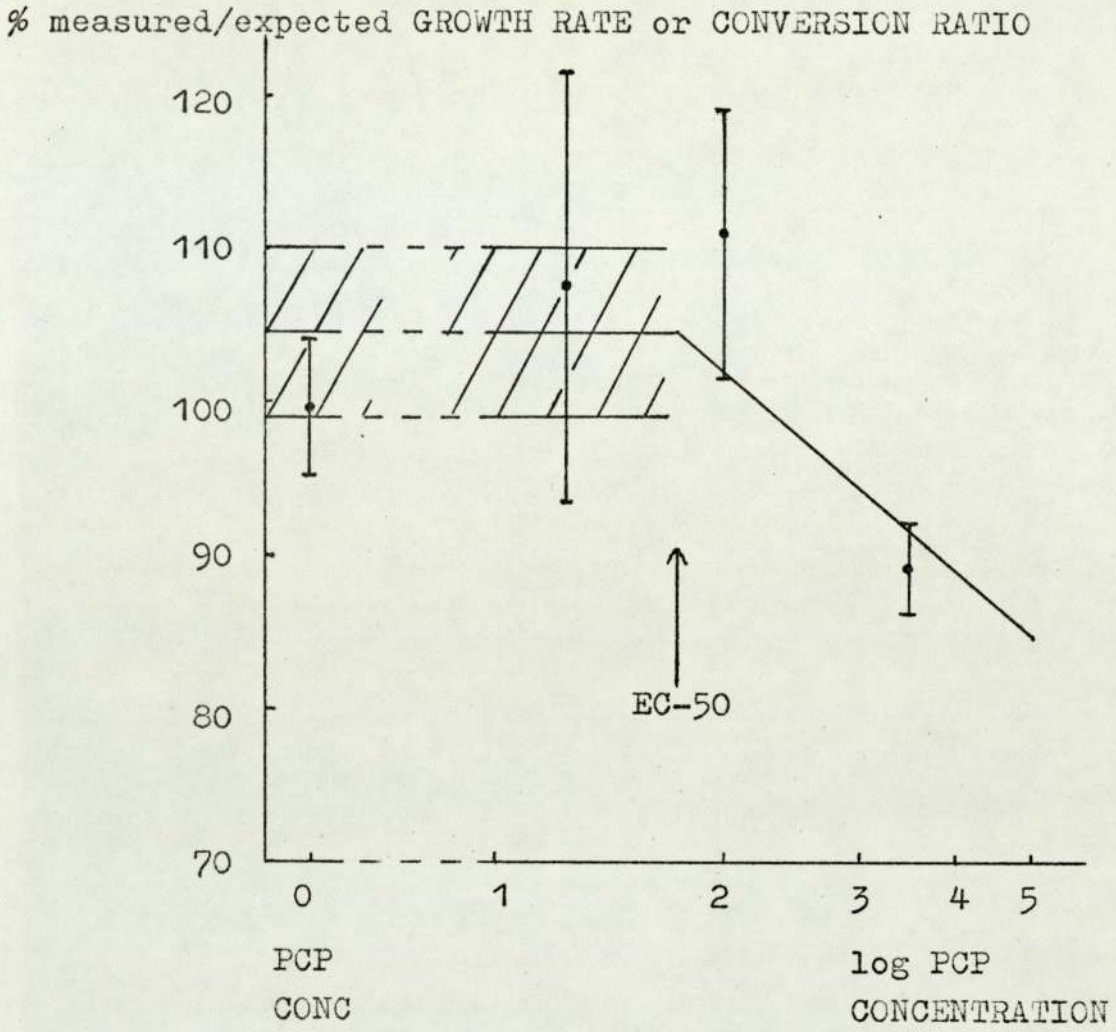
d) Temperature results are subject to proviso as in para.9.36.

e) N indicates not statistically tested.

depends to some extent on the former, but the thorough aeration that took place in the faecal traps probably rules out the former. A direct pH effect on growth rates seems unlikely, there being no evidence to date of such small pH shifts causing growth effects (in contrast to ammonia or excretory solutes), but future work would ideally be better undertaken under buffered conditions in order to remove this doubt.

9.65 Of the major measured effects only growth rate, in TO1, shows a significant difference between experimentals and controls, and this in an unexpected direction, i.e. experimental rates are significantly greater than controls, not less. Although TO2 does not statistically support this, the consideration of % weight increase and conversion ratios by phase shows a striking tendency, in both experiments, for SREF treated fish to be growing faster than controls of the same weight. Thus there is, contrary to expectations, some indication of a positive effect of SREF on growth patterns. In this respect it is interesting to note evidence from Sprague (1971) (quoting Pickering [1968]), that mild exposures to zinc may have a growth-stimulating effect; and further, from Webb & Brett (1973) the observation that this is a "not uncommon feature", which they show again in the context of sodium pentachlorophenate (PCP). Webb & Brett's figure is discussed in Appendix D; here it is presented (Fig.9.8) in order to point out that at concentrations of PCP below the EC-50, the ratio of measured:expected growth rate (treated:control) was definitely found to be greater than 100%. The same effect was found for conversion ratio: i.e. fish at "no stress" concentrations of the chemical (<EC-50) were in fact slightly stimulated in their conversion efficiency.

9.67 In the current studies, this slight growth stimulation could agree with the growth rate results of TO1 and the growth pattern differences of TO2, given the limited information available. This would indicate that SREF, as measured at levels of about 0.36 and 0.70 mg ℓ^{-1} TOA, is below the EC-50, and may be having a slight growth stimulation effect.



Shaded area shows ± 2 SEM about the zero response level, which is slightly elevated above 100% to about 104%

Figure 9.8 Elevation of zero response level for PCP effect on growth rate or conversion ratio (modified from Webb & Brett (1973))

9.68 In support of this, both Smith (1972) and Schulze-Wiehenbrauck (1974) were able to note definite positive factors due to ammonia exposure. Smith notes that fish from a treated tank which were subsequently allowed a 48-hour "rinse" in fresh water showed a 22% increase in performance capability, which raised them well above controls. Schulze-Wiehenbrauck (1974), besides noting that ammonia lessened weighing-stress in his fish, also found that a distinct increase in resistance to lethal UIA levels was achieved by culture in mild concentrations (130-170 $\mu g\ \ell^{-1}$). This agrees with Lloyd & Orr's (1969) observations of acclimation to low UIA, and it may be that the processes of acclimation and of slight growth stimulation could be different physiological expressions of the same underlying effect.

9.69 Brett (1974) has recently discussed growth rates of Canadian salmon, and has noted that size and age may be quite distinct in their influences on growth, so that fish weight alone may not determine growth rate. It has frequently been noted that the growth rate slows down with age, and similarly the conversion efficiency gradually falls: for the purposes of these experiments, all fish have been arbitrarily assumed to be on the same portions of these respective graphs since the period involved was short. Brett's observation suggests that it is truly a function of <u>age</u> in determining growth patterns, and not just weight. In this respect the results of TO1 and TO2 could be interpreted as a "rejuvenation" effect, where fish of the same weight are displaying greater growth capabilities when treated with SREF than when not. (The effect is not due to age differences since all fish were sampled from a common population.) If their resistance to lethal toxicity is also thereby increased, this would agree with Schulze-Wiehenbrauck's observations of greater resistance in small trout than in large ones, and of less resistance in fish with high condition factors (condition factor usually increases with age and growth).

9.70 Schulze-Wiehenbrauck (1974) noted that while food consumption fell under the influence of UIA, no deterioration of conversion was caused, and his loss in growth rate of fish exposed to 130-170 $\mu g\, l^{-1}$ UIA can thus be explained in terms of loss of appetite. This finding disagrees with Sprague's (1971) contention that conversion may be a more sensitive indicator than growth rate: it would seem that this is unlikely, since growth rate is primarily a product of the combination of appetite and conversion rate. Webb & Brett's (1973) results show that conversion ratios reacted similarly in response to PCP, but their fish were fed a standard reduced ration. In the current T-series experiments, appetite cannot be said to provide any extra information, but it can be appreciated that in cases where conversion and growth rate indications differ greatly, appetite may well provide an explanation. Thus the concept of appetite as a response is worthy of further assessment when trying to draw up tolerance guidelines: differences in appetite may cause losses in growth rate even when conversion is not affected, and either of these two quantities may be more sensitive in a given situation. An EC-50 for loss of appetite would be an extremely useful quantity for a fish farmer.

9.71 In conclusion, it can be said that on this evidence neither at 0.36 $mg\, l^{-1}$ nor at 0.70 $mg\, l^{-1}$ TOA does SREF exceed its EC-50 for reduction of rainbow trout growth. If fish excretory products are truly responsible for growth losses, two further steps are clearly necessary; (a) the establishing of the EC-50 for SREF, and (b) the definite identification of the exact agent involved (in particular, it would be useful to find out the effects of NH_4^+ alone). Step (b) might provide a basis for simpler filtration systems for recycled water in the future (see Chapter 10).

PART 5

EXCRETORY PROBLEMS

IN FISH CULTURE

10. EXCRETORY PROBLEMS IN CIRCULATING SYSTEMS

DISCUSSION

10.1 This study has been conducted with one specific type of fish-rearing situation, i.e. juvenile rainbow trout in open system circular tanks at fairly high loading. It is therefore important to emphasise that it is not admissible to generalise from this work to other types of situation unless there is appropriate supporting experimental evidence. Further, direct extrapolation to other magnitudes of the same situation (e.g. commercial circular rearing tanks) is also inadvisable. The work is thus presented as a line of approach to the problems rather than as an authoritative statement.

10.2 This said, it would be equally unacceptable to withhold speculation on interpretations of the methods used and the data collected.

Excretory productivity

10.3 The heart of the problem of describing excretory productivity in quantitative terms in a multivariable situation lies in an assessment of the factors determining excretory rate. This study has not been concerned with the internal biochemistry and physiology of excretion as a response to changes within the organism. The approach has been to treat the organism as a 'black box', and to measure external inputs and outputs. The belief underlying this is that with methods available to date, any attempt at internal (intra-organism) assessment of effects would automatically lead to errors simply because of the stresses and disturbance caused by the unavoidable handling, operating techniques, monitoring requirements, etc. involved. In the context of excretion, any such departure from normal culture conditions might provide spurious data. This might seem to invalidate all attempts at intra-organism assessment, but recent advances in miniaturisation of electronic equipment do allow one to conceive of instruments sufficiently small to measure internal parameters with minimal disturbance, the

disturbance limited to the operations required for instrument insertion (which could be followed by recovery and re-adaptation periods). So far, advances in this direction have been made in the context of fish-tagging in ecological work on fish movements (Holliday 1975), but there remains considerable technical progress to be made before devices can be developed which are sufficiently small, disturbance-free and multiple-sensing as to allow adequate internal monitoring for assessment of environmental effects on excretion.

10.4 Given that an extra-organism assessment is thus necessitated, a series of central problems arise relating to measurement of excretory rate:

(a) Is it possible to identify, and then measure, all input variables affecting the system?

(b) Is is possible to measure true excretory rate?

(c) Can the measured excretory rate be satisfactorily and meaningfully related to the input variables, so that future prediction is possible, this providing a firmer basis for fish culture techniques?

10.5 With respect to (a) above, it seems on balance that it is not possible to state that all possible input variables have been identified. Were that so, it would seem likely that (supposing they could be measured), it would be possible to describe and predict excretion in terms of them. With this in mind, the approach of this current work has been to aim at the following objectives: (i) to measure readily identifiable input variables undergoing normal culture-situation fluctuations, (ii) to build a predictive model, and (iii) thus to define the magnitude of effect of the unidentified input variables; i.e. to try to answer question (c), and thereby throw light on question (a). In stating (Chapter 7) that the models generated explain the data "well", the implication is that compared to the input variables measured, the unidentified ones have a small effect on excretion. This may only be true

for the current study - and even then only for populations greater than 100, or for fish of the size ranges studied (in particular, very small fish (<5g) may behave differently, having extremely different feed rates). Clearly, more information is required, but given the limits of applicability, the approach to the problem (i.e. fundamentally a multivariate system attempting to mimic the working fish-culture operation) seems to be justified, and might (in improved form) be worthy of repetition in future studies.

