

THE HUSBANDRY AND NUTRITION OF CARP LARVAE AND FRY.

Paul Lyndon Bryant BSc.

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

September, 1980.

11 FEB 1982

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SUMMARY.

In an investigation of the possibility of developing an artificial substitute for live food during first stage rearing of mirror carp (Cyprinus carpio) larvae, diet presentation and related husbandry techniques and selected nutritional requirements of carp juveniles were studied.

Carp larvae held at a temperature of 24°C in continuous light showed optimal growth and food conversion during the first five days after commencement of feeding when given Artemia nauplii in amounts ranging from 200 - 250% of body weight per day, reducing to 100 - 120% of body weight per day during the following five days.

It was found that carp larvae could be successfully reared on a dry, compounded commercial fry diet from a live body weight of approximately 15 mg, and that carp post-larvae of 15 - 100 mg body weight fed this diet showed optimal growth and food conversion for feeding rates of 15.0 - 17.5% of body weight per day. Carp fry of 100 - 500 mg body weight showed optimal growth and food conversion for feeding rates of 10 - 15% of body weight per day. These feed quantities were best administered in 12 equal amounts over 24 hours. It was also shown that presentation of green coloured diets to carp fry held on matching coloured backgrounds improved growth and food conversion.

It was postulated that a dietary crude protein content of 45% was optimal for carp fry of 100 - 500 mg live body weight, and that inclusion of lipid at levels above 5% of the dry diet did not appear to offer any advantages in terms of growth.

Manufacture of water-stable diets by the use of various binding agents and micro-encapsulation techniques did not substantially improve the performance of diets offered to carp larvae immediately after hatching, and it was concluded that further research will be required before it will be possible to eliminate the use of live food as a first food for carp larvae.

KEY WORDS: Cyprinus carpio - Carp larvae - Nutrition - Husbandry

ACKNOWLEDGEMENTS.

I am greatly indebted to Professor A.J. Matty for providing the opportunity to carry out this research, and would like to thank all of those friends and colleagues who provided assistance and encouragement throughout its course. I am particularly grateful to Mr. R. Watret of Edward Baker Ltd., to Mr. K.B.O. Maitland of the Caledonian Fishmeal Co.Ltd., to Dr. A.P. Scott of the MAFF Fisheries Laboratory at Lowestoft, to Dr. J. Adron of the NERC Institute of Marine Biochemistry, to Dr. P. Smith of BP Nutrition (UK) Ltd., and to Professor S.P. Meyers of Louisiana State University for their generous donations of research materials. I should also like to thank Dr. K. Dabrowski and Mrs. M. Varley for their assistance in the translation of foreign language publications.

I am specially grateful to Drs. A. Jackson and A.G.J. Tacon for their friendship and the many lively and helpful discussions in which they participated, and to Dr. A. Jackson for his critical appraisal of the manuscript. To my wife Pam I extend my most sincere thanks for the unstinting moral and practical support given in the last three years.

P.L.Bryant

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CHAPTER 1.

Chapter 1. Introduction.

1.1. Constraints on Aquaculture.

While it is only in this century that the term aquaculture has become associated with an animal husbandry industry seeking to produce aquatic organisms, especially fish, largely for human consumption, it is now widely appreciated that the foundations of this practice were laid in pre-Christian times. Documentary evidence of managed fish and shellfish populations attest the existence of some forms of aquaculture in Greek and Roman times, and which have been continued in various parts of the world until the present day (Sarig, 1966; Bardach et al, 1972).

Following the pattern set by terrestrial animal husbandry industries, fish culture in the developed countries has tended to become increasingly intensive in approach, with increased reliance on sophisticated technology to maximise water use and yields, in contrast with the more traditional methods of pond fish farming which largely rely on increasing the natural productivity of enclosed water bodies. The modern, intensive approach to fish farming has been predominantly associated with the culture of salmonid species because these fish are best adapted to the climatic conditions which prevail in much of the developed world. Thus a considerable proportion of the research relating to the physiological and nutritional requirements of fish under intensive culture conditions has been directed towards the salmonid species, despite the fact that the major part of the currently estimated 6 million tonne annual production of the global fin- and shellfish farming industries is comprised of warm water, non-salmonid species derived mainly from the less-developed countries.

A concensus of opinion predicts that production of farmed fish

will have greatly increased by the end of the century, some estimates suggesting a figure of 30 million tonnes per annum. Whether this production will come about by more intensive use of water resources or by an expansion of traditional pond farming remains to be seen, but it is certain that a greatly increased production is expected from the developing countries with emphasis on the culture of warm water species, as well as from mariculture.

The intensive culture of salmonid species has been greatly facilitated by the ease with which they may be bred and reared in captivity. This is not the case for a large number of commercially important warm water and marine fish species, and some established fish culture industries such as the farming of the Asiatic cyprinids have in the recent past relied heavily on recruitment of juveniles from natural fish populations. While the modern technique of induced breeding has made the mass propagation of such refractory species possible, considerable difficulties concerning the rearing of the larvae still exist. The massive projected increases in farmed fish production by the end of the century will therefore require substantial improvements to be made in the methods of rearing certain larval and juvenile fish species. That intensive mass production of juveniles is necessary is evidenced by the rapidly expanding number of specialised warm water fish hatcheries producing such species as Cyprinus carpio, Esox spp. and Coregonus spp., previously supplied in adequate quantity by reproduction under natural conditions (Woynarovich, 1973). Controlled reproduction is not only necessary to meet the demand for fish seed, it also makes possible the genetic selection of breeding stock and production of improved strains (Purdom, 1972).

An important potential constraint on the continuing expansion of aquaculture is thus the provision of large numbers of juvenile

fish for stocking purposes, particularly of certain warm water and marine species of proven or potential value.

1.2. Artificial Propagation.

It is now some fifty years since the pioneering work on control of reproduction in fishes was begun. Despite the large number of investigations into the physiology of the pituitary gland and its role in the reproduction of fish, it is only in the past twenty years that this knowledge has been incorporated into practical fish culture procedures on a regular basis, and its impact has been profound.

The first researchers to investigate the effects of pituitary hormone extracts on fish used mammalian and synthetic hormones and were not able to obtain very encouraging results (Young and Bellerby, 1935; Owen, 1936; Johnson and Riddle, 1939). The first to use piscine pituitary extracts in such work were the Brazilians Houssay (1931), Cardoso (1934) and Von Ihering and Azevedo (1934), who showed that it was possible to bring about maturation and spawning in freshwater fish of the families Characidae and Doradidae, and subsequent experimental data such as that of Hasler et al (1939), Ball and Bacon (1954) and Palmer et al (1954) showed the relatively higher efficiency of fish pituitary extracts over those of mammalian origin when injected into fishes. The early literature on the physiology of the pituitary gland and induced spawning of fishes was reviewed by Atz and Pickford (1959).

One of the earliest attempts to spawn carp was made by Khan (1938) in India. Using the Indian major carp Cirrhina mrigala, he administered extracts of mammalian pituitary at a concentration of 3.2 - 6.4 mg/Kg once weekly, and the commercial synthetic hormones Prolan and Antuitrin S at a concentration of 20 - 40 Rat Units daily and on alternate days. Although all treated fish ovulated

no viable eggs were obtained. Successful spawning of the Indian major carps was not achieved until some years later, when Chaudhuri and Alikunhi (1957) reported the breeding and rearing of large numbers of fry of the species Catla catla, Labeo rohita, Cirrhina mrigala, Hypothalmichthys molitrix, Ctenopharyngodon idella, Cirrhina molitorella, Mylopharyngodon piceus and Cyprinus carpio. With the exception of Cyprinus carpio, none of these species had ever been domesticated on any significant commercial scale before. The successful introduction of hypophysation on a regular basis into Indian piscicultural procedures has enabled the valuable Indian major carps to extend their range, and permitted considerable progress to be made in the selective breeding of these species. There can be no doubt that the practice of induced breeding has made a useful contribution to Indian fish culture.

The method of induced breeding is important because it permits hatching and rearing of larvae and fry under controlled, weather-independent conditions, improvement of stocks by genetic manipulation and prevention of the spread of diseases and parasites from parents to progeny. The technique of hypophysation is now well-established throughout Europe, the Middle East and the Americas (Shehadeh, 1973). However, despite its widespread adoption, relatively little is known about the precise mode of action of piscine gonadotrophins and most commercial hatcheries rely on the use of crude homogenates of whole fish pituitaries to bring about forced ovulation of a variety of fish species. Since the success of hypophysation depends upon both the potency of the fresh or preserved pituitary material and the vitellogenic stage of maturity of the female spawner, neither of which are invariably closely controlled, successful ovulation is frequently only obtained in approximately 50% of attempts (Shehadeh, 1973). There is thus ample scope for improvement of the tech-

nique.

Collection of fish pituitaries for the production of a crude gonadotropin extract is an extremely time-consuming process. Moreover, the commonly used acetone-dried pituitary powder contains a large number of other hormones in variable proportions and their intervention may not always be beneficial to the physiology of fish and especially to the quality of the genital products. Efforts have therefore been made to isolate the gonadotropic hormone or hormones from the pituitary of fish.

The available evidence seems to favour the existence of only one gonadotropic hormone in teleosts, rather than the two present in higher vertebrates. Purification of both carp and salmon gonadotropin from pituitary extracts has yielded just one fraction of high gonadotropic activity, differing from both mammalian luteinising hormone (LH) and follicle stimulating hormone (FSH). Evidence for the existence of two piscine gonadotropins derives from the observation of two gonadotropic cellular types in the pituitaries of some teleosts, such as the eel, goldfish and carp (Fontaine, 1976). However, immunofluorescence studies of purified carp gonadotropin (c-GTH) have revealed only a single population of gonadotropes in carp pituitaries (Billard et al, 1971).