10.6 However, the fact that unexplained variability has been shown to exist, as expected previously, leads to a consideration of whether possible components of that variability can ever be fully identified, or even then whether they are measurable.

10.7 Question (b) above has been partly dealt with in Chapter 5; it is clear that the actual quantity measured is TOA (a concentration), which is related to apparent SER (derived by calculation) thus:-

$$SER = \frac{\text{flow} \times \text{TOA}}{\text{fish mass}}$$

However, TOA itself is governed by a slightly different relationship, thus:-

$$TOA = \frac{TSR \times \text{fish mass}}{\text{flow}} \left(1 - e^{-\frac{\text{flow} \times t}{\text{volume}}}\right)$$

where TSR is the true specific excretory rate (see Chapter 5). The exponential function determines the difference between SER and TSR, and this function in turn will depend mainly on t (time) when flow and volume are steady. If the time required for equilibration is appreciable (see Table 5.2), then TSR may well change before the equilibration has occurred, hence TSR and SER will not be similar. In practice SER is the only useful quantity, but the reasons for TSR change, and the rates of change due to them, are mostly speculation. These reasons would thus conveniently fill the role of unidentified variables mentioned in para.10.6. Some possible reasons are listed below:-

a) diurnal cycles (due to biological clock and/or outside stimulation such as temperature, photoperiod)
b) seasonal cycles (due to similar effects)
c) age (see growth effects quoted from Brett in Chapter 9)
d) feedback from output variables
e) "stress"
f) social effects (e.g. hierarchy).

Clearly, techniques for measuring such possible variables might be lengthy and complex to develop and validate, even supposing that the variable can be quantitatively pinned down (difficult with stress and social effects).

10.8 The possible feedback effect quoted above underlines the fact that ammonia is not the only aspect of excretion (and thus not the only output variable). Other output variables must include production of carbon dioxide and urea (and other excretory compounds), oxygen depletion,and pH shift, besides quantities like production of heat and solid waste, activity, growth and such difficult possibilities as pheromones, if they exist in this situation. It is possible, and even probable, that many of these quantities are related, especially those measurable as chemical effects, but the constancy of the relationships is not necessarily proven. In this respect, the assumption of the current work, that urea is a relatively constant, low proportion of excretory production, would certainly invite thorough investigation, as would the status of CO_2 production.

10.9 In summary, it can be said that the methods used in the current work allow a suitable line of approach to the problem of predicting ammonia excretion in the fish-culture situation used; that improvement and refinement of methods both to identify and to measure input variables would allow a better framework for prediction; and that this kind of multivariate approach would be worthy of application to other fish-culture situations, especially commercial-size circulating systems. Thus a number of future experiments could be envisaged, as detailed later.

10.10 In essence, the ideal approach suggested is (a) to set up a functioning full-size fish culture circulating system, (b) to monitor constantly over an extended period all identifiable and measurable input and output variables, and (c) to perform suitably sophisticated multivariate analysis on the data so produced, in order to generate the shapes and limits of reliable predictive functions for excretory outputs. The methodological problems of this are substantial but not insoluble: suitable equipment for constantly monitoring, multiplexing, logging and analysing a number of data sources (especially if served by electrode probe-type sensors) is already available, although the co-operation of statistics, electronics and programming experts would probably be advisable in building such a system.

10.11 One major experimental problem is the monitoring of weight and length of fish. Theoretically, these quantities should be measured continuously, accurately, and without fish disturbance. In practice, at present, the best that can be done is to disturb the fish periodically by removal for weighing and measuring, and to estimate (interpolate) intermediate values by some method such as that described in Chapter 7. This should undoubtedly be seen as the weakest part of the method used, introducing a further level of error into the system in addition to that of actual measurements. It does seem conceivable that there should exist some method of accurately determining length and mass of moving objects underwater by remote-sensing means, and if this is so it ought to be possible to design such a system to operate at tank scale on a population of fish. If this were possible, the greatest operational problem of scientific fish husbandry would be removed.

10.12 Of the two final models for excretion prediction derived in Chapter 7 (OM1 model (3) and OM2 model (4)), each has interesting features, briefly summarised in the statement: that model (3) (OM1) may be more practically (or commercially) useful in

that prediction is based on envisaging the tank as one large ammonia-producing machine, with individual fish differences unaccounted for (or, strictly averaged out) and with independent variables readily measurable; whereas model (4) (OM2) is perhaps more scientifically interesting in that it includes components which reflect individual differences and possible social effects, and this provides plenty of scope for further definition, elucidation and measurement of contributing input variables. Both types of model would therefore seem worthy of trial application in future experiments.

10.13 In either case, the reduction of the multivariate situation to one descriptive relationship means that it is possible for a fish culturist to fit his own particular values into the equation (e.g. using values of temperature, number, mass and volume for model (4)), and to compute a predicted excretory rate. The process could be made operationally simpler by preparation of suitable tables or graphs for reference, or by a specially prepared slide-rule embodying the equation.

10.14 The kind of constant-monitoring, multivariate approach suggested could provide the basis for a further step: the elucidation of cumulative effects in a closed system. This would be an alternative way, to that used in the current work, of assessing tolerance to excretory products, and would have the advantage of allowing several important input variables (oxygen, pH, temperature) to be held at optimum levels.

10.15 In the context of closed system fish culture, a reliable model for predicting excretion is a fundamental requirement, since characterisation of the effluent governs the treatment facilities that are needed, once a reliable criterion of tolerance/toxicity is available. Current processes of closed system design (e.g. Speece 1973, Liao & Mayo 1972, 1974) thus stand or fall by the accuracy of the expected excretory output for the particular situation under consideration. It is felt

that current knowledge of micro-environmental effects on excretion in fish-culture systems, especially intensive ones, is not as comprehensive as it might be, and so current estimates of excretory productivity are open to doubt; this is not to say that the rules-of-thumb used so far may not turn out to be valid, but simply that they need to be either shown to be valid authoritatively, or discredited and replaced by better-substantiated ones.

10.16 In a wider context still, a reliable model of excretory output would be particularly useful in allowing a fish-farmer to design his operations (whether open- or closed-system) so as to meet local water quality requirements in the final effluent discharged. This consideration becomes more important as effluent control regulations become tighter and quality standards higher, especially when the ability to meet such standards may involve substantial land area, and/or equipment and cost.

Tolerance to dissolved excretory products

10.17 It is important to stress that tolerance as discussed here was only investigated in limited circumstances, as preliminary work aimed at finding an experimental approach to the problem of setting a reliable water quality guide for treatment of fish effluent for recycling. As such, the work used only a limited size range of fish, a limited temperature range, one type of effluent (SREF of soft-water quality with low ammonia dissociation), and only two strengths of SREF. Thus the quantitative results obtained apply only in this situation, and cannot be unduly extrapolated.

10.18 The approach used for this work hinges on the search for a water quality criterion based on tolerance rather than on toxicity; i.e. the objective is a concentration up to which the excretory pollutant can be allowed to accumulate before growth or conversion are affected, not a concentration down to which pollutant must be reduced before physiological effects

are negligible. Secondly, the work has assumed that SREF is worthy of investigation as one total effect. Thus the effluent used to dose experimental tanks was essentially uncharacterised in the terms of any excretion productivity model. It was treated as a homogeneous effector, whose strength could be gauged by measuring TOA as an indicator: thus independent effects due to UIA, TOA, urea or any other component were not separated out. Given however, the same assumptions as were made for the excretory productivity work i.e. that other dissolved excretion products are in a relatively constant, low proportion to ammonia, it is felt that the information generated could ideally be used, in combination with an excretion prediction model, to determine the treatment efficiency required of a filter unit.

10.19 Implied in this is the assumption that some measure of SREF persistence in a closed system might not be incompatible with maintenance of growth and conversion rates (although accumulation, as opposed to persistence, would be a different matter).

10.20 The system of SREF dosage used ruled out possible interference from feedback or hierarchy effects, although interference from pheromones cannot be ruled out (neither can they be positively shown to be present as distinct from excretory output).

10.21 Within these experimental limits, it can be said that both ranges of SREF strength tested were below the EC-50 for growth rate or conversion effect; as the data also tend to agree with previous indications of a slight positive growth effect at $<$EC-50 levels, there may be two aspects of advantage to a less stringent water-treatment policy: (a) possible lower cost for a potential filter, and (b) possible slight beneficial effect on growth.

10.22 Experimentally, the tolerance experiments would benefit in future application from the buffering of temperature and pH:

in practice, this need not necessarily mean constant values, only that at any given instant the same value was true for both experimental and control tanks. An efficient heat exchanger between the two lines might be sufficient to absorb small temperature differences; buffering to a constant value would probably be the simplest solution in the case of pH.

10.23 The chemical nature of recycled effluent is determined by two factors, (a) the chemical nature of the water independent of fish, but affected by such things as deliberate environmental control measures, chemical effects of filtration, etc.; and (b) the excreted material of the fish. Thus recycled effluent is an extremely variable quantity. Hence, any meaningful tests of excretion tolerance in fish-culture situations must take into account the original chemical nature of the water independent of excretion effects. The most reliable way of doing this is to use control and experimental tanks supplied by the same water and to standardise EC-50 measurements individually, for the characteristic water supply.

10.24 This means that in order to establish meaningful local quality standards, the following steps would be logical:-

(a) a series of experimental and control tests to determine an EC-50 under given acclimation conditions;
(b) assuming that the LC-50/EC-50 relationship is roughly constant (see Appendix C), a series of LC-50 tests to allow estimation of EC-50 for other conditions.

10.25 In the long run, it is desirable to attempt to investigate the tolerance/toxicity properties of all excretory products individually, and much work has already been done on ammonia as detailed in Chapter 8. Nevertheless there is still a lack of firm evidence on EC-50 levels for UIA, TOA, urea and other excretory compounds.

10.26 However, it must be remembered that in a fish effluent these agents do not act alone, and ultimately individual recycled effluents are the real criteria, since they represent the

sum of individual effects and interactions. It is rash to assume that growth processes in fish will be affected by recycled effluent in the same way that they are by straightforward ammonia solutions, whether the ammonia is present as TOA or UIA: in other words, interaction effects must be catered for. (Further, if biological filtration is envisaged as a water treatment method in closed fish systems, then the effect of fish effluent on filters may well be different from that of ammonia solutions, due to interaction of simple nitrogenous and other organic compounds, e.g. see Bruce, Merkens & Haynes [1975]).

SUGGESTED FUTURE EXPERIMENTAL POSSIBILITIES

10.27 Excretory productivity

1. The complete instrumentation, by electronic sensing devices with suitable measurement, data logging and analytical equipment, of experimental operating fish-culture tanks in order to continually monitor as many input and output variables of the intra-tank environment as possible, including at least oxygen, pH, temperature, ammonia, volume, flow, illumination, photoperiod, feeding and time. The analysis of such data, in company with all other measurable variables (mass, length, number, chemical quantities which require a sampling approach) in order to build up as complete a picture as possible of the situation in the operational fish-culture system; thus to provide the basis for a truly reliable predictive model for excretory effects due to ammonia, urea and any other excretory compounds.

2. The technical development of remote, accurate, non-stressful measurement methods for estimating at frequent intervals the individual mass and length, and the number, of fish in a culture tank: thus allowing study of other aspects of the situation (environment, growth) without interference.

10.28 Tolerance to recycled water

3. The establishing of EC-50's for loss of growth rate and/or conversion and/or appetite according to strength of recycled

effluent (as measured by an indicator substance such as TOA), for a wide variety of appropriate water supplies; thus to derive working limits for operational closed systems.

4. The investigation of other pertinent criteria of sub-lethal effect e.g. stamina, disease resistance, healthy appearance, as measured by suitably validated rating scales, and the establishment of appropriate EC-50 criteria.

5. The elucidation of the effects of sublethal concentrations of individual components of recycled water e.g. TOA, UIA, urea.

6. The investigation of possible growth or conversion enhancement effects at non-lethal sub-EC-50 concentrations of effluents or effluent components; and the clarification of their modes of action if confirmed.

7. The further analysis of, and formulation of a definitive theoretical basis for, the concept of stress as applicable in fish-culture environments; the investigation of both internal (e.g. endocrinological) and external (e.g. respiratory) aspects of stress in this context.

10.29 General

8. The advancement of intra-organism and extra-organism multiple-channel assessments of environmental effects, including stress situations.