The pituitary itself is under the control of the hypothalamus. In the more primitive cyclostomes, the pituitary seems capable of autonomous function, or is at least controlled by a blood borne factor in the systemic circulation. In teleosts, however, the pituitary is under direct nervous control as in higher vertebrates, from the nucleus lateralis tuberis. There does not appear to be any zoological specificity in the action of the gonadotropin releasing factor (GTH-RH). The peptide containing nine amino acids isolated from mammalia is capable of releasing gonadotropin in carp and

brown trout, and similarly carp GTH-RH is capable of releasing GTH from sheep pituitaries in vitro (Fontaine, 1976).

This lack of specificity is not shown by the piscine gonadotropins themselves. To date, two hormones of high activity have been isolated from Oncorhynchus tshawytscha (Donaldson et al, 1972) and Cyprinus carpio (Sinha, 1971; Burzawa-Gerard, 1971); both are capable of restoring all the diverse stages of maturation in hypophysectomised teleosts. However, these two gonadotropins differ in their ability to stimulate adenyl cyclase activity in goldfish ovary homogenates, the salmon GTH being some 36 times less effective than c-GTH in this assay (Fontaine et al, 1972). Furthermore, purified sturgeon GTH has shown a difference in activity in amphibian assays from c-GTH, together with a different amino acid profile and sub-unit structure (Burzawa-Gerard et al, 1976). It has long been known that homoplastic pituitaries are the most effective for spawning fish of a particular species, and piscine GTH may thus possess some species specificity.

Physical characterisation of the carp and salmon gonadotropins has shown that both possess a fairly similar molecular weight. Carp GTH has been identified as consisting of two sub-units possessing molecular weights of approximately 27,000 and 15,000, the larger molecule retaining some potency after separation, while the smaller molecule loses all effect (Donaldson, 1973). Salmon GTH has been found to possess a molecular weight of approximately 29,000 with a sub-unit weight of 13,000 (Chaudhuri, 1976). Both carp and salmon GTH are acidic in nature, resembling FSH more than LH in this respect, and c-GTH furthermore resembles FSH in terms of its amino acid residues (Sinha, 1971). However, when mammalian hormones are observed to have a positive affect on spermiation and ovulation, LH activity predominates. Anti-ovine LH also complexes with

carp and salmon gonadotropes in the pituitary, whereas anti-ovine FSH does not, but neither LH nor FSH reacts with rabbit anti-serum to partially purified c-GTH (Fontaine, 1976).

It is presumed that the fish gonadotropins exert their effect at certain stages in the reproductive cycle through sexual steroids produced by the endocrine tissues of the gonad. The evidence for the role of sexual steroids is, however, rather contradictory. Androgens such as methyl testosterone and testosterone have been found capable of inducing spermiation in some species, but in others, for example Heteropneustes fossilis, testosterone actually suppresses spermatogenesis (Sundararaj et al, 1971). In the female, similar problems have been encountered: estradiol has been shown to have both a stimulatory and inhibitory effect on vitellogenesis (Schreck and Scanlon, 1977). Of all the steroids tested, the steroid 17α - hydroxy 20β dihydroprogesterone seems to be the most effective agent for induction of ovulation, and has been isolated from the plasma of spawning Atlantic salmon (Schreck and Scanlon, 1977).

The corticosteroids seem to have more affect on ovulation than the gonadal steroids. These can be synthesised by both the ovary and the interrenal. Cortisol and cortisone have been shown to synergise 17α hydroxy 20β dihydroprogesterone, but gonadotropin induced corticosteroid biosynthesis has not been demonstrated in the salmon, although it occurs in the catfish Heteropneustes fossilis (Jalabert, 1975). LH stimulates the interrenal, but this may be a pharmacological action rather than a normal physiological mechanism (Fontaine, 1976).

Recently, Jalabert et al (1977) have used 17α hydroxy 20β dihydroprogesterone as an aid in the induced spawning of carp. They noticed that a priming dose of pituitary extract was still necessary to bring about maturation of the oocytes from stage 3

to stage 4, the migration of the nucleus towards the micropyle (see section 1.2.1); 17α hydroxy 20β dihydroprogesterone apparently had no effect until this stage had been reached in carp and a number of other species. Once this maturity stage was achieved however, administration of the steroid could bring about ovulation at a lower temperature than is normally necessary for the successful induction of ovulation in carp using pituitary extract alone. Jala-bert et al (1977) postulated that the failure of the pituitary extract or c-GTH to bring about ovulation at low temperature was due to the high temperature requirement for biosynthesis of the natural steroid mediator which brings about ovulation. They suggested that 17α hydroxy 20β dihydroprogesterone mimicked the action of this steroid, if it was not the steroid itself, and that once the steroid was synthesised it did not need a high temperature to act. How the pituitary extract worked to prime the oocytes was not clear, but it must involve some other substance unlike 17α hydroxy 20β dihydroprogesterone.

While there is thus still some controversy surrounding the exact mode of action of piscine gonadotropins, considerable progress has been made and it would appear only a matter of time before more effective, synthetic alternatives replace the use of crude pituitary extracts for the induction of ovulation in fish. However, in order to guarantee a high quality of genital product, it will also be necessary to regulate the timing of hormone administration to coincide with the most receptive stage of vitellogenesis.

1.2.1. Optimisation of Hypophysation.

Many researchers have observed that selection of "ripe" female fish for spawning induction is crucial to the success of the operation, but few have attempted to define the required degree of "ripeness" in objective terms. Selection on the basis of such characters

as the colouration of the vent and the softness of the abdomen (Chen et al,1969) are unreliable and subjective and result in poor success rates for hypophysation in the field. A practical and reliable method for assessing the state of maturity of female mullet (Mugil cephalus) has been developed (Kuo et al,1974),which could be applied to many other commercially important species requiring hypophysation as a routine procedure.

Oogenesis in the mullet has been divided into five general stages according to the histological appearance of the oocytes. The first two stages relate to immature oocytes which are characterised by the presence of a nucleus occupying the greater proportion of the oocyte and the gradual accumulation of yolk vesicles. The third stage of development is associated with the accumulation of yolk,the appearance of the zona radiata and the disappearance of the nuclear membrane. At the end of this stage,the oocytes are mature and will respond to injected pituitary extracts or purified piscine GTH. The oocytes of the fourth,ripe stage have only been observed in hypophysised females immediately before ovulation,and are characterised by the migration of the nucleus toward the animal pole and the fusion of yolk globules and oil droplets. Many short villi also appear on the surface of the egg. The fifth stage describes oocytes undergoing atresia.

It was found by Kuo et al (1974) that the diameter of oocytes was linearly related to their stage of maturity,and that the sexual maturity of the fish could be expressed in terms of mean oocyte diameter. This could be calculated from the egg diameter frequency of samples collected in vivo from unanaesthetised females through a polyethylene cannula inserted into the oviduct of the fish (Shehadeh et al,1973). Furthermore,it was found that the dose of partially purified salmon gonadotropin required to induce spawning

was linearly related to the initial mean egg diameter, ranging from 12 - 21 $\mu\text{g/g}$ body weight for oocytes ranging in diameter from 600 - 730 μm . The dose was applied in two injections, one third of the total dose being injected 48 hours before the remainder; this sequence was found to be necessary to avoid partial spawning. Oocytes with diameters below 600 μm would not respond to this treatment, but maturation of oocytes with mean diameters of 500 - 600 μm could be brought about by a sequence of injections increasing from 0.12 to 3.4 $\mu\text{g/g}$ body weight daily over a period of six to eight days.

The synchronous development of the oocytes of the mullet facilitates the measurement of the stage of sexual maturity of the female. However, in Cyprinus carpio, the species used in the course of the research reported here, ovarian development proceeds differently. Six stages of oocyte development are recognised in the carp (Gupta, 1975; Bieniarz and Epler, 1976), which may be summarised as follows:

- (1) Stage I; immature oocytes characterised by a large nucleus at the centre of the cell and surrounded by a single layer of follicle cells; 50 - 150 μm in diameter.
- (2) Stage II; oocytes characterised by yolk accumulation at the periphery of the ooplasm and a peripherally migrating nucleus less than half-way to the edge of the cell; 150 - 300 μm in diameter.
- (3) Stage III; advanced maturing oocytes characterised by yolk globule accumulation in the inner ooplasm eventually fusing to form a continuous mass, disintegration of the nuclear membrane and migration of the nucleus more than half-way to the periphery, and development of oolemma and follicular and granulosa layers; 300 - 700 μm in diameter.

- (4) Stage IV; mature oocytes characterised by a nucleus adjacent to the micropyle and complete disintegration of the nuclear membrane; 700 - 850 μm in diameter.
- (5) Stage V; ripe oocytes characterised by disappearance of nuclear contents and development of short villi on the surface, and only observed after hypophysation immediately before ovulation; 800 - 900 μm in diameter.
- (6) Stage VI; atretic oocytes characterised by fragmentation of granulosa layer and breakdown of the oolemma.