10.30 Overall

9. The truly efficient design of the simplest and cheapest form of closed rearing system which will allow optimal productivity of cultured fish.

NOTE - All of these suggestions have been conceived in the context of rainbow trout work, but clearly all apply equally to any species which is potentially capable of closed system culture, including marine and warm-water types, although these may have additional environmental problems providing further scope for investigation.

CONCLUSION

10.31 This study has attempted to formulate experimental

approaches to two fundamental aspects of fish-culture in circulating systems: the gauging of excretory productivity on an open system, and the investigation of the tolerance of fish to recycled effluent in a closed system. Both are fundamental requirements for informing the efficient design of the treatment equipment so often required in order to recycle safely. During the pursuit of these overall objectives, it has become clear that the fish-culture environment holds a dual challenge for investigators; firstly, because of its immediacy in humanitarian, technological and commercial application, which lends pragmatism and excitement to the endeavour; but secondly, less obviously and yet finally of equal weight to the scientific sense of the investigator, because of its complexity, scope for future co-operation between workers of different disciplines, and sense of being at the beginning of a new field, with much that seems worthy of investigation, and, in particular, the opportunities for new syntheses of information. Whether or not the opportunities are grasped depends on cost, technical advance, and the foresight and inter-disciplinary co-operation of decision-makers; but ultimately on having the will to do it.

APPENDICES

AND BIBLIOGRAPHY

A1. PILOT EXPERIMENT RESULTS

CONTENTS:-

Table A1.1 P01 results: TOA, temperature, pH, UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
2/9/72	1200	X04	20	17.0	7.07	0.075
		BCK	445	17.0	6.26	0.258
	1600	BCK	455	17.5	6.21	0.244
3/9	1150	X02	20	16.5	6.84	0.042
		X04	10	17.0	6.93	0.027
		BCK	450	17.0	6.21	0.233
	1600	X02	30	17.0	7.02	0.100
		X04	30	17.0	7.08	0.115
		BCK	440	17.0	6.26	0.255
4/9	0925	X01	30	17.5	6.81	0.064
		X02	20	17.0	6.88	0.048
		X04	10	16.5	6.87	0.023
		X06	10	16.5	6.88	0.023
	MEAN	X	20	16.9	6.93	0.057
	MEAN	BCK	447	17.1	6.23	0.247
4/9	1715	01	70	16.5	6.80	0.135
		02	70	17.0	6.84	0.154
		04	90	17.0	6.69	0.140
		06	110	17.0	6.55	0.124
5/9	1200	01	60	16.5	6.86	0.133
		02	60	16.5	6.84	0.127
		04	120	16.5	6.71	0.189
		06	110	16.5	6.62	0.141
	1600	01	70	16.5	6.81	0.139
		02	70	16.5	6.91	0.174
		04	120	16.5	6.72	0.193
		06	140	16.5	6.61	0.175
6/9	1000	01	89	16.5	6.80	0.172
		02	72	16.5	6.87	0.164
		04	132	16.5	6.65	0.181
		06	123	16.5	6.60	0.150
	1400	01	80	17.0	6.89	0.198
		02	76	17.0	6.96	0.220
		04	127	17.0	6.74	0.222
		06	132	17.0	6.77	0.247
	1800	01	36	17.0	6.95	0.102
		02	32	17.0	6.94	0.089
		04	87	17.0	6.84	0.192
		06	79	17.0	6.74	0.138
	2200	01	62	17.0	6.98	0.188
		02	47	17.0	7.02	0.156
		04	104	17.0	6.93	0.282
		06	104	17.0	6.81	0.214
7/9	0600	01	22	17.0	6.89	0.054
		02	22	17.0	6.93	0.060
		04	60	16.5	6.81	0.119
		06	67	16.5	6.75	0.116

Table A1.1 continued

DATE	TIME	SAMPLE	TOA	TEMP	pH	UIA
	1010	01	42	17.0	6.88	0.101
		02	31	17.0	6.87	0.073
		04	82	17.0	6.73	0.140
		06	101	17.0	6.66	0.147
	1400	01	50	17.5	6.89	0.128
		02	43	17.5	6.92	0.118
		04	122	17.5	6.71	0.207
		06	109	17.5	6.70	0.181
	1800	01	56	17.5	6.90	0.147
		02	50	17.5	6.96	0.151
		04	112	17.5	6.83	0.250
		06	84	17.5	6.80	0.175
	2200	01	62	17.0	6.96	0.180
		02	56	17.0	7.00	0.178
		04	122	17.0	6.86	0.281
		06	102	17.0	6.85	0.230
8/9	0600	01	40	16.0	6.91	0.096
		02	30	16.0	6.95	0.079
		04	79	16.0	6.82	0.154
		06	86	16.0	6.83	0.171
	1000	01	43	16.5	6.78	0.079
		02	30	16.0	6.92	0.073
		04	99	16.0	6.78	0.176
		06	92	16.0	6.69	0.133
	MEAN	01	53	16.8	6.88	0.131
	MEAN	02	44	16.8	6.92	0.124
	MEAN	04	102	16.8	6.77	0.200
	MEAN	06	100	16.8	6.71	0.171

Notes:-

a) BCK indicates background (holding tank) sample.

b) Sample references are the ST number (preceding X indicates pre-fish sample).

c) TOA and UIA means are calculated from the last 11 samples (Phase 2 of the experiment) except for ST 06 where the last 3 are ignored due to mortality at 2000, 7/9. The values used correspond to spectrophotometer absorbance readings (see Chapter 4).

Table A1.2 P01 results: flow, volume

	DATE	TIME	TANK (ST)			
			01	02	04	06
MEASURED	4/9	AM	33.5	33.0	32.5	32.5
FLOW:	5/9	1200	33.0	32.75	32.75	33.5
time(s)	6/9	1200	33.5	32.5	32.75	33.0
for	7/9	1200	34.0	33.0	32.0	33.0
500 ml	8/9	1200	33.5	33.0	33.5	32.5
	MEAN		33.50	32.85	32.70	32.90
MEAN FLOW	(ℓ min^{-1})		0.896	0.913	0.917	0.912
VOLUME	(ℓ)		2.3	8.2	8.6	2.6

P01 mortality

One fish was lost (no apparent cause) at 2000 on 7/9/72 from ST 06. All discussion of weight, stocking and loading conditions in Chapter 6 is on the basis of the fish weight prior to this loss (i.e. final tank weight plus weight of dead fish).

Table A1.3 PO2 results: TOA, temperature, pH UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ \ell^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ \ell^{-1}$)
9/1/73	1200	XO9	46	8.8	7.21	0.126
		X10	35	8.8	7.25	0.105
		X11	10	8.8	7.17	0.025
		X12	18	8.8	7.19	0.047
		BCK	410	8.0	6.44	0.180
	1600	XO9	21	-	-	-
		X10	18	-	-	-
		X11	28	-	-	-
		X12	24	-	-	-
		BCK	640	-	-	-
10/1	1000	XO9	46	8.8	7.21	0.126
		X10	36	8.8	7.28	0.116
		X11	37	8.9	7.19	0.098
		X12	36	8.8	7.20	0.097
		BCK	1150	8.0	6.50	0.579
	MEAN	X	30	8.8	7.21	0.092
	MEAN	BCK	733	8.0	6.47	0.379
10/1	2200	09	100	8.9	6.51	0.055
		10	76	8.9	6.57	0.048
		11	135	8.9	6.42	0.061
		12	109	8.9	6.33	0.040
		IS	20	8.8	7.29	0.066
11/1	0200	09	66	8.9	6.63	0.048
		10	84	8.9	6.68	0.069
		11	94	8.9	6.54	0.056
		12	87	8.9	6.47	0.044
		IS	15	8.8	7.12	0.033
	0600	09	35	8.8	6.63	0.025
		10	34	8.8	6.70	0.029
		11	56	8.8	6.49	0.029
		12	77	8.8	6.52	0.043
		IS	24	8.8	7.18	0.062
	1000	09	37	8.7	6.64	0.027
		10	34	8.7	6.50	0.018
		11	40	8.7	6.46	0.019
		12	57	8.7	6.33	0.020
		IS	-	8.6	7.10	-
	1400	09	92	8.6	6.73	0.082
		10	76	8.6	6.67	0.059
		11	145	8.6	6.36	0.055
		12	75	8.6	6.42	0.033
		IS	23	8.5	7.15	0.054
	1800	09	96	8.6	6.72	0.084
		10	54	8.6	6.75	0.051
		11	127	8.6	6.57	0.079
		12	91	8.6	6.40	0.038
		IS	26	8.5	7.12	0.057

Table A1.3 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
12/1	1000	09	35	8.6	6.83	0.040
		10	46	8.6	6.73	0.041
		11	65	8.6	6.62	0.045
		12	56	8.6	6.48	0.028
		IS	20	8.3	7.29	0.063
	1400	09	76	8.4	6.43	0.034
		10	72	8.4	6.57	0.044
		11	96	8.4	6.36	0.036
		12	83	8.4	6.31	0.028
		IS	27	7.9	7.21	0.069
	1800	09	31	8.8	6.78	0.032
		10	30	8.8	6.71	0.026
		11	49	8.8	6.62	0.035
		12	42	8.8	6.65	0.032
		IS	15	8.2	7.19	0.037
	2200	09	36	8.9	6.87	0.046
		10	41	8.9	6.88	0.053
		11	59	8.9	6.64	0.044
		12	57	8.9	6.79	0.051
		IS	21	8.4	7.18	0.052
13/1	0200	09	38	8.5	6.96	0.057
		10	36	8.5	6.87	0.044
		11	54	8.5	6.74	0.049
		12	60	8.5	6.71	0.051
		IS	19	8.4	7.20	0.049
	0600	09	34	8.5	6.92	0.047
		10	34	8.5	6.83	0.038
		11	67	8.5	6.81	0.072
		12	103	8.5	6.77	0.100
		IS	10	8.4	7.17	0.024
	1000	09	42	9.0	6.95	0.064
		10	55	9.0	6.93	0.081
		11	109	9.0	6.51	0.061
		12	82	9.0	6.74	0.078
		IS	-	8.2	7.25	-
	1400	09	75	9.0	6.48	0.039
		10	70	9.0	6.73	0.065
		11	140	9.0	6.59	0.094
		12	111	9.0	6.40	0.048
		IS	25	8.2	7.31	0.082
14/1	1000	09	33	9.0	6.94	0.049
		10	39	9.0	6.83	0.045
		11	67	9.0	6.58	0.044
		12	62	9.0	6.54	0.037
		IS	19	8.2	7.23	0.052
	1400	09	38	9.0	6.93	0.056
		10	51	9.0	6.60	0.035
		11	94	9.0	6.60	0.064
		12	95	9.0	6.49	0.051
		IS	23	8.4	7.24	0.066

Table A1.3 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
14/1	1800	09	37	9.0	6.83	0.043
		10	52	9.0	6.78	0.054
		11	84	9.0	6.64	0.063
		12	74	9.0	6.68	0.061
		IS	24	8.3	7.14	0.054
15/1	1000	09	65	9.0	6.91	0.091
		10	82	9.0	6.75	0.079
		11	96	8.9	6.70	0.082
		12	94	8.8	6.77	0.094
		IS	35	8.1	7.28	0.107
	1400	09	52	8.9	6.85	0.063
		10	50	8.9	6.76	0.049
		11	106	8.9	6.60	0.072
		12	133	8.9	6.63	0.097
		IS	32	8.0	7.20	0.081
	2200	09	54	9.1	6.80	0.059
		10	34	9.1	6.97	0.055
		11	110	9.1	6.75	0.107
		12	121	9.1	6.49	0.065
		IS	24	8.5	7.20	0.063
16/1	0200	09	52	9.1	6.90	0.072
		10	43	9.1	6.83	0.050
		11	110	9.1	6.78	0.115
		12	75	9.1	6.61	0.053
		IS	29	8.7	7.17	0.072
	0600	09	58	9.1	6.90	0.080
		10	55	9.1	6.84	0.066
		11	102	9.1	6.80	0.112
		12	102	9.1	6.75	0.100
		IS	35	8.5	7.22	0.096
	1000	09	58	9.0	6.90	0.079
		10	67	8.9	6.88	0.087
		11	120	9.0	6.50	0.065
		12	100	8.9	6.55	0.061
		IS	32	8.1	7.23	0.087
	MEAN	09	54	8.8	6.78	0.055
	MEAN	10	53	8.8	6.75	0.052
	MEAN	11	92	8.8	6.59	0.063
	MEAN	12	85	8.8	6.56	0.054
	MEAN	IS	24	8.4	7.20	0.063

Notes:-

a) Sample references as in Table A1.1; IS indicates inlet sample.

b) Means are calculated from all tabulated data after fish-introduction.