In vivo ovarian biopsy techniques were developed for carp by Bieniarz and Epler (1976), and application of these to a study of ovarian development in sexually mature carp throughout the yearly cycle (Bieniarz et al, 1977, 1979) has revealed that all stages of oocyte development, with the exception of Stage V, may be observed at any time. Mature oocytes in Stage IV of development predominate throughout the yearly cycle except immediately after spawning, when oocytes in Stages I and II of development proliferate in order to replace oocytes lost during ovulation. Changes occurring in the ovaries during the yearly cycle consist of a gradual development of a resorption process in the oocytes after a completed vitellogenesis, the store of oocytes being progressively supplemented by those in earlier stages of development (Bieniarz et al, 1979), irrespective of whether spawning occurs. This process is also independent of temperature. Gupta (1975) found that mature oocytes were present in considerable numbers even after what was considered a reasonably complete spawning, and Bieniarz et al (1979) showed that these residual oocytes would respond to hypophysation, by demonstrating nucleus migration, within two weeks after a previous induction of ovulation, although further ovulation did not necessarily result.

There is thus no endogenous sexual rhythm in carp and induced spawning can be accomplished several times and throughout the year (Meske et al,1968). While multiple spawnings of carp are facilitated by maintenance of year-round high temperatures (Kossmann,1973), the temperature-independent development of the ovary implies that this is not strictly necessary; Huisman (1973) recorded that carp from outdoor ponds at very low temperatures could be successfully spawned immediately after acclimation to hatchery temperatures.

Because of the confusing picture which may be obtained from ovarian biopsy samples, it is difficult to apply mean egg diameter criteria to carp as a means of assessing the receptiveness of the recipient female to hypophysation, as is possible for the mullet. Since carp females would appear receptive to hypophysation at all times except immediately after spawning, it is difficult to explain why some fish do not respond at all to pituitary injections. Much more information about the process of resorption of oocytes, and how this affects the response of other apparently mature oocytes to hypophysation, is required before it will be possible to optimise the induced spawning procedure for Cyprinus carpio. Available information would suggest that it is possible to obtain some eggs from carp females held under constant environmental conditions after every spawning attempt, but that the quantity and quality of these will be dependent on the vitellogenic stage prevailing at the time of the attempt, which will be different for each female. Synchronous ripening under natural conditions is presumably influenced by external factors, which are well-documented (Sarig,1966), but exactly how these act at a biochemical level is unknown.

1.2.2. Egg Incubation.

Losses of eggs and larvae after natural spawning are often

very great, even when semi-controlled spawning is effected in specially constructed ponds or enclosures. The potential benefits of incubation under aseptic and manageable conditions are therefore considerable, reducing losses to poor fertilisation, disease and predation.

While many fish eggs can be incubated successfully en masse in simple flowing water containers, until techniques were developed some twenty years ago the artificial incubation of carp eggs, among those of a few other species, presented serious problems. In nature, carp eggs possess a sticky surface mucin which attaches them to plants and other floating or submerged substrates. When incubated in containers, the eggs stick together to form clumps, which may deprive eggs at the centre of the clump of oxygen. These dead eggs are then frequently attacked by fungi which may spread to adjacent eggs and kill them. Until a method was devised to inactivate the sticky layer, artificial incubation tended to be accompanied by higher mortalities than were encountered in ponds.

Woyнарovich (1955) introduced a commercially applicable technique for overcoming this problem, which originally consisted of mixing eggs and milt in small amounts of water, whereupon fertilisation took place, followed by separation and spreading onto sack cloth. Eggs treated in this manner could withstand transport for up to twelve hours if kept in damp moss. The sheets of sack cloth with attached eggs were then suspended in a special spray room, where a fine water mist produced by high-pressure spray heads kept them moist; just prior to hatching, the eggs were transferred to hatching ponds and culture continued in the normal fashion. However, this was a tedious and expensive technique and has since been superceded by better methods.

Woyнарovich (1960, 1962) devised another technique whereby

permanent de-gumming and fertilisation of carp eggs could be effected simultaneously. Investigation of the affect of various salt solutions on the mobility of carp spermatozoa, with reference to their mobility in fresh water revealed that a solution containing 0.3% urea and 0.6% sodium chloride both prolonged the activity of the spermatozoa and reversibly inhibited the stickiness of the eggs. After treatment with this solution for approximately one hour, further immersion in a 0.85% urea solution permanently removed the glycoprotein adhesive layer from the eggs.

Konradt and Sakharow (1966) also developed an alternative technique which proved faster than the above, but which is not widely used. They found that the glycoprotein layer could be removed by treatment of the eggs with a crude hyaluronidase solution prepared by acetone extraction of pig testicles; a freshly prepared solution of the extract was effective within 25 - 35 minutes. They also found that dilute tannic acid solutions were effective for removal of the glycoprotein layer surrounding beluga sturgeon eggs, and a combination of the Woynarovich (1962) technique and a tannic acid treatment after Konradt and Sakharow (1966) is now most often used during the preparation of carp eggs for incubation.

1.3. Larval Rearing.

The gradual supplanting of pond rearing techniques for fish larvae and juveniles by intensive production in hatcheries has meant that food must be taken to the fish rather than vice versa. This practice presents some serious problems for many species of fish larvae, particularly for those which derive from smaller eggs than are produced by the Salmonidae. Under natural conditions, all fish larvae are carnivorous during the early part of their life cycle and feed upon various kinds of zooplankton, the provision of which in the large quantities required for a commercial hatchery

can be problematical. Thus mass-rearing of fish larvae has required the development of mass-rearing techniques for living fish food sources for those species of fish larvae which cannot currently be cultured on alternative feeds. Only a relatively few species of invertebrates have proved themselves amenable to mass culture as a source of food for fish larvae, although fortunately these have been acceptable to a large number of fish species. The most notable among these living food organisms are the rotifer Brachionus plicatilis and the branchiopod Artemia salina.

The success of complete, compounded artificial diets for the rearing of post-juvenile fish in commercial fish culture has suggested that similar formulations may be employed during the larval phase, particularly since the costs of living feed production are high (Girin, 1979). While it has proved possible to rear salmonid larvae in this way, preparation of artificial diets for other species of fish larvae, especially those of marine origin, has presented considerable technical difficulty often ascribable to their small size and feeding behaviour (van Limborgh, 1979).

A number of commercially important warm water and marine fish species produce eggs of 1 mm or less in diameter (eg. Cyprinus carpio, Scophthalmus maximus and Dicentrarchus labrax) which produce correspondingly small larvae in comparison with those of many salmonid species. Carp are unable to ingest living feeds having a diameter of greater than 200 to 300 μm , and the production of similarly sized or smaller artificial feed particles is practically difficult. Firstly such small particles present a very large surface area to the water, which may cause their disintegration or substantial loss of water soluble ingredients. Secondly, these feed particles must individually represent the overall formulation of the diet, else the fish may ingest an unbalanced ration,

which necessitates the use of finely comminuted or dissolved ingredients. Thirdly, larvae must be able to recognise such particles as food and eat them, which means they must combine both physical and possibly chemical attractiveness.

With the recognition of these problems have come several ingenious suggestions for their solution (Meyers and Butler, 1971; Meyers, 1979; Metailler et al, 1979), such as the micro-encapsulation of dietary ingredients within an impermeable but digestible or rupturable capsular material, or their stabilisation by natural or synthetic binding agents. While the difficulties associated with manufacture of larval diets may therefore be superable, very little success has so far been achieved with the rearing of larvae that have accepted such artificial diets and it has become clear that a better understanding of larval nutrition must be gained before this will be possible.

1.3.1. The Rearing of Carp Larvae.

Carp eggs usually range from 0.9 - 1.1 mm in diameter before post-fertilisation water absorption and the newly hatched larvae measure some 6 - 7 mm in length and weigh approximately 1 mg. The yolk sac is small and its absorption is complete after 48 hours at a temperature of 21 - 24°C (Neudecker, 1976), when the larvae commence exogenous feeding. The gape at this stage is approximately 550 µm (Shirota, 1970) and the larvae are capable of immediate ingestion of Artemia nauplii, which have proved to be a suitable food for the first few weeks of rearing (Woynarovich and Kausch, 1967; Kossmann, 1973; Huisman, 1973).

It has been found that carp larvae will ingest inert diet particles of a suitable size quite readily and as soon as they commence active feed intake (Appelbaum, 1976), therefore acceptance of artificial diets by this species does not constitute a problem

as it does for some other species. Carp larvae also feed throughout the water column and from the base of a rearing container, thus this favourable feeding behaviour has prompted several researchers to investigate the possibilities of rearing carp larvae on various artificial diets.

Early experiments concentrated on the use of readily available substances for feeding carp larvae immediately after hatching, such as chicken egg, beef liver, cottage cheese and several commercial trout and salmon fry diets and aquarium fish feeds (Meske, 1973; Kainz, 1974). None of these were successful without additional live food in the form of zooplankton or Artemia, most causing considerable mortalities which were partly a result of severe water pollution.

Subsequent experiments by other researchers provided more encouraging results (Anwand et al, 1976; Lukowicz and Rutkowski, 1976), but mortalities were still high and growth poor in comparison with fish fed live food. These more suitable diets were formulated from a large number of ingredients and lyophilised protein sources and were subjected to chemical analyses in order to assess their nutritional value. The relatively poor performance of the diets could not be easily explained in terms of their analysed nutritional value.

More recent experiments (Dabrowski et al, 1978, 1979; Dabrowska et al, 1979) sought to improve the performance of compounded larval diets for carp by addition of enzyme extracts and control of pH, which had been shown by other researchers to affect assimilation of nutrients in young fish (Nose et al, 1974; Wilson et al, 1977; Dabrowski and Glogowski, 1977a, c), but these measures did not have any appreciable effect on the growth or survival of the experimental larvae, which were markedly inferior to larvae fed on live food.