Table A1.4 PO2 results: flow, volume

	DATE	TIME	TANK (ST) 09	10	11	12
FLOW (s ℓ^{-1})	10/1	1730	72	71	73.5	72
		2300	74	72	74	73.5
	11/1	1200	73	71.5	73.5	70.5
	12/1	1100	73	73	73.5	71.5
		2400	72	72	73.5	73.5
	13/1	1430	73.5	73.5	72.5	72.5
	14/1	1100	73.5	73.5	73	71
	15/1	1145	74.5	73	72	74.5
	16/1	1100	73.5	73.5	72	73.5
	MEAN		73.2	72.6	73.1	72.5
MEAN FLOW (ℓ min^{-1})			0.819	0.827	0.821	0.828
VOLUME (ℓ)			3.6	7.8	7.7	3.8

Table A1.5 PO3 results: TOA, temperature, pH, UIA

DATE	TIME	SAMPLE	TOA ($\mu g\ \ell^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ \ell^{-1}$)
5/2/73	1000	09	100	9.4	6.21	0.029
		10	74	9.3	6.37	0.031
		11	98	9.2	6.39	0.042
		12	115	9.1	6.27	0.037
		IS	15	8.6	7.35	0.056
	1200	09	83	9.2	6.22	0.024
		10	101	9.2	6.41	0.045
		11	93	9.1	6.29	0.031
		12	146	9.1	6.26	0.046
		IS	14	8.7	7.27	0.044
	1400	09	88	9.2	6.29	0.030
		10	143	9.2	6.44	0.069
		11	122	9.2	6.34	0.047
		12	171	9.2	6.38	0.072
		IS	-	8.7	7.39	-
	1600	09	93	9.2	6.34	0.036
		10	163	9.2	6.42	0.075
		11	141	9.2	6.37	0.058
		12	152	9.1	6.39	0.065
		IS	15	8.4	7.33	0.053
6/2	1000	09	131	9.3	6.42	0.061
		10	157	9.2	6.45	0.077
		11	201	9.2	6.37	0.082
		12	233	9.2	6.33	0.087
		IS	22	8.6	7.36	0.084
	1200	09	144	9.3	6.31	0.052
		10	167	9.2	6.46	0.084
		11	233	9.2	6.30	0.081
		12	305	9.3	6.31	0.110
		IS	21	8.7	7.51	0.114
	1400	09	162	9.3	6.39	0.070
		10	202	9.2	6.48	0.107
		11	265	9.2	6.34	0.106
		12	335	9.2	6.39	0.144
		IS	22	8.4	7.35	0.081
	1600	09	143	9.3	6.44	0.069
		10	217	9.0	6.59	0.145
		11	269	9.2	6.35	0.105
		12	385	9.3	6.29	0.132
		IS	22	8.6	7.40	0.092
7/2	1000	09	150	9.5	6.48	0.081
		10	202	9.4	6.45	0.101
		11	212	9.4	6.30	0.075
		12	258	9.4	6.31	0.094
		IS	24	8.7	7.35	0.090

Table A1.5 continued

DATE	TIME	SAMPLE	TOA ($\mu g\ l^{-1}$)	TEMP (^{o}C)	pH	UIA ($\mu g\ l^{-1}$)
7/2	1200	09	175	9.5	6.35	0.070
		10	236	9.4	6.41	0.108
		11	187	9.4	6.33	0.071
		12	297	9.5	6.31	0.109
		IS	-	8.6	7.35	-
	1400	09	196	9.4	6.44	0.096
		10	285	9.3	6.38	0.121
		11	207	9.3	6.27	0.068
		12	305	9.2	6.28	0.102
		IS	25	8.0	7.32	0.083
	1600	09	183	8.7	6.43	0.083
		10	295	8.7	6.53	0.168
		11	199	8.7	6.27	0.062
		12	340	8.8	6.30	0.115
		IS	21	8.0	7.39	0.082
8/2	1000	09	182	9.1	6.49	0.098
		10	272	9.1	6.50	0.149
		11	265	9.0	6.39	0.112
		12	323	9.1	6.35	0.126
		IS	34	8.1	7.37	0.127
	1200	09	210	9.0	6.39	0.089
		10	323	8.8	6.41	0.141
		11	292	8.9	6.33	0.107
		12	303	8.9	6.27	0.096
		IS	26	8.2	7.37	0.098
	1400	09	228	8.8	6.47	0.114
		10	405	8.8	6.41	0.176
		11	285	8.8	6.33	0.103
		12	365	8.8	6.39	0.152
		IS	22	8.1	7.34	0.077
	1600	09	252	8.8	6.46	0.123
		10	435	8.8	6.47	0.218
		11	278	8.8	6.39	0.116
		12	365	8.8	6.43	0.167
		IS	20	8.0	7.34	0.069
	MEAN	09	137	9.3	6.36	0.058
	MEAN	10	230	9.1	6.45	0.113
	MEAN	11	200	9.1	6.33	0.079
	MEAN	12	275	9.1	6.33	0.103
	MEAN	IS	22	8.4	7.36	0.082

Notes:-

a) Sample references as in Table A1.3.

b) Means are calculated from all tabulated data except ST 09; for this tank values subsequent to mortality at 0845, 8/2 are ignored.

Table A1.6 PO3 results: food consumption

DATE	TIME	TANK (ST) 09 NETX	09 %	10 NETX	10 %	11 NETX	11 %	12 NETX	12 %
4/2/73	1100	29	79.4	61	56.7	76	56.6	63	64.0
	1300	55	61.0	56	60.3	52	70.3	52	70.3
	1500	40	71.6	70	50.4	59	66.3	57	67.4
	1700	64	54.6	68	51.8	37	78.9	67	61.7
5/2	0900	28	80.1	0	100.0	22	87.4	0	100.0
	1100	68	51.8	32	77.3	45	74.3	58	66.9
	1300	56	60.3	18	87.2	66	62.3	36	79.4
	1500	61	56.7	1	99.3	52	70.3	97	44.6
	1700	69	51.1	5	96.5	29	83.4	31	82.3
	1900	78	44.7	17	87.9	53	69.7	0	100.0
6/2	0945	44	68.8	0	100.0	14	92.0	0	100.0
	1100	58	58.9	3	97.9	38	78.3	12	93.1
	1330	49	65.2	0	100.0	26	85.1	24	86.3
	1500	74	47.5	0	100.0	81	53.7	77	56.0
	1700	21	85.1	0	100.0	76	56.6	97	44.6
7/2	0915	7	95.0	0	100.0	50	71.4	0	100.0
	1100	30	78.7	0	100.0	56	68.0	20	88.6
	1300	54	61.7	0	100.0	93	46.9	56	68.0
	1500	40	71.6	0	100.0	101	42.3	45	74.3
	1700	43	69.5	0	100.0	87	50.3	60	65.7
	1900	52	63.1	0	100.0	80	54.3	84	52.0
8/2	0855	2	98.6	0	100.0	123	29.7	68	61.1
	1110	51	63.8	0	100.0	92	47.4	53	69.7
	1300	43	69.5	0	100.0	70	60.0	84	52.0
	1515	45	68.1	0	100.0	76	56.6	40	77.1
	1725	67	52.5	0	100.0	63	64.0	0	100.0
	MEAN	47	66.5	13	91.0	62	64.5	45	74.0

Notes:-

a) NETX = number of pellets uneaten;*

b) % = percentage of offered food eaten.

c) Means are calculated from all tabulated data; ignoring 4/2 (on which no ammonia measurements were made) has little effect on the means except in ST 10 where the mean NETX drops to 3 and the mean % rises to 97.6.

*rations are ST 09, 10: 141 pellets and ST 11, 12: 175 pellets.

Table A1.7 PO3 results: flow, volume

	DATE	TIME	TANK (ST) 09	10	11	12
FLOW ($s\ \ell^{-1}$)	5/2	1130	94.5	89	92	93
		1915	90.5	104.5	92	92.5
	6/2	1120	94	85	94.5	101
		1910	-	75	-	-
	7/2	0900	90	119	89	98.5
		1145	95.5	85.5	95	97.5
		1540	96	88.5	95	96.5
	8/2	1125	96.5	99	93.5	96
		1705	95	99.5	96.5	97
	MEAN		94	94	93.4	96.5
MEAN FLOW ($\ell\ min^{-1}$)			0.638	0.638	0.642	0.622
VOLUME (ℓ)			6.0	7.6	5.7	8.0

PO3 mortality

One fish was removed from ST 09 at 0845 on 8/2/73. The fish displayed flank and tail wounds and abrasions of the kind associated with cannibalistic bullying by other fish, and died soon afterwards. All weight, stocking and loading conditions discussed in Chapter 6 imply the fish weight prior to this loss (i.e. final tank weight plus weight of dead fish) as the final weight.

A2. E-SERIES PRELIMINARY WORK RESULTS

CONTENTS:-

Table A2.1 Summary of BMP results

BMP	FCAT	No.of FISH		TOTAL WEIGHT (g)		MEAN LENGTH (cm)		CONDITION FACTOR		FOOD FED (g)	FINAL CR	VOL (ℓ)	FINAL STOK (g ℓ$^{-1}$)	MEAN TEMP (°C)
		INT	FIN	INT	FIN	INT	FIN	INT	FIN					
1	8	34	34	4503	5909	-	22.9	-	-	769	0.55	126	46.9	
	9	54	49	9634	10794	-	24.9	-	-	1555	0.74	134	80.6	12.0
	10	18	18	4373	5125	-	27.1	-	-	625	0.83	105	48.8	
2	8	13	13	2043	2301	22.1	22.8	1.46	1.48	322	1.25	92	25.0	
	9A	22	22	4042	4560	23.4	24.2	1.43	1.46	596	1.15	100	45.6	
	9B	32	31	6680	7324	24.2	25.2	1.43	1.48	968	1.14	147	49.8	11.7
	10	35	35	9239	10156	26.4	27.1	1.43	1.46	1265	1.38	155	65.5	
3	8	6	6	963	1049	22.3	22.9	1.44	1.45	148	1.72	84	12.5	
	9A	21	21	4136	4479	22.8	24.2	1.65	1.50	587	1.71	117	38.3	
	9B	25	25	5558	6095	24.7	25.4	1.47	1.48	789	1.47	126	48.4	10.7
	10A	26	26	6766	7221	26.1	27.8	1.47	1.45	886	1.95	147	49.1	
	10B	23	23	6918	7436	27.5	28.0	1.44	1.48	894	1.73	142	52.4	
4	9A	16	16	3098	3113	23.4	23.8	1.50	1.45	312	20.81	84	37.1	
	9B	19	19	4211	4310	24.7	25.2	1.46	1.43	425	4.29	84	51.3	
	10A	28	27	6853	7031	25.0	26.2	1.46	1.44	619	3.48	142	49.5	7.3
	10B	28	28	8364	8691	27.2	27.5	1.48	1.50	752	2.30	147	59.1	
	10C	10	10	3478	3557	28.9	29.2	1.45	1.44	314	3.97	105	33.9	

Notes:-

a) BMP: 1-4 represent the four two-week periods.

b) INT = Initial, FIN = final.

c) CR = Conversion rate = food fed per weight gain.

d) STOK = Stocking = weight per volume.

e) Conversions for BMP period 1 are so low as to be particularly suspicious; given that conversions below 1.0 are possible (water uptake can account for excess weight gain) nevertheless a value of 0.55 seems highly unlikely. The most probable error is in the feed record.

f) In BMP 4 conversions are at the opposite extreme; fish were growing very large (although stocking was no higher than in earlier periods) and this combined with a sharp fall in temperature probably accounts for a lack in feeding and a great fall off in growth, indicated by poor conversion (high values) and a deterioration in condition factor.

g) BMP lasted from 10/10/73 to 5/12/73.