The most encouraging results to date have been obtained by Appelbaum (1977) who found that carp larvae could be successfully reared on a diet consisting largely of the petro-protein yeast, Candida lipolytica, with vitamin and mineral supplements. Although growth was still poor in comparison with Artemia-fed carp larvae, it was better than has been reported by any other researcher and the survival of the larvae fed on the yeast diet was apparently excellent (> 70%). Thus it is clear that the rearing of carp larvae on artificial diets is not an impossibility.

1.4. Research Objectives.

Because of their favourable feeding behaviour, carp larvae present fewer problems as an experimental animal than many other species of fish larvae which are difficult to rear on artificial diets, while the adult carp are amenable to culture and controlled reproduction under laboratory conditions. It would also seem plausible to suggest that the nutritional and practical difficulties associated with the rearing of carp larvae on artificial larval diets may be representative of other freshwater and perhaps even marine species. Thus carp larvae would appear to be a suitable experimental animal for which to study these difficulties, with reasonable expectations for extrapolation of results to other larval fish species. With these considerations in mind, together with the commercial importance of carp as a species in their own right, it was decided to use carp as the experimental animal in the following studies of the practicalities of rearing their larvae entirely on artificial diets.

At the commencement of the research, considerable emphasis was being placed by other researchers on the importance of the physical presentation of larval diets, thus close attention was initially paid to this subject. The research was begun by an

investigation of the effects of diet preparation and presentation on the acceptance and utilisation of such diets by carp larvae (Chapter 8). However, the disappointing results of this work indicated that the physical presentation of the diet was not in itself the most important factor governing the success of an artificial diet, and that many undefined variables precluded a rational approach to the problem of developing a successful artificial diet for carp larvae.

It was not known with any accuracy what the growth potential of carp larvae fed on the best available diet (Artemia) was, and to what extent larvae fed on artificial diets were falling short of this potential. Therefore it was decided to define the correct feeding rates for the live food employed before proceeding further (Chapter 3). In addition, it was realised that presentation of arbitrary diet formulations largely based on the nutritional requirements of adult fish to larvae was an unsound approach. Since it was impossible to nutritionally optimise the diet formulation without first developing a successful larval diet which could be used to define the nutritional requirements during the larval stage - a circular argument - it was decided to adopt a more logical approach to the problem.

Since it had been demonstrated that fish larvae could be reared on diets initially unsuited to post-hatching larvae after a relatively short period of growth (Barahona-Fernandes and Girin, 1976; Bromley, 1977), it was reasoned that investigation of the earliest weight at which carp larvae could be transferred to an arbitrary diet formulation with acceptable growth and survival would provide a more logical starting point for an investigation of the nutritional requirements of the larvae (Chapter 4). It was considered that nutritional optimisation of the diet for these older

fish would permit manufacture of a diet which would allow rearing to be commenced at a lower body weight with essentially the same growth and survival as obtained before.

Before commencing on this programme of research, it was decided to define certain other factors which could conceivably have exerted a negative influence on the performance of artificial diets, which may be classified as husbandry techniques, namely the optimal quantities of artificial diet compatible with maximum growth and minimum wastage (Chapter 5) and the feeding frequency and diet colour promoting optimal particle acceptance and utilisation (Chapter 6). Only then was an investigation of the nutritional requirements of artificial diet-adapted carp juveniles begun (Chapter 7). It was hoped that definition of the optimum ratio of crude protein to lipid in diets for these juveniles would permit the formulation of a diet producing better growth and allowing earlier adaptation than the arbitrary diet initially used to define the adaptation weight (a commercial trout fry diet). It was then intended to begin the process of nutritional optimisation again, in order to progressively reduce the weight at which larvae could be successfully adapted to an artificial diet, eventually resulting in a diet which could be used from the beginning of food intake.

CHAPTER 2.

Chapter 2. General Materials and Methods.

2.1. Brood Fish Facility.

Due to the requirement for a regular supply of experimental carp larvae, it was necessary to construct a facility capable of maintaining a resident stock of adult carp, together with facilities for spawning induction and egg incubation. Initially, brood fish were held in an ambient temperature recycling system, temperature acclimation being brought about by transfer to a separate warm water recycling system for a short period before hormonal induction of spawning was begun.

With the acquisition of a greater number of brood fish, it was decided to convert the original ambient temperature recycling system into a warm water facility with increased carrying capacity, in order to hold the fish continually at a temperature of between 18 and 25°C. This eliminated the need for a temperature acclimation period before induction of spawning could be begun and enabled multiple spawnings of individual fish to be effected during the course of a year.

The arrangement of the components of this system is shown schematically in Fig.1. Brood fish were held in a 2000 litre glass fibre tank, which also contained a 3 Kw stainless steel immersion heater (Bunting Titanium Co., Ltd) screened from the fish by an insert of galvanised iron mesh (2.5 cm aperture). The output of this heater, which operated continually, was balanced against an inflow of Birmingham tap water to maintain a temperature of approximately 22°C in the system. A thermostat set to switch off the immersion heater at 28°C was included in the holding tank as a fail-safe device to safeguard against any cessation of inflow into the system. The fresh water inflow required to maintain the desired system temperature varied from 3 - 20 litres per minute,

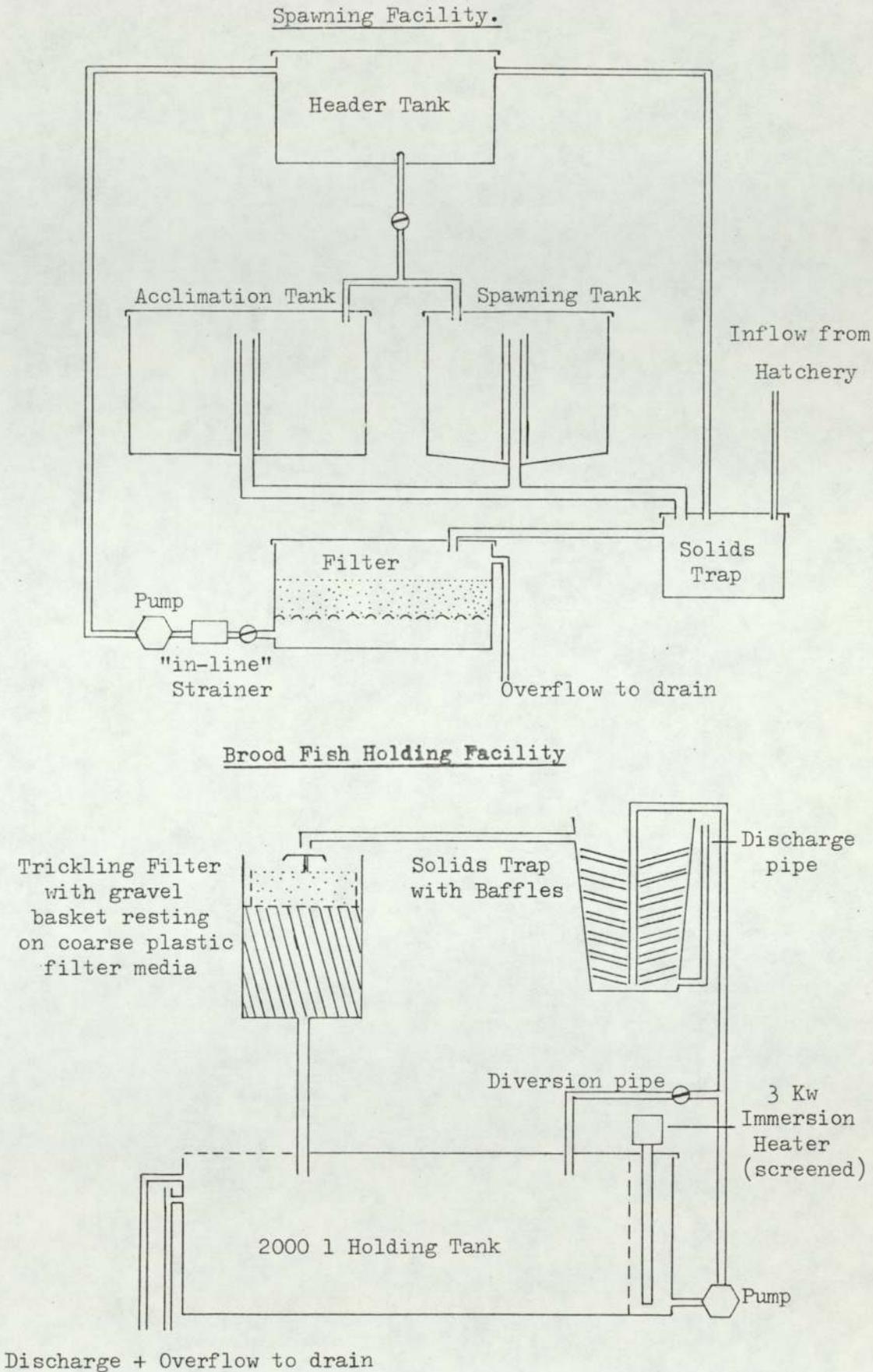
dependent upon season.

Water for the partial recycling loop was withdrawn from the base of the holding tank within the immersion heater enclosure via a 0.2 Kw centrifugal pump (James Beresford & Son Ltd.) delivering approximately 40 litres of water per minute. A proportion of this output was returned to the holding tank immediately to promote oxygenation of the water, the remainder being discharged into the base of a solids trap containing short lengths of pipe wired into circular bundles and directed towards the base of the solids trap. Seven layers of these pipe bundles were stacked one above another, their purpose being to increase the sedimenting efficiency of the trap. The solids trap could be easily drained and cleaned by inversion of a moveable stand pipe inserted at its base and to one side.

Overflow water from the solids trap then traversed a trickling filter containing two types of media before return to the holding tank. After dispersion by a diffusor plate, filter water passed first through a layer of 10 mm gravel 30 cm deep, contained in a plastic netting basket to facilitate removal and cleaning, followed by a 60 cm depth of coarse plastic media consisting of 5 x 3 cm ribbed plastic cylinders. Filter outflow water returned to the holding tank through a length of perforated pipe to enhance oxygenation.

The holding tank was fitted with a heavy wooden lid to prevent fish escaping, containing a netting insertion to permit observation and feeding of the fish. Waste water was removed from the tank by means of a constant level overflow device. Water quality within the system was routinely determined at 3 - 4 day intervals; over a seventeen month utilisation period, temperature ranged from 18.5 - 23.8°C, pH from 6.2 - 7.1, total ammonia from 0.1 - 0.8

Figure 1. Schematic Arrangement of Holding and Spawning Facilities.



mg/litre and dissolved oxygen from 3.2 - 7.4 mg/litre, nitrate and nitrite measured together from 2.5 - 9.0 mg/litre and total hardness from 20.5 - 39.5 mg/litre CaCO_3 .

2.2. Spawning Facility.

A separate warm water recycling system was constructed to serve a dual purpose, firstly to allow temperature acclimation of brood fish transferred to the system from one at ambient temperature as well as to allow segregation of the sexes during spawning induction, and secondly to provide an extra holding facility for non-experimental fry outgrowing the hatchery.

The arrangement of the components of this system is shown schematically in Fig.1. Two double-skinned, insulated glass fibre tanks of 400 and 200 litres capacity were used to hold selected brood fish, female spawners usually being confined to the smaller tank when injected to reduce the risk of premature egg release. Outflow water from these tanks traversed a 40 litre capacity solids trap before draining into the biological filter.

The filter was of the submerged type and contained 200 litres of 20 mm gravel and crushed cockle shells, the latter providing some buffering capacity. The filter media rested on a filter plate consisting of a sheet of perforated, corrugated plastic, itself supported on an arrangement of bricks in order to create a continuous water space beneath the gravel from which the pump could draw. The filter plate was sealed to the sides of the filter tank with a surround of polyurethane foam, preventing any gravel from falling into the pump water space below. A water depth of 20 cm was maintained above the surface of the filter gravel by means of an overflow at the required height.

Water was pumped through the filter by a 0.2 Kw centrifugal pump (James Beresford & Son Ltd), protected by an "in-line" strainer,

and passed to a 200 litre polyethylene header tank before being returned to the holding tanks. The header tank, filter and solids trap were insulated with a 2 cm layer of expanded polystyrene and all of the tanks were fitted with lids to prevent excessive water and heat loss through evaporation.

The temperature of the spawning facility was maintained at $23 \pm 0.5^{\circ}\text{C}$ by means of the waste water from the incubation and experimental facility, which was not operated on a recycling basis. Waste water discharged into the solids trap of the spawning facility in order to effect some purification before re-use, the good insulation of the system reducing temperature losses to approximately 1°C . Without any heat input, the spawning facility lost 2.5°C per hour when the external air temperature was 13°C .

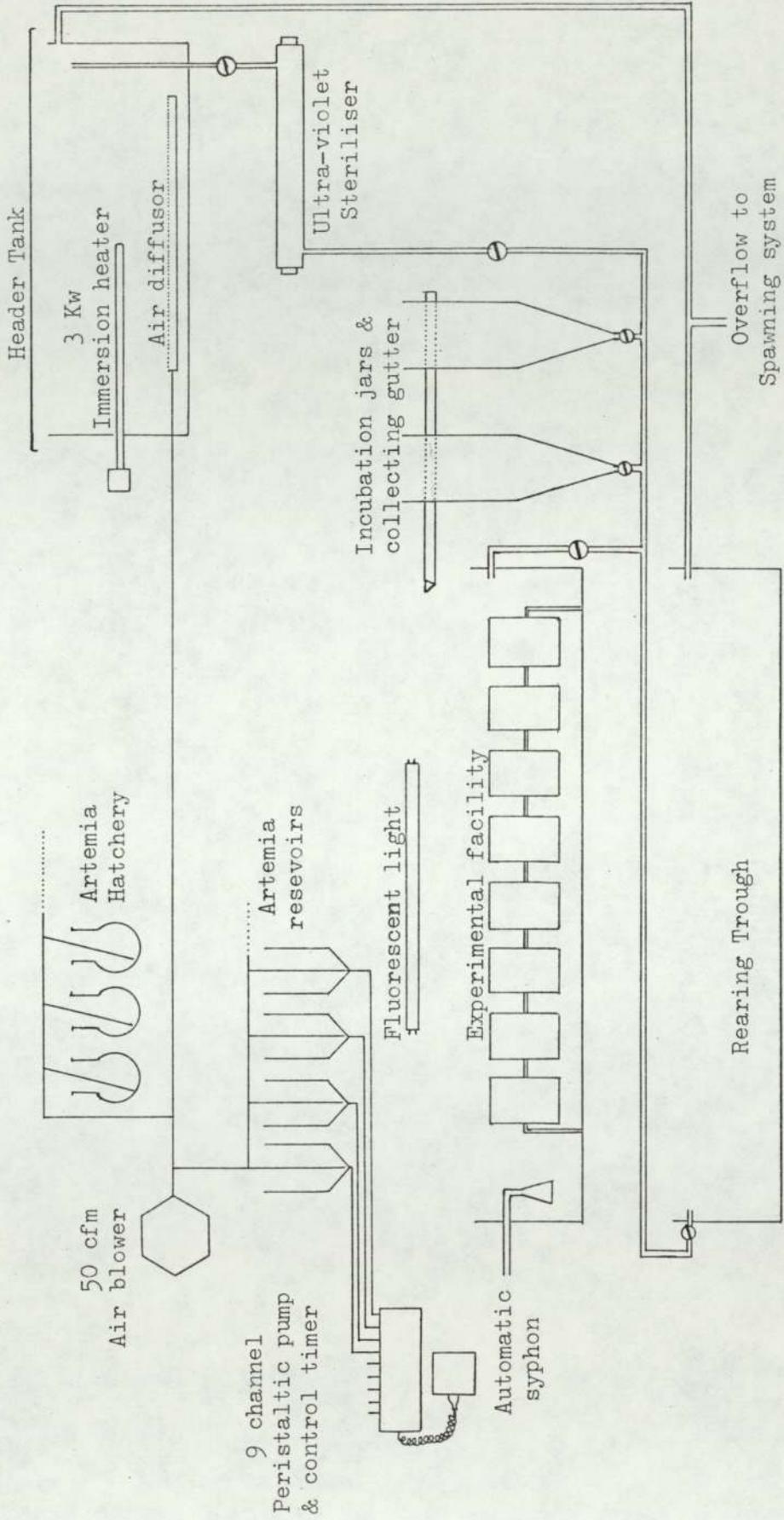
Water quality parameters were measured routinely when the system was in use (an intermittent occurrence), but were never a cause for concern due to the low stocking densities employed.

2.3. Incubation and Experimental Facility.

Considerable modification of this facility was made during the course of the research, as equipment requirements altered and design faults were encountered.

Egg incubation and all experiments with larvae and fry were carried out at a temperature of $24 \pm 0.5^{\circ}\text{C}$, which represented both a useful compromise between the heating capacity of the system and water flow, and a temperature frequently selected by other researchers in experiments with similarly sized carp. All water supplied to the incubation and experimental facility was used only once before discharge, in order to maintain the highest standard of water quality, and was supplied from a 300 litre capacity insulated glass fibre header tank containing a 3 Kw stainless steel immersion heater (Bunting Titanium Co., Ltd) operated continuously. Water temperature

Figure 2. Schematic Arrangement of Incubation and Experimental Facility.



was regulated by balancing an inflow of Birmingham tap water at ambient temperature against the output of the immersion heater, a safety override being provided by a thermostat set to switch off the immersion heater at 28°C.

This system of water heating was able to provide between 8 and 12 litres of water per minute at the desired temperature to the incubation and experimental facility, dependent on the temperature of the incoming water, with an average temperature fluctuation of $\pm 0.5^{\circ}\text{C}$. However, it suffered from one major disadvantage. Due to the rapid warming of the cold incoming water, outflow water from the header tank had a tendency to be supersaturated, which posed a threat to the delicate larvae and interfered with the operation of the incubation facility. A partial solution to the problem was effected by passage of inflowing water through a heat-exchange piping loop immersed in the header tank, but it proved necessary to vigorously aerate the header tank with a 50 cfm air blower to eliminate the supersaturation.

As an additional safety precaution water was withdrawn from the header tank through a stand pipe to ensure that the operating immersion heater was never exposed to the atmosphere. The header tank was fitted with an insulated glass fibre lid to reduce heat losses. Water supply to the incubation and experimental facility, which is depicted schematically in Fig.2, was separately controllable so that the experimental system could be run independently of the incubation jars.

2.4. Egg Incubation.

After fertilisation, all eggs were chemically treated in order to remove the adhesive glycoprotein from their surfaces and allow incubation in the classical fashion developed by Woynarovich (1960). This entailed gentle agitation of the eggs in an upwelling current

of water, which was carried out in a specially designed vessel known as a "zouger" jar consisting of a conical glass jar with sides inclined at approximately 30° to the vertical. Water entered this jar from its base and after traversing the egg mass, was discharged from the top of the jar into a collecting gutter leading to the hatching tray. Hatching of the eggs took place in the jar and free swimming larvae were carried with the water outflow into the hatching tray.