Table A2.2 Summary of PO4 results

Number of fish		initial		16
		final		10
Weight	total	initial	(g)	886
		final	(g)	591
	mean	initial	(g)	55.375
		final	(g)	59.100
		increase	(g)	3.725
Length;	mean	initial	(cm)	16.469
		final	(cm)	16.600
		increase	(cm)	0.131
Condition factor		initial		1.24
		final		1.29
Total food fed			(g)	205.8
Estimated overall conversion*				5.525
Temperature		initial	(^{o}C)	16.3
		final	(^{o}C)	14.4
		mean	(^{o}C)	14.9

* estimated from:-

$$\frac{\text{total food fed}}{(\text{mean weight increase}) \times (\text{final number of fish})}$$

(This assumes that the 6 fish which died consumed no food.)

Table A2.3 Summary of EOO results

				MT 7	MT 8
FCAT				4	3
Number of fish		initial		30	30
		final		27	30
Weight	total	initial	(g)	489	278
		final	(g)	458	289
	mean	initial	(g)	16.30	9.27
		final	(g)	16.96	9.63
		increase	(g)	0.66	0.36
Length;	mean	initial	(cm)	10.93	8.90
		final	(cm)	10.95	9.05
		increase	(cm)	0.02	0.15
Condition factor		initial		1.25	1.31
		final		1.29	1.30
Total food fed			(g)	30.1	21.5
Conversion ratio*				1.479	1.955
				1.523	
				1.507	
				1.900	
Temperature		initial	(^{o}C)	5.5	5.5
		final	(^{o}C)	6.1	6.1
		mean	(^{o}C)	6.0	6.0
Average TOA			($\mu g\ \ell^{-1}$)	107	55
Average SER			(mg $kg^{-1}\ h^{-1}$)	9.57	5.42
Average flow			($\ell\ min^{-1}$)	0.972	0.945
Average loading			(kg min ℓ^{-1})	0.495	0.299
Estimated average stocking			(g ℓ^{-1})	40	24

* The conversions quoted were calculated by the standard weight estimation routine used in the E-series (see DATA ANALYSIS section) and those in MT 7 represent phases of the experiment before, between, and after mortalities.

Notes:-

a) The stocking values given in the table assume a volume of 12 ℓ in each tank; this was approximately correct by eye observation

b) Poor growth in MT 8 (FCAT 3) is indicated by the fall in condition factor.

A3. E-SERIES EXPERIMENT RAW RESULTS

CONTENTS:-

BASIC DATA

A3.1 The data are computer-listed in Table A3.1 and follow a particular convention in 8 columns. The first column consists of experimental data string codings, made up of 5 sections, thus:-

E / W / X / YY / Z

where W, X, Y, Z represent digits. The last 7 columns each bear a heading which indicated the quantity (as described in Chapter 7 para. 7.75).

A3.2 Codings:-

E = experiment type; E indicates environment/excretion experiment (as opposed to T, used in tolerance experiments).

W = series of experiments; this takes values 0,1 or 2 where 0 represents a pilot experiment, 1 an experiment using MTS, and 2 an experiment using LTS.

X = experiment number; this takes values 0,1,2,3,4,5,6, 7,8,9 according to the number of experiments in the particular series. A value of 0 was found only in series 1 (MTS experiments) and corresponds to the run-up experiment E00. X is the last digit of experiment titles as in Chapters 6 and 7.

YY = experimental DAY; values were from 01 to 14 (see Chapter 7).

Z = experimental tank; this takes values from 1 to 8 (since the MTS, the largest system, held 8 tanks). For pilot experiments P02 and P03, St Nos. 09,10,11,12 were designated tanks 1,2,3,4.

A3.3 For example, the coding

E03042

indicates:-

E - environment/excretion

O - pilot series experiment
3 - pilot experiment 3 (PO3)
O4 - DAY O4
2 - tank 2 (ST lO in this case)

Similarly, the coding

E1OO38

indicates:-

E1 - E-series experiment, MTS
O - run-up experiment (EOO)
O3 - DAY O3
8 - MT8

A3.4 Values of zero in the columns headed VØLUME, SIZELN and AVERLN correspond to situations where these values were not measured. The full matrix comprises 264 data strings.

A3.5 Units are as follows:-

TØTAMM - $\mu g\ \ell^{-1}$
ENUMBR - (number)
FLØWRT - $\ell\ min^{-1}$
VØLUME - ℓ
WEIGHT - g
SIZELN - cm
AVERLN - cm

A3.6 All PO3 data except for ST lO are omitted due to the difficulties in food consumption mentioned in Chapter 6.

Table A3.1/see over

XX

	TOTAMM	ENUMBR	FLOWRT	VOLUMF	WEIGHT	SIZELN	AVERLN
XX							
E01011	80.	4.	0.896	2.3	61.00	0.00	0.000
E01012	76.	5.	0.909	8.2	57.00	0.00	0.000
E01014	127.	13.	0.916	8.6	170.00	0.00	0.000
E01016	132.	18.	0.909	2.6	211.00	0.00	0.000
E01021	50.	4.	0.896	2.3	61.00	0.00	0.000
E01022	43.	5.	0.909	8.2	57.00	0.00	0.000
E01024	122.	13.	0.916	8.6	170.00	0.00	0.000
E01026	109.	18.	0.909	2.6	211.00	0.00	0.000
E02021	92.	7.	0.822	3.6	306.00	0.00	0.000
E02022	76.	6.	0.839	7.8	325.00	0.00	0.000
E02023	145.	13.	0.816	7.7	609.00	0.00	0.000
E02024	75.	13.	0.851	3.8	591.00	0.00	0.000
E02031	76.	7.	0.822	3.6	306.00	0.00	0.000
E02032	72.	6.	0.822	7.8	325.00	0.00	0.000
E02033	96.	13.	0.816	7.7	609.00	0.00	0.000
E02034	83.	13.	0.839	3.8	591.00	0.00	0.000
E02041	75.	7.	0.816	3.6	306.00	0.00	0.000
E02043	140.	13.	0.828	7.7	609.00	0.00	0.000
E02044	111.	13.	0.828	3.8	591.00	0.00	0.000
E02051	38.	7.	0.816	3.6	306.00	0.00	0.000
E02052	51.	6.	0.816	7.8	325.00	0.00	0.000
E02053	94.	13.	0.822	7.7	609.00	0.00	0.000
E02054	95.	13.	0.845	3.8	591.00	0.00	0.000
E02061	52.	7.	0.805	3.6	306.00	0.00	0.000
E02062	50.	6.	0.822	7.8	325.00	0.00	0.000
E02063	106.	13.	0.833	7.7	609.00	0.00	0.000
E02064	133.	13.	0.805	3.8	591.00	0.00	0.000
E03032	202.	18.	0.706	7.6	1035.00	0.00	0.000
E03042	285.	18.	0.702	7.6	1035.00	0.00	0.000
E03052	405.	18.	0.606	7.6	1035.00	0.00	0.000
E04047	119.	16.	3.243	14.8	900.93	264.03	16.502
E04057	122.	15.	3.333	12.9	837.69	247.22	16.481
E04067	107.	15.	3.333	14.1	843.66	247.44	16.496
E04077	116.	14.	3.429	14.3	795.85	231.58	16.542
E04127	89.	11.	3.429	14.9	645.77	182.70	16.609
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E10037	107.	29.	0.972	0.0	481.41	317.21	10.938
E10038	55.	30.	0.945	0.0	283.08	269.08	8.969
E11051	282.	30.	0.193	0.0	294.67	271.60	9.053
E11052	246.	20.	0.348	0.0	385.88	228.42	11.421
E11054	328.	29.	0.171	0.0	301.08	265.05	9.140
E11055	280.	20.	0.297	0.0	368.54	226.42	11.521
E11057	294.	30.	0.212	0.0	297.00	272.37	9.079
E11058	378.	20.	0.277	0.0	410.84	231.16	11.558
E11081	104.	30.	0.452	0.0	296.83	272.90	9.097
E11082	191.	19.	0.282	0.0	371.05	217.71	11.458
E11084	82.	29.	0.513	0.0	303.21	265.65	9.160
E11085	164.	19.	0.344	0.0	356.40	216.07	11.372
E11087	165.	30.	0.257	0.0	297.00	273.15	9.105
E11088	165.	20.	0.392	0.0	414.49	232.42	11.621
E11131	257.	30.	0.205	0.0	301.04	275.42	9.181
E11132	189.	19.	0.299	0.0	374.42	218.81	11.516
E11134	335.	29.	0.195	0.0	307.17	266.77	9.199
E11135	263.	19.	0.267	0.0	367.07	217.19	11.431
E11137	272.	30.	0.193	0.0	297.00	274.27	9.142
E11138	415.	20.	0.230	0.0	420.55	234.50	11.725
E12031	172.	30.	0.284	8.8	288.49	272.76	9.092
E12032	237.	20.	0.330	9.5	327.79	218.71	10.935
E12033	307.	30.	0.191	9.4	285.08	270.60	9.020
E12034	259.	20.	0.464	9.3	377.25	217.58	11.452
E12035	201.	29.	0.346	9.3	265.02	258.35	8.909
E12036	255.	20.	0.421	9.9	363.70	227.46	11.373
E12037	247.	29.	0.323	10.0	252.99	256.04	8.829
E12038	367.	20.	0.438	10.6	393.21	231.22	11.561
E12071	410.	30.	0.237	8.4	303.27	276.15	9.205
E12072	292.	20.	0.248	9.5	340.82	221.02	11.051
E12073	429.	30.	0.176	9.3	296.60	274.23	9.141
E12074	344.	20.	0.364	9.2	394.01	220.65	11.613
E12075	384.	29.	0.292	9.2	277.17	262.18	9.041
E12076	640.	20.	0.208	9.6	378.62	229.12	11.456
E12077	477.	29.	0.225	10.0	260.13	258.88	8.927

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E12078	837.	20.	0.249	10.6	399.67	233.68	11.684
E12091	330.	30.	0.241	8.4	310.99	277.91	9.264
E12092	325.	20.	0.230	9.4	347.59	222.23	11.111
E12093	397.	30.	0.186	9.1	303.03	276.26	9.209
E12094	292.	20.	0.370	9.3	402.54	222.21	11.695
E12095	377.	29.	0.293	9.2	283.71	264.24	9.112
E12096	565.	20.	0.208	9.4	386.00	229.93	11.497
E12097	497.	29.	0.233	10.0	263.62	260.27	8.975
E12098	495.	20.	0.216	10.5	403.07	234.98	11.749
E12101	445.	30.	0.228	8.3	317.09	279.31	9.310
E12102	590.	20.	0.241	9.4	352.73	223.14	11.157
E12103	535.	30.	0.214	9.1	307.95	277.82	9.261
E12104	472.	20.	0.360	9.3	409.56	223.49	11.763
E12105	447.	29.	0.293	9.2	289.09	265.93	9.170
E12106	795.	19.	0.230	9.4	370.84	219.10	11.532
E12107	580.	29.	0.214	10.0	266.27	261.32	9.011
E12108	502.	20.	0.200	10.4	405.34	235.85	11.792
E13031	245.	27.	0.214	8.8	131.27	200.32	7.419
E13032	146.	40.	0.870	10.1	425.06	382.06	9.551
E13033	137.	30.	1.000	9.9	445.05	321.69	10.723
E13034	233.	40.	0.571	9.5	422.11	380.54	9.513
E13035	226.	30.	0.732	11.0	439.83	323.94	10.798
E13036	215.	40.	0.811	11.8	426.24	385.83	9.646
E13037	248.	29.	0.755	10.5	588.61	351.41	12.117
E13038	307.	20.	0.529	11.6	553.79	267.81	13.391
E13041	225.	27.	0.207	8.9	135.36	201.85	7.476
E13042	138.	40.	0.889	10.4	435.02	384.21	9.605
E13043	117.	30.	1.000	9.9	454.24	322.88	10.763
E13044	197.	40.	0.612	9.6	430.62	382.30	9.557
E13045	202.	30.	0.741	11.2	448.40	326.08	10.869
E13046	176.	40.	0.779	11.8	435.70	387.72	9.693
E13047	233.	29.	0.694	10.5	601.27	352.64	12.160
E13048	224.	20.	0.494	12.1	557.04	268.20	13.410
E13051	141.	27.	0.217	8.9	137.09	202.49	7.500
E13052	111.	39.	1.043	10.7	432.61	376.58	9.656