Simple "zouger" jars constructed from inverted glass winchester bottles with their bases removed were initially used to incubate the eggs. However, these proved to be totally inadequate and were later replaced with two 10 litre capacity "zouger" jars manufactured to specification by the university glassblowing department, which served their purpose excellently.

All water used in the incubation and experimental facility was pre-treated by passage through a 0.25 Kw ultra-violet sterilising unit (Coast Air Ltd). This was incorporated in the system after outbreak of a gill disease amongst experimental larvae, when microscopical examination revealed gill clubbing and necrosis associated with large numbers of bacteria. After installation of the sterilising unit this problem did not recur, but microbiological testing of water samples before and after irradiation revealed no apparent reduction in the negligible numbers of bacteria naturally present in the water. With hindsight, it would appear that the beneficial action of the ultra-violet unit was most probably due to its detoxifying action on free chlorine and chloramine residues in the water supply, present as a consequence of Water Authority treatment of a potable water supply (Seegert and Brooks, 1978). Possibly the irritant action of chlorine residues on delicate gill tissue was the primary cause of the disease observed, bacterial colonisation of the injured tissue representing a secondary infec-

ion.

2.5. Experimental Facility.

After hatching, larvae were collected in one of a pair of 150 litre capacity glass fibre rearing troughs mounted in an angle-iron frame one above the other and insulated with a 1 cm layer of expanded polystyrene. Both tanks were fitted with a removeable nylon screen at one end, a false plastic base extending to the screen and fitting flush against the screen frame. This allowed a certain amount of debris to pass through the screen without obstruction from the screen frame. Newly hatched larvae were separated from hatching debris in the upper rearing trough and transferred to the lower trough where rearing of non-experimental fish was carried out. The upper rearing trough was used as the experimental facility, larvae being transferred from the lower trough as required.

Originally, the experimental fish were held in a series of nylon nets suspended in the upper rearing trough, but this arrangement was quickly abandoned due to great difficulty in cleaning the nets. A second experimental facility consisting of 28 adjoining rectangular compartments formed from interlocking strips of 2 mm gauge high-density polyethylene sheeting was constructed (Fig.3). The floor of each compartment was formed from fixed 0.5 mm aperture nylon netting. The whole facility was immersed in the upper rearing trough and could be removed in one piece.

In order to make the compartments partially self-cleaning, and to ensure a uniform and continuous water exchange, the rearing trough was fitted with an automatic syphon device. This rapidly drained the water from the trough to a set level, whereupon an air break interrupted the syphon action and allowed the trough to refill to a level which again operated the syphon. The volume of the

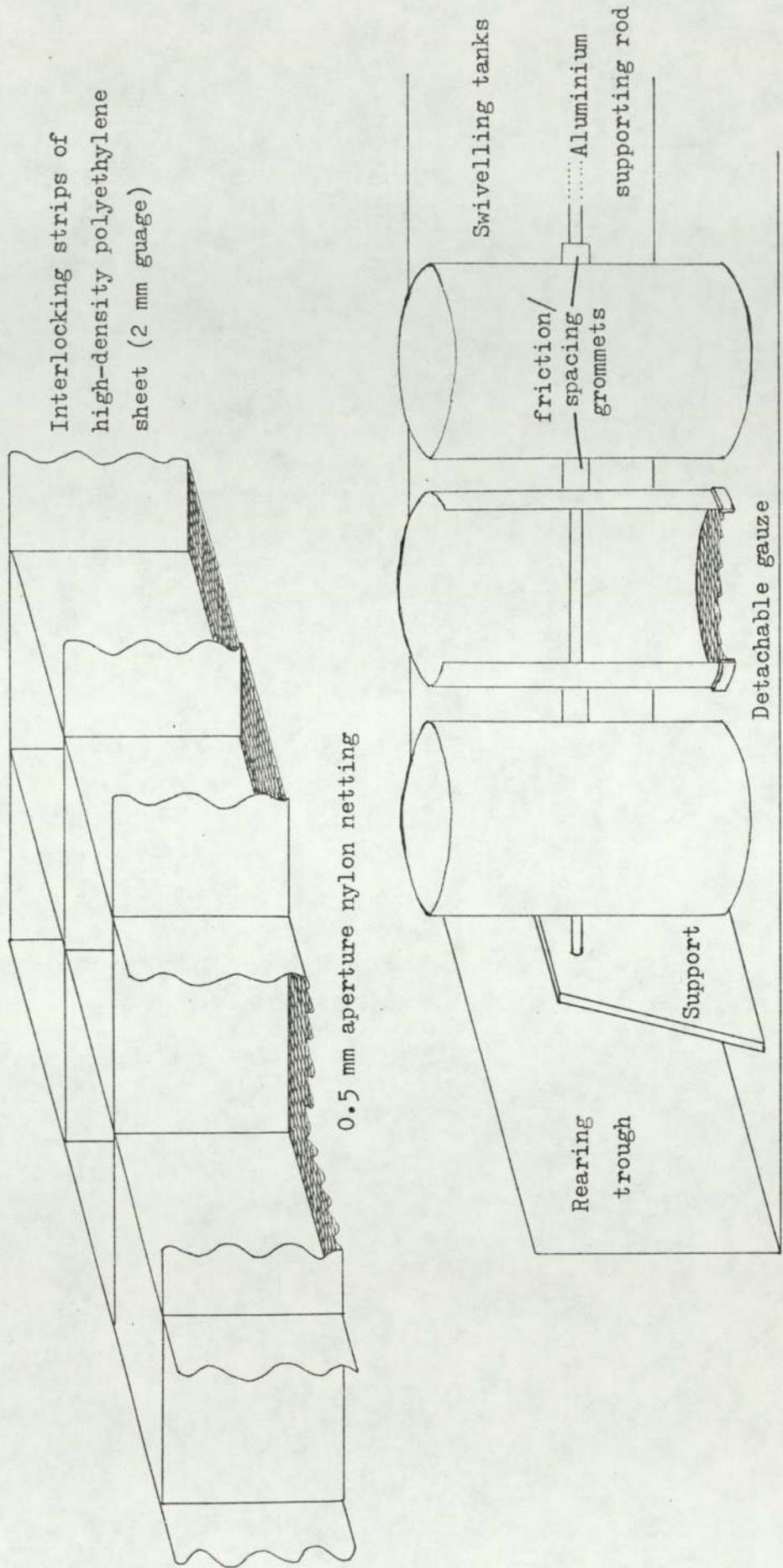
compartments was adjusted such that each contained 1 litre at the upper water level in the rearing trough and 0.5 litre at the lower. Each tank received a complete water change at approximately five minute intervals, controllable by the water inflow to the trough.

This system again proved very difficult to keep clean when artificial diets were being used, and was abandoned in favour of a much more effective system based on an idea of Adron et al (1974). This is illustrated in Fig.3, and eventually consisted of two series of eight tanks constructed from 20 cm lengths of 12 cm diameter plastic drain pipe. These were supported and pivoted at their centres on a length of 10 mm diameter aluminium rod and each rack of eight tanks was supported 2 cm above the rearing trough base by a stand locked to the aluminium bar at either end. Rings of plastic drain pipe were cut from a length that had been heat expanded and forced over another piece of drain pipe, and these rings were covered with nylon netting to produce detachable screens for each of the experimental tanks.

These tanks could be cleaned simply with minimum disturbance of the experimental fish by attachment of a new gauze to the top of the tank followed by tank inversion and removal of the fouled screen. The automatic syphon device was retained as a means of ensuring a uniform water exchange in the tanks, which conveniently had a volume of 1 and 2 litres at the lower and upper water levels in the rearing trough respectively.

The experimental tanks were painted white internally with a non-toxic waterproof paint ("Humbrol" enamel) to increase the visibility of the larvae and so aid the detection of larval mortalities, which were otherwise difficult to see against the normally grey pipe. The system was continually illuminated by a fluorescent tube giving a light intensity of approximately 1200 lux at

Figure 3. Construction of Experimental Facilities.



the water surface, although some variation in light intensity amongst the tanks was inevitable.

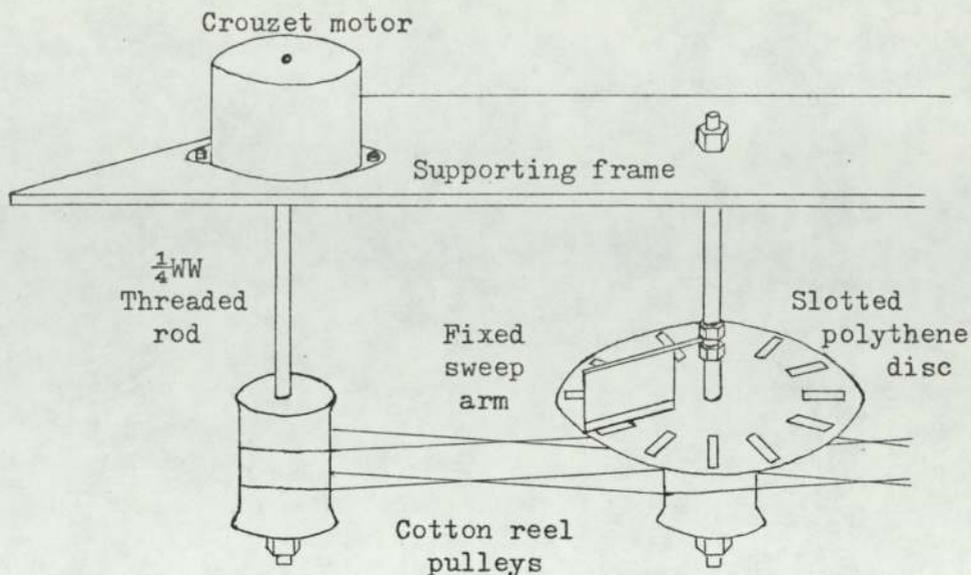
2.6. Automatic Feeding Equipment.

Early experiments where larvae were hand-fed up to six times per day convincingly demonstrated the need for some manner of automatic feeding equipment which would improve the unsatisfactory growth rates of control fish fed on live food and permit some standardisation of presentation of artificial diets. Two systems of automatic food delivery were therefore adopted to cope with the antithetical problem of administering measured quantities of live and dry artificial diets.

Live Artemia and moist diets such as aqueous micro-capsules were delivered to the experimental tanks from a stock suspension via a 9 channel peristaltic pump. Known volumes of a stock suspension of Artemia or micro-capsules could be placed in any of nine feeder reservoirs consisting of inverted glass winchester bottles with their bases removed, and were then made up to a volume of 2 litres with the aqueous medium employed (saline in the case of Artemia). The reservoirs were aerated by individual bleeds from the main header tank airline, which was fed by a 50 cfm air blower, in order to keep their contents in suspension. The 2 litre volume was then delivered to the experimental tanks incrementally over 24 hours. The peristaltic pump was controlled by an electric timer consisting of an interrupted 240v mains circuit controlled by two linked "Crouzet" electro-mechanical timers, infinitely adjustable between 0 and 40 hours. The timers independently governed the "on" and "off" periods of the peristaltic pump, so that it was possible to accurately control the amount and duration of feeding within a 24 hour period.

Dry food was administered by an array of mechanical feeders

Figure 4. Detail of Automatic Feeders.



mounted in a frame positioned above the experimental tanks. Each feeder consisted of a 12 cm diameter disc of 2 mm gauge high-density polythene, perforated with 12 equi-distant slits and attached to a wooden cotton reel pulley (Fig.4). The pulley was free to rotate about a spindle affixed to the supporting framework and was driven by twin strings from a motor-powered master pulley. Two rows of eight feeding discs were powered by independent "Crouzet" motors geared to perform one revolution every 24 hours. A sweep arm fixed to the pulley spindle, consisting of a 3 x 2 cm rectangle of 1 mm guage ABS sheet with a 7 mm strip of rubber sheet glued to its lower edge, pushed food piles placed between the slits through the slits into the experimental tanks.

Each feeder was capable of delivering up to approximately 2 - 3g of food over a 24 hour period at one hour intervals with very little wastage. Despite their rather crude construction the feeders performed satisfactorily during use, with only one minor breakdown.

2.7. Artemia Hatchery.

Freshly hatched nauplii of the brine shrimp, Artemia salina, were used in all experiments with carp larvae requiring a recognised control diet and for general early rearing of non-experimental larvae. Artemia are widely used commercially for rearing larval fishes because of the ease with which they may be cultured and their nutritional suitability for a wide variety of species. Consequently, a considerable amount of recent research has focussed on their production and culture requirements (Sorgeloos et al, 1977a; Sorgeloos et al, 1977b).

All Artemia nauplii were derived from a single batch (No.198) of San Francisco Bay Brand (Metaframe Corporation) Artemia cysts. Following the information provided by Sorgeloos and Persoone (1975) and Persoone and Sorgeloos (1975) regarding salinity, density, oxygenation and illumination requirements for optimal cyst incubation and hatching, Artemia cysts were incubated at densities of up to 10 g/litre in saline of Specific Gravity 1.025. The culture vessels comprised six 2 litre capacity round bottomed flasks, which were continuously aerated either by 2 mm bore bleeds from the main header tank airline (Fig.2) or by independent aquarium air pumps. No airstones were used on the 2 mm bore airline outlets because a stream of large air bubbles was found to be more effective for maintaining suspension of the hydrated cysts and provided adequate oxygenation of the medium.

All nauplii were harvested after 48 hours incubation in continuous daylight at a temperature of $21 \pm 2^{\circ}\text{C}$ to ensure constancy of composition and maximal nutritional value (Benijts et al, 1975). Adequate separation of the nauplii from hatching debris was achieved by allowing the contents of a culture vessel to settle, when the nauplii could be syphoned from the base of the vessel as empty cysts

floated to the surface. Three consecutive separations were made in order to obtain an uncontaminated suspension of nauplii. Although an Artemia separating device was manufactured according to the specifications of Persoone and Sorgeloos (1975), which operated by exploiting the phototactic behaviour of the nauplii, the above separation technique was much simpler and faster than the separating device and gave better results.

Hatching success utilising the above incubation apparatus and harvesting method was acceptable, ranging from 55 - 85%, and was largely dependent on the degree of temperature fluctuation encountered during incubation. The best hatching rates occurred when the incubation jars were held in a water bath fed by the overflow from the main header tank, the poorest when incubation occurred in a "warm room" with an air temperature of 22°C. Much greater temperature fluctuation occurred in this latter facility.

2.8. Induced Spawning Technique.

All carp larvae originated from an unknown strain of mirror carp parents that had been induced to spawn with injections of carp pituitary material. Natural spawning of carp confined in tanks is extremely unlikely to occur under normal circumstances, although the fish become sexually mature to a point where it is possible to bring about complete maturation and release of oocytes with a series of hormone injections. Furthermore, the natural adhesiveness of the fertilised eggs complicates their artificial incubation and it is necessary to remove the sticky mucoprotein layer from the eggs to ensure successful incubation.

The administration of a crude pituitary extract to the fish in order to bring about release of mature oocytes is a relatively straightforward technique, but the selection of carp females which will respond to this treatment requires a certain amount of exper-

ience before replicable success can be assured. An improving ability to produce larvae as and when required was therefore developed as the research progressed, and the spawning technique described below represents the most successful method adopted.

Before the construction of a warm water broodstock facility, two females and three males at a time were transferred to the spawning facility (section 2.2) and acclimatised to a temperature of 23 °C at a rate of 2 °C per day. The brood fish were sexed according to their external appearance, females having a softer abdomen and more rounded abdominal profile than males. At a later date, similar numbers of fish were transferred directly to the spawning system from the warm water broodstock facility without the need for a temperature acclimation period. Sexing was also facilitated at this time by the continuous production of milt by the males and a better developed profile of the females, despite the smaller size of the brood fish. Females were chosen for spawning on the basis of their ovary size and appearance, no attempt being made to assess the stage of maturity of the oocytes by ovarian biopsy because of the risk of damage to a limited number of brood fish. As only a small number of eggs were required, maximum fecundity was not needed.

A commercially produced acetone-dried carp pituitary extract (Stoller Fisheries Inc.) was injected into the dorsal muscles of the fish just below the dorsal fin, after anaesthetisation in a 0.01 g/litre benzocaine solution. The pituitary dosage rates advocated by Huisman (1973) were eventually adopted, consisting of an initial priming dose to the female of 0.3 mg/Kg of acetone-dried material, followed after 18 hours by the main dose to the female of 3 mg/Kg and accompanied by a priming dose to the male(s) of 0.3 mg/Kg. The acetone-dried pituitary material was homogenised in distilled water, centrifuged, and 1 ml of the supernatant cont-

aining the correct dosage injected into the fish. Consecutive doses to the female were made on opposite sides of the dorsal fin.

Between four and five hours after the second injection to the female, a close watch was maintained on the fish with periodic checks on spawning condition. Eggs were usually released some 6 - 9 hours after the second injection and were stripped into a plastic bowl for fertilisation and subsequent treatment. Males were held in a net above the eggs and milt was stripped from them onto the eggs, the milt from two males normally being used for each batch of eggs. Males and females were lightly dried on a towel before stripping to prevent any water mixing with the eggs, which would have activated the adhesive glycoprotein coating of the eggs and caused clumping.

After some practice, it was possible to arrange for eggs to be collected at approximately 18.00 hours and incubation pre-treatment to be completed by 20.00 hours when the eggs could conveniently be left overnight. The initial priming dose to the female was therefore given at approximately 17.00 hours on the previous day and the second, main dose at 11.00 hours on the desired day of spawning. Hatching was completed some 72 hours after the commencement of incubation.

In order to gain an estimate of the number of eggs obtained during a spawning attempt, and to quantify subsequent fertilisation success, the eggs were weighed immediately before fertilisation to the nearest 0.01 g on a balance tared for the weight of the collecting vessel. The net weight of the eggs was then multiplied by 1000, since the average weight of an unfertilised carp egg was found to be 1 mg, to obtain an approximation of egg numbers. No attempt was made to remove ovarian fluid from the eggs before weighing, thus the estimates were liable to be excessive. Fertilisation

success never exceeded 85%, which was a rather poor result in comparison to the fertilisation rates recorded by other researchers (Woynarovich and Kausch, 1967; Meske et al, 1968; Huisman, 1973).

Fertilisation success improved with experience, so it is likely that a large proportion of early disappointing results were due to inexperience in selecting females for spawning induction and pre-incubation treatment of the eggs.

2.9. Preparation of Eggs for Incubation.

After fertilisation of the eggs was effected by gentle stirring of eggs and milt with a feather, the eggs were treated in the manner described by Woynarovich (1960), with a modification suggested by Sassmann (1969), in order to inactivate the adhesive layer and permit incubation in the equipment described in Section 2.4.