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E13053	126.	29.	1.000	10.0	446.90	313.37	10.806
E13054	199.	40.	0.591	9.6	438.69	383.97	9.599
E13055	163.	30.	0.750	11.1	455.82	327.92	10.931
E13056	154.	40.	0.800	11.8	443.97	389.36	9.734
E13057	195.	29.	0.632	10.5	611.84	353.68	12.196
E13058	246.	20.	0.476	11.9	559.83	268.54	13.427
E13061	110.	27.	0.209	8.8	138.11	202.87	7.514
E13062	93.	39.	1.008	10.8	439.44	378.05	9.693
E13063	108.	29.	1.000	10.2	455.22	314.41	10.842
E13064	168.	40.	0.563	11.2	445.27	385.33	9.633
E13065	149.	30.	0.719	11.1	461.97	329.46	10.982
E13066	153.	40.	0.774	11.8	450.12	390.59	9.765
E13067	192.	29.	0.642	10.5	620.42	354.52	12.225
E13068	209.	20.	0.460	11.9	562.11	268.81	13.440
E13071	151.	27.	0.208	8.6	139.57	203.41	7.534
E13072	142.	38.	0.960	12.2	438.80	370.90	9.761
E13073	141.	28.	0.992	13.1	454.58	305.53	10.912
E13074	229.	40.	0.585	11.2	453.89	387.11	9.678
E13075	203.	29.	0.779	12.2	455.01	320.78	11.061
E13076	163.	40.	0.795	11.8	458.20	392.20	9.805
E13077	236.	26.	0.625	11.7	567.45	319.41	12.285
E13078	253.	20.	0.476	12.0	565.07	269.16	13.458
E13111	181.	27.	0.375	9.1	156.05	209.55	7.761
E13112	154.	37.	0.902	13.6	464.89	369.13	9.976
E13113	146.	27.	0.976	13.2	467.92	298.30	11.048
E13114	227.	38.	0.606	11.4	465.82	375.00	9.869
E13115	152.	24.	0.759	13.3	397.26	272.03	11.334
E13116	143.	39.	0.811	11.8	480.67	389.13	9.978
E13117	202.	25.	0.566	11.6	584.29	311.07	12.443
E13118	217.	19.	0.426	11.9	548.30	257.34	13.544
E13121	225.	27.	0.374	9.2	160.70	211.28	7.825
E13122	175.	37.	0.896	14.0	472.76	370.74	10.020
E13123	150.	26.	0.984	13.3	456.37	288.29	11.088
E13124	262.	38.	0.553	11.3	474.47	376.76	9.915
E13125	167.	24.	0.784	13.6	405.11	273.37	11.391

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E13126	207.	39.	0.800	11.8	487.96	390.59	10.015
E13127	304.	25.	0.558	11.4	592.55	311.63	12.465
E13128	367.	19.	0.403	11.8	551.23	257.59	13.557
E14064	119.	29.	1.250	10.5	357.80	287.21	9.904
E14065	161.	19.	0.706	12.6	295.95	205.74	10.829
E14066	120.	30.	1.304	10.8	365.82	296.06	9.869
E14067	97.	19.	1.379	12.4	295.26	204.83	10.781
E14068	86.	19.	1.739	12.8	349.35	218.10	11.479
E14074	125.	29.	1.538	11.2	366.39	289.27	9.975
E14075	185.	19.	0.857	13.0	302.72	206.67	10.877
E14076	180.	30.	1.212	11.2	375.68	298.05	9.935
E14077	191.	19.	0.889	11.8	303.03	205.82	10.832
E14078	153.	19.	1.250	12.4	356.86	219.11	11.532
E14084	112.	29.	1.538	11.1	374.96	291.33	10.046
E14085	145.	19.	0.863	13.2	309.06	207.53	10.923
E14086	123.	30.	1.154	11.2	384.76	299.88	9.996
E14087	156.	19.	0.851	12.1	310.18	206.72	10.880
E14088	102.	18.	1.081	12.3	347.94	208.87	11.604
E14094	185.	29.	0.800	10.1	382.13	293.06	10.105
E14095	145.	19.	0.833	13.2	314.29	208.24	10.960
E14096	126.	30.	1.154	11.2	393.80	301.70	10.057
E14097	137.	19.	0.952	12.2	315.63	207.41	10.916
E14098	124.	16.	0.938	12.1	320.02	187.18	11.699
E14104	204.	29.	0.816	10.3	388.31	294.54	10.157
E14105	163.	19.	0.839	13.3	319.46	208.95	10.997
E14106	163.	30.	1.165	11.4	402.68	303.49	10.116
E14107	116.	19.	0.960	12.2	320.49	208.03	10.949
E14108	142.	16.	0.976	12.1	321.93	187.42	11.714
E15036	177.	31.	1.611	14.1	898.26	431.66	13.925
E15046	170.	31.	1.589	14.1	914.91	432.52	13.952
E15076	109.	31.	1.875	15.5	950.01	434.33	14.011
E15086	99.	31.	1.529	14.5	962.36	434.97	14.031
E15096	149.	30.	1.538	14.5	948.60	421.28	14.043
E15106	184.	30.	1.538	14.7	961.48	422.07	14.069
E15037	173.	30.	1.529	12.8	919.32	418.07	13.936

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E15047	189.	30.	1.644	13.0	940.00	419.36	13.979
E15077	141.	30.	1.860	13.7	980.49	421.88	14.063
E15087	135.	30.	1.500	12.8	994.93	422.78	14.093
E15097	175.	30.	1.548	12.8	1011.72	423.83	14.128
E15107	165.	30.	1.500	12.9	1028.01	424.84	14.161
E15038	257.	26.	1.622	13.7	1607.50	442.80	17.031
E15048	237.	26.	1.558	13.7	1618.24	443.66	17.064
E15078	190.	26.	1.765	14.5	1645.66	445.85	17.148
E15088	239.	26.	1.319	13.7	1655.54	446.64	17.179
E15098	276.	26.	1.326	13.7	1666.32	447.51	17.212
E15108	261.	26.	1.364	13.7	1676.25	448.30	17.242
E16025	140.	30.	0.427	12.5	541.43	347.43	11.581
E16035	162.	30.	0.492	12.5	548.26	347.99	11.600
E16085	136.	30.	0.594	12.8	586.18	351.09	11.703
E16095	232.	30.	0.471	12.3	594.89	351.80	11.727
E16135	190.	30.	0.449	12.3	623.54	354.14	11.805
E16026	201.	30.	0.492	9.2	585.29	356.48	11.883
E16036	152.	30.	0.462	9.2	593.38	357.13	11.904
E16086	151.	30.	0.600	9.4	631.13	360.15	12.005
E16096	242.	29.	0.515	9.9	616.28	348.14	12.005
E16136	189.	29.	0.533	9.6	646.91	351.02	12.104
E16027	217.	30.	0.548	11.2	584.06	352.67	11.756
E16037	157.	30.	0.531	11.6	590.95	353.28	11.776
E16087	111.	29.	0.800	12.0	615.36	345.59	11.917
E16097	225.	29.	0.649	11.7	623.86	346.32	11.942
E16137	159.	29.	0.682	11.9	650.95	348.65	12.022
E16028	198.	30.	0.536	11.2	558.96	348.97	11.632
E16038	134.	30.	0.682	11.6	565.41	349.50	11.650
E16088	161.	30.	0.469	11.2	592.30	351.69	11.723
E16098	285.	30.	0.504	11.2	601.85	352.46	11.749
E16138	228.	29.	0.407	11.0	611.57	343.14	11.832
E27062	365.	63.	1.579	84.0	3351.38	1027.76	16.314
E27072	550.	63.	1.702	84.0	3383.96	1030.56	16.358
E27102	447.	63.	1.690	89.0	3462.81	1037.33	16.466
E27112	440.	63.	1.404	84.0	3490.06	1039.67	16.503

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E27122	390.	63.	1.644	84.0	3517.15	1041.99	16.540
E27132	330.	63.	1.297	84.0	3542.20	1044.14	16.574
E27064	427.	212.	1.905	97.0	7129.01	3001.37	14.157
E27074	630.	212.	2.202	93.0	7200.37	3006.49	14.182
E27104	397.	212.	2.637	97.0	7377.09	3019.16	14.241
E27114	452.	212.	2.051	101.0	7445.35	3024.05	14.264
E27124	380.	212.	2.143	97.0	7513.60	3028.95	14.287
E27134	332.	212.	2.069	101.0	7571.95	3033.13	14.307
E27065	347.	147.	2.069	84.0	3170.35	1762.59	11.990
E27075	870.	147.	1.231	76.0	3226.06	1768.37	12.030
E27105	565.	147.	1.600	72.0	3362.02	1782.49	12.126
E27115	650.	147.	1.081	72.0	3407.34	1787.20	12.158
E27125	480.	147.	1.148	76.0	3452.26	1791.86	12.190
E27135	470.	147.	1.021	76.0	3495.27	1796.33	12.220
E27066	505.	350.	2.143	55.0	3183.53	3102.36	8.864
E27076	1190.	350.	1.379	50.0	3243.06	3114.52	8.899
E27106	690.	350.	1.569	50.0	3393.31	3145.20	8.986
E27116	800.	350.	1.096	50.0	3445.25	3155.80	9.017
E27126	620.	350.	1.159	50.0	3496.39	3166.24	9.046
E27136	610.	350.	1.096	50.0	3545.93	3176.36	9.075
E18065	139.	40.	0.676	12.5	856.64	472.56	11.814
E18075	156.	40.	0.623	12.5	864.20	473.22	11.831
E18115	128.	40.	0.608	12.5	894.44	475.87	11.897
E18125	143.	40.	0.611	12.5	901.41	476.48	11.912
E18135	172.	40.	0.606	12.5	908.45	477.10	11.928
E18066	158.	40.	0.637	9.8	923.16	481.94	12.049
E18076	224.	40.	0.562	9.7	929.96	482.73	12.068
E18116	149.	38.	0.569	9.7	907.00	460.90	12.129
E18126	183.	38.	0.580	9.8	914.72	461.95	12.157
E18136	290.	38.	0.557	9.8	922.29	462.99	12.184
E18067	137.	40.	0.614	11.5	815.04	467.60	11.690
E18077	240.	40.	0.526	11.5	821.30	468.36	11.709
E18117	167.	40.	0.460	11.7	846.18	471.35	11.784
E18127	238.	40.	0.545	11.7	852.98	472.17	11.804
E18137	287.	40.	0.485	11.7	860.29	473.05	11.826

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E18068	106.	40.	0.787	11.3	863.19	476.64	11.916
E18078	185.	39.	0.605	11.2	844.15	464.64	11.914
E18118	164.	38.	0.600	11.3	845.64	454.49	11.960
E18128	203.	38.	0.620	11.1	852.17	455.09	11.976
E18138	382.	35.	0.585	11.4	792.56	419.48	11.985
E29042	313.	92.	1.967	89.0	4737.95	1490.31	16.199
E29052	305.	92.	1.765	89.0	4766.39	1491.95	16.217
E29062	257.	92.	1.633	89.0	4794.68	1493.58	16.235
E29072	284.	92.	1.805	89.0	4823.76	1495.26	16.253
E29102	274.	92.	1.818	89.0	4915.52	1500.56	16.310
E29112	246.	92.	1.778	89.0	4946.85	1502.37	16.330
E29132	325.	92.	1.860	93.0	5008.48	1505.93	16.369
E29044	260.	234.	2.474	109.0	7798.81	3278.82	14.012
E29054	245.	234.	2.500	109.0	7846.23	3283.53	14.032
E29064	229.	234.	2.727	114.0	7896.50	3288.53	14.054
E29074	244.	234.	2.581	114.0	7950.41	3293.90	14.076
E29104	245.	233.	3.000	114.0	8069.83	3294.77	14.141
E29114	190.	233.	2.727	114.0	8119.94	3299.84	14.162
E29134	276.	233.	2.727	109.0	8219.69	3309.93	14.206

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CALCULATED DATA

A3.5 Calculated data are listed in Table A3.2 in a similar way to Table A3.1, under the same data string codings. Values of zero indicate absence of measurement, and the units are as follows:-

SPEXRT - mg kg^{-1} h^{-1}

TEMPRT - ^{o}C

DENSTY - (number) ℓ^{-1}

DNLØAD - kg min ℓ^{-1}

DNSTØK - g ℓ^{-1}

FACTØR - kg min ℓ^{-1} m^{-1}

GRUBFD - g

Table A3.2/see over

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	SPEXRT	TEMPRT	DENSTY	DNLOAD	DNSTOK	FACTOR	GRUBFD	FDMEAL
XX								
E01011	70,464	17,0	1,739	0,06812	26,52	0,000	0,00	0,00
E01012	72,727	17,0	0,610	0,06270	6,95	0,000	0,00	0,00
E01014	41,060	17,0	1,512	0,18558	19,77	0,000	0,00	0,00
E01016	34,123	17,0	6,923	0,23210	81,15	0,000	0,00	0,00
E01021	44,040	17,5	1,739	0,06812	26,52	0,000	0,00	0,00
E01022	41,148	17,5	0,610	0,06270	6,95	0,000	0,00	0,00
E01024	39,444	17,5	1,512	0,18558	19,77	0,000	0,00	0,00
E01026	28,178	17,5	6,923	0,23210	81,15	0,000	0,00	0,00
E02021	14,827	8,6	1,944	0,37230	85,00	0,000	0,00	0,00
E02022	11,774	8,6	0,769	0,38729	41,67	0,000	0,00	0,00
E02023	11,662	8,6	1,688	0,74602	79,09	0,000	0,00	0,00
E02024	6,480	8,6	3,421	0,69443	155,53	0,000	0,00	0,00
E02031	12,248	8,4	1,944	0,37230	85,00	0,000	0,00	0,00
E02032	10,925	8,4	0,769	0,39542	41,67	0,000	0,00	0,00
E02033	7,721	8,4	1,688	0,74602	79,09	0,000	0,00	0,00
E02034	7,071	8,4	3,421	0,70428	155,53	0,000	0,00	0,00
E02041	12,005	9,0	1,944	0,37485	85,00	0,000	0,00	0,00
E02043	11,415	9,0	1,688	0,73587	79,09	0,000	0,00	0,00
E02044	9,326	9,0	3,421	0,71412	155,53	0,000	0,00	0,00
E02051	6,082	9,0	1,944	0,37485	85,00	0,000	0,00	0,00
E02052	7,686	9,0	0,769	0,39812	41,67	0,000	0,00	0,00
E02053	7,612	9,0	1,688	0,74095	79,09	0,000	0,00	0,00
E02054	8,150	9,0	3,421	0,69935	155,53	0,000	0,00	0,00
E02061	8,212	8,9	1,944	0,37995	85,00	0,000	0,00	0,00
E02062	7,587	8,9	0,769	0,39542	41,67	0,000	0,00	0,00
E02063	8,703	8,9	1,688	0,75080	79,09	0,000	0,00	0,00
E02064	10,875	8,9	3,421	0,73382	155,53	0,000	0,00	0,00
E03032	8,266	9,2	2,368	1,46625	136,18	0,000	0,00	0,00
E03042	11,594	9,3	2,368	1,47488	136,18	0,000	0,00	0,00
E03052	14,229	8,8	2,368	1,70775	136,18	0,000	0,00	0,00
E04047	25,703	15,7	1,081	0,27779	60,87	1,683	5,76	14,40
E04057	29,127	15,5	1,163	0,25131	64,94	1,525	6,36	15,00
E04067	25,365	15,2	1,064	0,25310	59,83	1,534	7,48	17,02
E04077	29,984	15,0	0,979	0,23212	55,65	1,403	7,48	18,70
E04127	28,351	14,1	0,738	0,18835	43,34	1,134	6,88	16,12

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E10037	12,958	6.1	0.000	0.49545	0.00	4,530	2.64	5.64
E10038	11,015	6.1	0.000	0.29959	0.00	3,340	1.64	4.10
E11051	11,096	7.0	0.000	1.52491	0.00	16,844	1.35	3.90
E11052	13,305	6.6	0.000	1.10939	0.00	9,714	1.60	4.60
E11054	11,189	7.0	0.000	1.75882	0.00	19,244	1.30	3.02
E11055	13,557	6.7	0.000	1.23920	0.00	10,946	1.50	4.28
E11057	12,581	6.9	0.000	1.40209	0.00	15,443	1.75	3.95
E11058	15,299	6.9	0.000	1.48243	0.00	12,826	1.50	4.28
E11081	9,501	7.0	0.000	0.65674	0.00	7,220	1.84	3.44
E11082	8,700	7.1	0.000	1.31723	0.00	11,496	1.68	3.43
E11084	8,321	7.0	0.000	0.59125	0.00	6,454	1.96	3.26
E11085	9,495	7.0	0.000	1.03652	0.00	9,115	1.92	3.22
E11087	8,574	7.2	0.000	1.15459	0.00	12,681	1.56	3.16
E11088	9,366	7.1	0.000	1.05696	0.00	9,095	1.72	3.67
E11131	10,480	7.1	0.000	1.47134	0.00	16,026	2.04	4.28
E11132	9,052	6.9	0.000	1.25274	0.00	10,878	1.40	3.70
E11134	12,768	7.1	0.000	1.57426	0.00	17,114	1.94	4.13
E11135	11,489	7.0	0.000	1.37345	0.00	12,015	2.00	3.91
E11137	10,593	7.2	0.000	1.54069	0.00	16,852	1.64	4.21
E11138	13,611	7.1	0.000	1.82940	0.00	15,602	2.14	4.95
E12031	10,148	10.0	3,409	1.01694	32,78	11,185	2.04	3.96
E12032	14,321	10.0	2,105	0.99294	34,50	9,080	2.08	5.14
E12033	12,366	10.0	3,191	1.48954	30,33	16,514	1.64	4.10
E12034	19,123	9.9	2,151	0.81265	40,56	7,096	2.88	7.14
E12035	15,737	9.9	3,118	0.76636	28,50	8,602	2.24	5.42
E12036	17,712	9.9	2,020	0.86380	36,74	7,595	2.44	5.92
E12037	18,922	10.0	2,900	0.78321	25,30	8,871	2.32	5.86
E12038	24,526	10.0	1,887	0.89783	37,10	7,766	2.60	5.54
E12071	19,199	9.9	3,571	1.28132	36,10	13,920	2.64	6.60
E12072	12,758	9.9	2,105	1.37321	35,88	12,426	2.36	5.90
E12073	15,315	10.0	3,226	1.68071	31,89	18,386	2.20	5.56
E12074	19,049	9.9	2,174	1.08354	42,83	9,330	2.32	5.86
E12075	24,271	9.9	3,152	0.94930	30,13	10,500	2.12	5.36
E12076	21,056	9.9	2,083	1.82367	39,44	15,919	2.12	5.36
E12077	24,795	9.9	2,900	1.15434	26,01	12,931	2.24	5.60

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E12078	31,349	9.9	1,887	1.60199	37,70	13,711	2,52	6.30
E12091	15,375	10.5	3,571	1.28800	37,02	13,904	4,20	7.26
E12092	12,897	10.5	2,128	1.51201	36,98	13,608	3,60	6.42
E12093	14,647	10.5	3,297	1.62628	33,30	17,660	3,72	6.84
E12094	16,120	10.4	2,151	1.08685	43,28	9,295	4,36	7.60
E12095	23,393	10.3	3,152	0.96697	30,84	10,613	4,04	7.10
E12096	18,265	10.5	2,128	1.85602	41,06	16,144	4,24	7.24
E12097	26,383	10.5	2,900	1.13027	26,36	12,594	3,56	6.44
E12098	15,946	10.6	1,905	1.86253	38,39	15,853	3,72	7.62
E12101	19,173	10.6	3,614	1.39257	38,20	14,957	4,20	10.50
E12102	24,159	10.5	2,128	1.46532	37,52	13,134	3,60	9.00
E12103	22,336	10.7	3,297	1.43712	33,84	15,519	3,76	9.34
E12104	24,918	10.5	2,151	1.13652	44,04	9,662	4,36	10.90
E12105	27,220	10.5	3,152	0.98532	31,42	10,745	4,08	10.14
E12106	29,626	10.6	2,021	1.61005	39,45	13,962	4,28	10.64
E12107	27,931	10.7	2,900	1.24591	26,63	13,826	3,56	8.90
E12108	14,861	10.9	1,923	2.02672	38,98	17,187	3,76	9.34
E13031	23,997	15.6	3,068	0.61257	14,92	8,256	2,64	5.16
E13032	17,921	15.4	3,960	0.48882	42,09	5,118	5,28	11.76
E13033	18,470	15.4	3,030	0.44505	44,95	4,150	5,68	11.50
E13034	18,925	15.4	4,211	0.73869	44,43	7,765	5,44	11.92
E13035	22,559	15.4	2,727	0.60110	39,98	5,567	5,64	12.12
E13036	24,539	15.4	3,390	0.52569	36,12	5,450	6,04	13.36
E13037	19,079	15.4	2,762	0.77990	56,06	6,436	7,28	16.34
E13038	17,583	15.4	1,724	1.04758	47,74	7,823	5,84	12.32
E13041	20,671	15.6	3,034	0.65310	15,21	8,736	0,96	4.92
E13042	16,919	15.4	3,846	0.48939	41,83	5,095	4,60	12.52
E13043	15,454	15.3	3,030	0.45424	45,88	4,221	4,88	13.40
E13044	16,806	15.4	4,167	0.70334	44,86	7,359	5,04	13.20
E13045	20,022	15.3	2,679	0.60534	40,04	5,569	4,40	12.86
E13046	18,886	15.4	3,390	0.55915	36,92	5,769	5,28	14.34
E13047	16,128	15.4	2,762	0.86683	57,26	7,128	5,60	16.52
E13048	11,915	15.4	1,653	1.12800	46,04	8,412	4,56	13.32
E13051	13,367	15.1	3,034	0.63289	15,40	8,439	0,64	2.08
E13052	16,064	15.1	3,645	0.41458	40,43	4,294	4,28	11.18

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E13053	16,917	15.1	2.900	0.44690	44.69	4.136	4.68	12.00
E13054	16,089	15.1	4.167	0.74211	45.70	7.731	4.96	12.52
E13055	16,092	15.1	2.703	0.60775	41.06	5.560	4.52	11.12
E13056	16,650	15.1	3.390	0.55496	37.62	5.701	4.60	12.52
E13057	12,077	15.1	2.762	0.96875	58.27	7.943	5.40	13.80
E13058	12.555	15.1	1.681	1.17564	47.04	8.756	4.60	11.44
E13061	9,990	15.2	5.068	0.66063	15.69	8.792	0.27	1.23
E13062	12,805	15.1	3.611	0.43578	40.69	4.496	2.37	8.79
E13063	14.235	15.0	2.843	0.45522	44.63	4.199	5.50	12.52
E13064	12,754	15.1	3.571	0.79035	39.76	8.205	2.77	10.21
E13065	13,906	15.0	2.703	0.64291	41.62	5.854	2.45	9.23
E13066	13,725	15.0	3.390	0.58141	38.15	5.954	2.42	9.32
E13067	11,915	15.1	2.762	0.96683	59.09	7.909	5.10	11.20
E13068	10.257	15.1	1.681	1.22259	47.24	9.096	2.47	9.37
E13071	13,477	15.3	5.140	0.67225	16.23	8.923	0.92	1.75
E13072	18,640	15.0	3.115	0.45708	35.97	4.683	4.88	12.01
E13073	18,457	15.0	2.137	0.45837	34.70	4.201	4.56	21.06
E13074	17,720	15.0	3.571	0.77539	40.53	8.012	5.04	13.37
E13075	20,859	15.0	2.377	0.58393	37.30	5.279	4.40	11.75
E13076	16,962	15.0	3.390	0.57657	38.83	5.880	4.96	12.24
E13077	15,596	15.0	2.222	0.90792	48.50	7.390	5.40	14.70
E13078	12,792	15.0	1.667	1.18664	47.09	8.817	4.68	12.11
E13111	26,096	15.9	2.967	0.41615	17.15	5.362	2.04	5.52
E13112	17,933	15.8	2.721	0.51526	34.18	5.165	5.00	11.96
E13113	18,264	15.7	2.045	0.47962	35.45	4.341	4.20	11.10
E13114	17,721	15.8	3.333	0.76860	40.86	7.788	5.64	13.92
E13115	17,436	15.8	1.805	0.52306	29.87	4.615	4.44	11.46
E13116	14,473	15.8	3.305	0.59283	40.73	5.942	4.72	12.10
E13117	11,741	15.8	2.155	1.03224	50.37	8.296	5.40	13.32
E13118	10,105	15.8	1.597	1.28850	46.08	9.513	4.76	11.78
E13121	31,406	16.0	2.935	0.42986	17.47	5.493	2.52	5.58
E13122	19,889	16.0	2.643	0.52792	33.77	5.269	4.88	12.38
E13123	19,397	15.9	1.955	0.46398	34.31	4.184	4.40	10.70
E13124	18,322	16.0	3.363	0.85799	41.99	8.654	5.44	13.90
E13125	19,399	15.9	1.765	0.51652	29.79	4.535	4.44	11.10

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E13126	20,362	15.9	3.305	0.60995	41.35	6.090	4.12	11.20
E13127	17,181	16.0	2.193	1.06164	51.98	8.517	5.44	13.54
E13128	16,086	16.0	1.610	1.36889	46.71	10.097	4.80	11.94
E14064	24,944	16.6	2.762	0.28624	34.08	2.890	4.28	10.58
E14065	23,041	16.5	1.508	0.41926	23.49	3.872	3.76	8.44
E14066	25,671	16.5	2.778	0.28047	33.87	2.842	5.16	10.92
E14067	27,187	16.5	1.532	0.21407	23.81	1.986	3.84	8.94
E14068	25,687	16.5	1.484	0.20088	27.29	1.750	4.72	10.24
E14074	31,493	16.4	2.589	0.23815	32.71	2.388	4.64	11.06
E14075	31,430	16.4	1.462	0.35317	23.29	3.247	3.84	9.48
E14076	34,845	16.4	2.679	0.30994	33.54	3.120	4.36	12.10
E14077	33,616	16.4	1.610	0.34091	25.68	3.147	4.04	9.80
E14078	32,156	16.5	1.532	0.28548	28.78	2.476	4.36	11.44
E14084	27,573	16.6	2.613	0.24372	33.78	2.426	4.08	11.04
E14085	24,302	16.6	1.439	0.35799	23.41	3.278	3.12	8.88
E14086	22,132	16.6	2.679	0.33346	34.35	3.336	4.60	11.14
E14087	25,682	16.6	1.570	0.36446	25.63	3.350	2.96	9.02
E14088	19,016	16.6	1.463	0.32184	28.29	2.773	4.00	10.54
E14094	23,238	16.9	2.871	0.47767	37.84	4.727	3.12	9.24
E14095	23,068	16.8	1.439	0.37714	23.81	3.441	2.64	7.32
E14096	22,151	16.8	2.679	0.34129	35.16	3.394	4.20	11.10
E14097	24,803	16.8	1.557	0.35141	25.87	3.036	2.44	6.88
E14098	21,795	16.8	1.322	0.34136	26.45	2.918	2.80	8.80
E14104	25,731	17.0	2.816	0.47569	37.70	4.683	3.28	7.96
E14105	25,690	17.0	1.429	0.38069	24.02	3.462	3.28	7.24
E14106	28,296	17.0	2.632	0.34563	35.32	3.417	4.60	10.90
E14107	20,848	17.0	1.557	0.33385	26.27	3.049	2.48	6.14
E14108	25,820	17.0	1.322	0.32998	26.61	2.817	2.84	7.04
E15036	19,044	12.7	2.199	0.55767	63.71	4.005	7.40	16.82
E15046	17,720	12.5	2.199	0.57563	64.89	4.126	5.88	16.98
E15076	12,908	11.8	2.000	0.50667	61.29	3.616	4.48	11.20
E15086	9,435	11.8	2.138	0.62954	66.37	4.487	5.88	12.60
E15096	14,499	11.7	2.069	0.61659	65.42	4.391	5.96	14.78
E15106	17,665	11.5	2.041	0.62496	65.41	4.442	5.44	14.38
E15037	17,260	12.7	2.344	0.60139	71.82	4.315	9.12	17.70

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E15047	19,831	12,5	2,308	0,57184	72,31	4,091	5,60	19,28
E15077	16,053	11,8	2,190	0,52701	71,57	3,748	4,84	12,16
E15087	12,212	11,7	2,344	0,66328	77,73	4,707	6,20	13,46
E15097	16,070	11,7	2,344	0,65341	79,04	4,625	6,36	15,66
E15107	14,445	11,5	2,326	0,68534	79,69	4,839	5,64	15,18
E15038	15,555	12,7	1,898	0,99129	117,34	5,821	7,40	16,16
E15048	13,695	12,5	1,898	1,03837	118,12	6,085	5,32	16,42
E15078	12,225	11,8	1,793	0,93254	113,49	5,438	5,64	14,10
E15088	11,422	11,8	1,898	1,25545	120,84	7,308	6,64	15,10
E15098	13,178	11,7	1,898	1,25668	121,63	7,301	6,52	16,48
E15108	12,739	11,5	1,898	1,22925	122,35	7,129	5,40	15,18
E16025	6,625	8,3	2,400	1,26785	43,31	10,948	2,68	7,72
E16035	8,719	8,0	2,400	1,11479	43,86	9,611	2,60	6,62
E16085	8,270	7,3	2,344	0,98674	45,80	8,432	1,46	5,66
E16095	11,012	7,4	2,439	1,26413	48,36	10,780	2,60	8,44
E16135	8,217	7,1	2,439	1,38738	50,69	11,753	2,88	6,78
E16026	10,134	8,3	3,261	1,19009	63,62	10,015	2,84	7,82
E16036	7,094	8,0	3,261	1,28567	64,50	10,800	3,08	7,34
E16086	8,613	7,3	3,191	1,05189	67,14	8,762	1,50	6,30
E16096	12,134	7,3	2,929	1,19661	62,25	9,968	2,96	8,96
E16136	9,349	7,1	3,021	1,21296	67,39	10,021	2,88	7,26
E16027	12,215	8,3	2,679	1,06590	52,15	9,067	2,24	7,76
E16037	8,464	8,0	2,586	1,11295	50,94	9,451	2,68	6,04
E16087	8,658	7,3	2,417	0,76920	51,28	6,455	1,48	6,04
E16097	14,036	7,3	2,479	0,96179	53,32	8,054	3,16	9,08
E16137	9,992	7,1	2,437	0,95473	54,70	7,941	2,88	6,96
E16028	11,386	8,2	2,679	1,04340	49,91	8,970	2,28	7,26
E16038	9,695	8,0	2,586	0,82927	48,74	7,118	2,28	5,70
E16088	7,645	7,3	2,679	1,26358	52,88	10,779	1,46	4,76
E16098	14,326	7,3	2,679	1,19366	53,74	10,160	2,60	8,44
E16138	9,099	7,1	2,636	1,50343	55,60	12,706	2,96	6,62
E27062	10,318	7,3	0,750	2,12254	39,90	13,011	6,16	23,26
E27072	16,599	7,2	0,750	1,98807	40,29	12,153	11,72	36,36
E27102	13,090	7,4	0,708	2,04883	38,91	12,443	11,64	28,38
E27112	10,617	7,1	0,750	2,48667	41,55	15,068	12,96	30,42

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E27122	10,937	6,9	0,750	2,13960	41,87	12,936	10,80	50,24
E27132	7,252	7,0	0,750	2,73044	42,17	16,475	11,76	27,96
E27064	6,845	7,3	2,186	3,74273	73,49	26,437	12,40	48,88
E27074	11,559	7,2	2,280	3,27017	77,42	23,059	23,60	73,20
E27104	8,516	7,2	2,186	2,79715	76,05	19,641	26,60	67,04
E27114	7,472	7,0	2,099	3,62961	73,72	25,445	30,12	70,02
E27124	6,502	6,9	2,186	3,50635	77,46	24,541	24,84	70,02
E27134	5,443	6,8	2,099	3,65978	74,97	25,580	22,60	59,86
E27065	13,587	7,3	1,750	1,53233	37,74	12,780	8,40	32,70
E27075	19,915	7,2	1,934	2,62117	42,45	21,789	16,16	49,76
E27105	16,133	7,3	2,042	2,10126	46,69	17,329	16,00	39,70
E27115	12,374	7,0	2,042	3,15179	47,32	25,924	16,48	40,48
E27125	9,580	6,9	1,934	3,00634	45,42	24,663	15,40	40,12
E27135	8,240	7,0	1,934	3,42245	45,99	28,007	15,32	38,42
E27066	20,395	7,1	6,364	1,48565	57,88	16,761	10,04	40,64
E27076	30,367	7,2	7,000	2,35122	64,86	26,422	20,88	61,04
E27106	19,138	7,3	7,000	2,16324	67,87	24,073	21,16	51,10
E27116	15,268	7,0	7,000	3,14379	68,90	34,867	21,52	53,26
E27126	12,336	6,9	7,000	3,01563	69,93	33,355	20,16	52,44
E27136	11,311	7,0	7,000	3,23566	70,92	35,653	20,56	50,80
E18065	6,582	7,5	3,200	1,26711	68,53	10,725	5,68	8,90
E18075	6,752	7,5	3,200	1,38631	69,14	11,718	5,44	8,96
E18115	5,217	7,5	3,200	1,47210	71,56	12,374	5,40	8,74
E18125	5,813	7,0	3,200	1,47606	72,11	12,391	5,16	8,26
E18135	6,885	6,9	3,200	1,49893	72,68	12,567	5,60	8,54
E18066	6,537	7,5	4,082	1,45014	94,20	12,036	5,76	9,16
E18076	8,123	7,5	4,124	1,65455	95,87	13,710	5,64	9,28
E18116	5,606	7,5	3,918	1,59481	93,51	13,149	4,00	8,14
E18126	6,959	7,0	3,878	1,57789	93,34	12,980	4,72	10,72
E18136	10,506	6,9	3,878	1,65627	94,11	13,594	5,44	10,52
E18067	6,191	7,5	3,478	1,32784	70,87	11,359	5,16	8,20
E18077	9,228	7,5	3,478	1,56047	71,42	13,327	5,56	8,30
E18117	5,444	7,5	3,419	1,84044	72,32	15,618	5,16	7,96
E18127	9,132	7,0	3,419	1,56379	72,90	13,248	4,28	9,02
E18137	9,705	6,9	3,419	1,77435	73,53	15,003	5,28	9,70

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E18068	5,798	7,5	3,540	1.09697	76,39	9,206	3,36	7,74
E18078	7,949	7,5	3,482	1.39637	75,37	11,720	3,08	8,12
E18118	6,982	7,5	3,363	1.40940	74,84	11,784	3,24	8,52
E18128	8,864	7,0	3,423	1.37412	76,77	11,474	3,12	7,98
E18138	16,928	6,9	3,070	1.35395	69,52	11,297	2,96	7,64
E29042	7,798	7,5	1,034	2.40846	53,24	14,868	12,88	30,16
E29052	6,775	7,0	1,034	2.70095	53,55	16,655	12,56	31,88
E29062	5,251	6,9	1,034	2.93674	53,87	18,089	12,88	31,72
E29072	6,374	6,5	1,034	2.67317	54,20	16,447	13,28	32,60
E29102	6,081	6,5	1,034	2.70354	55,23	16,575	13,84	34,54
E29112	5,304	6,4	1,034	2.78261	55,58	17,040	14,36	35,12
E29132	7,244	6,3	0,989	2.69206	53,85	16,446	13,84	40,80
E29044	4,949	7,4	2,147	3.15202	71,55	22,495	22,16	55,46
E29054	4,684	7,0	2,147	3.13849	71,98	22,366	21,84	55,08
E29064	4,745	6,8	2,053	2.89538	69,27	20,602	23,64	58,40
E29074	4,752	6,5	2,053	3.08078	69,74	21,886	24,16	62,62
E29104	5,465	6,5	2,044	2.68994	70,79	19,023	22,92	58,68
E29114	3,829	6,3	2,044	2.97731	71,23	21,023	24,24	58,62
E29134	5,495	6,1	2,138	3.01389	75,41	21,216	23,64	69,00

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