A solution comprising 0.4% sodium chloride and 0.3% urea was added to the eggs gradually over a period of approximately one hour as it was absorbed. This had the effect of temporarily inhibiting the development of adhesion while prolonging the fertilising capacity of the carp sperm, although the eggs required continual stirring for the solution to be effective. This was decanted and the eggs washed twice with fresh hatchery water before treatment with a 0.16% solution of tannic acid after Sassmann (1969). This solution was decanted after approximately 10 seconds, and the eggs washed three times with fresh hatchery water. A second tannin rinse was applied for a similar duration and further thorough washing of the eggs carried out before transfer to the "zouger" incubation jars. Provided stirring was continued throughout the treatment process, discrete eggs resulted and incubation could proceed without any problems.

Several unsuccessful attempts at egg separation were made before the method was perfected, resulting in considerable clumping of the eggs and fungal infection during incubation. This was attributable to lack of continuous stirring with a feather throughout the process, so that discrete eggs were not obtained before the application of the tannin rinse, and omission of the essential triple water rinses in between consecutive tannin treatments.

Slight fungal development on dead eggs during incubation was found to be controlled by the application of a 1 mg/litre treatment of malachite green. This was introduced into the water inflow to the "zouger" jar and was allowed to gradually flush out.

Samples of approximately 50 eggs were withdrawn by pipette from the "zouger" jar twice daily until the eggs were eyed and the number of living and dead eggs determined in each sample. It was thus possible to obtain an estimate of fertilisation success. No attempt was made to estimate hatching success, approximately 10,000 larvae being counted and transferred from the upper rearing trough to the lower, this being the maximum number of larvae with which the installed facilities could cope. The numbers of larvae obtained at each spawning were always in excess of this amount, and were most frequently donated to interested parties.

2.10. Larval Weighing Techniques.

The most important recorded parameter in all experiments was growth rate in response to differing treatments and growth was therefore quantified by measuring weight increments with time. Length was not considered to be a sufficiently accurate indicator of growth response, because it was observed that larvae of identical length could exhibit up to 50% variation in live body weight. The ratio between the weight and the length cubed, known as condition factor (Brown, 1957), has been advocated by several researchers as a

comparative statistic for fish larvae (Anwand et al, 1976; Beck and Bengston, 1979), but the rapid changes in body shape occurring during the early stages of carp growth (section 3.3) were considered to render it inapplicable.

After examination of several different methods, two weighing procedures were adopted, each being used for differently sized fish. In order to simplify the description of experiments involving different weight classes of carp juveniles, the following simple categorisation of the developmental stages of carp is adopted in this thesis, after Alikunhi (1966). The larval phase of carp is considered to extend from hatching until fin differentiation is complete, which point commonly corresponds to a body length of 12 mm and live weight of approximately 15 mg. The post-larval phase is considered to extend to that point when colouration is complete and scale formation well advanced, usually corresponding to a live weight of approximately 70 mg. The term fry is applied to fish weighing between approximately 70 mg and 5 g.

Because of the delicate nature of larvae and post-larvae, no satisfactory method could be devised whereby living larvae could be accurately weighed without considerable mortality. Larvae and post-larvae of up to 70 mg live weight were therefore weighed immediately after death, representative samples being withdrawn from the experimental populations at pre-determined intervals in order to gain an approximation of the population growth rate. Sampled fish were drained of water and gently blotted dry on filter paper, whereupon they were simultaneously killed by spinal severance and transferred to pre-weighed aluminium foil trays with forceps. The trays were weighed to an accuracy of 0.01 mg on an Oertling microbalance and placed in an oven maintained at 105°C overnight before being weighed again for moisture and dry weight determination.

Consistent results were obtained with this method of weighing provided that moribund larvae and post-larvae were not sampled. It was noticed that a very rapid absorption of water occurred when dead fish were allowed to remain in water, hence the practice of killing fish after they had been superficially dried. No significant difference ($P > 0.05$) in weight was observed when larvae of identical weight were treated in the above manner and weighed singly, or in combination to derive an average individual weight. Less consistent results were obtained when living larvae and post-larvae were immersed in 70% alcohol before weighing in order to remove adhering water, and this practice was never adopted. Sampled fish were usually weighed in triplicate batches with up to 10 fish per weighing tray and an overall average individual live weight obtained.

Because post-larvae and fry of 70 mg weight and upwards were considerably more resistant to handling, it was possible to adopt a non-sacrificial method of weighing. Only small populations of these larger fish were held in the experimental tanks, usually either ten or fifteen fish per tank, and these were weighed together to obtain an average individual weight and returned to their respective tanks.

The tank populations were captured in a nylon tea strainer by upending the individually swivelling tanks above the strainer and precipitating any fish adhering to the tank sides into the strainer with a water jet from a hypodermic syringe. Excess water was then removed from the fish by drying the outside of the strainer on a paper towel, which drew water through the mesh. The fish themselves were not brought in contact with the towel. The dried fish were then transferred to a tared, water-filled container and weighed to an accuracy of 1 mg before being returned to the experimental tanks. This technique proved fast and accurate, it being possible to deal with all sixteen experimental tanks in 45 minutes.

2.11. Analytical Techniques.

Where applicable, a proximate analysis of dietary ingredients and experimental fish was carried out, in order to permit formulation of semi-purified diets for experimental purposes and to compare the carcass compositions of experimental fish exposed to differing dietary treatments. Proximate analysis comprised of the determination of crude protein, crude lipid, ash and moisture.

Crude protein was determined by the micro-kjeldahl method for nitrogen analysis (A.O.A.C. Methods, 1970), followed by multiplication with the empirical factor 6.25 to convert total nitrogen to crude protein.

Crude lipid was determined by Soxhlet extraction of fresh material, using 40 - 60°C boiling range petroleum ether. Lipid content was then determined gravimetrically.

Ash was estimated gravimetrically by ignition of samples in a muffle furnace for 12 hours at 500°C.

Moisture was determined gravimetrically by drying samples for 12 hours in an oven at 105°C.

In addition, estimation of assumed carbohydrate was carried out by calculation of Nitrogen Free Extractives by difference, as $100 - (\% \text{ protein} + \% \text{ lipid} + \% \text{ ash} + \% \text{ moisture})$.

2.12. Diet Formulation.

All diets prepared in the laboratory contained one or two complex protein sources as their major nitrogen source. Diets were formulated on a dry weight basis with chosen ingredients of known proximate composition, allowance being made for the water content of the raw ingredients during weighing. Pure oils of marine and vegetable origin were blended and added to the diets during manufacture to complement any lipid supplied incidentally by other ingredients and bring the overall lipid content of the

diets within the desired range. Vitamin and mineral supplements were added to all the diets, together with a binding agent. Mineral supplements were added to fishmeal-containing diets in amounts calculated to balance the total ash content of the diets on a dry weight basis because of the mineral contribution from the fishmeal, although this practice generally resulted in lower total ash content of the finished diet than calculated due to the decomposition of a portion of the added minerals during ashing. Various colouring agents were also added to the diets.

After manufacture, all diets were subjected to proximate analyses in order to check on formulation. Since several different methods of manufacture were used in the course of the research, all formulations and manufacturing techniques are described in detail in the relevant chapters.

2.13. Analysis of Experimental Data.

In order to make comparisons of growth rate between different treatments, particularly when fish of differing sizes were involved, growth was viewed in terms of the Specific Growth Rate (Brown, 1957), defined as:

$$\text{SGR} = 100 \times \frac{\log_e W_T - \log_e W_t}{T - t} \quad \%/ \text{day},$$

where W_T is the final live body weight at time T and W_t is the initial live body weight at time t, T and t usually being expressed in days. Specific Growth Rate was assessed over as short a time period as practicable because of the rapid changes in growth rate shown by the smallest sizes of fish used in the research (Chapter 3).

The efficiency of food utilisation was measured, where applicable, by one or all of three parameters, the Food Conversion Ratio, Protein Efficiency Ratio and the Apparent Net Protein Utilisation.

Food Conversion Ratio (FCR), defined as:

$$\text{FCR} = \frac{\text{Weight of Food Consumed}}{\text{Live Weight Gain}}$$

was calculated for all experiments using semi-dry artificial diets from the true dry weight of food administered, correction being made for the water content of the diets as analysed. Due to practical difficulties in administering the food, it was impossible to ensure that all administered food was eaten and the calculated Food Conversion Ratios were derived from the weight of food offered to the fish without correction for wastage. Food Conversion Ratios for carp larvae offered live Artemia were not corrected for the water content of the Artemia.

The efficiency with which the fish were able to utilise dietary protein was assessed by calculation of values for Protein Efficiency Ratio (PER), defined as the gain in wet weight of fish per unit weight of crude protein consumed (Osborne et al, 1919). Again, protein consumption was estimated from the amount of food offered without correction for wastage.

The apparent efficiency of deposition of dietary protein as body tissue was determined by the carcass analysis method of Bender and Miller (1953) and Miller and Bender (1955), without correction for endogenous nitrogen loss so that the results are expressed as Apparent Net Protein Utilisation (NPUapp.), defined as:

$$\text{NPUapp. \%} = \frac{N_b - N_a}{N_i} \times 100$$

where N_b is the body nitrogen (or protein) at the end of the test, N_a the body nitrogen (or protein) at the start of the test and N_i the amount of nitrogen (or protein) ingested throughout the test, and where protein is derived from the analysed nitrogen content

of carcasses and diets.

Statistical interpretation of results was largely confined to analysis of statistically significant differences between treatment means. After testing for homogeneity of variance among sample statistics by the application of Bartlett's test (Campbell, 1967), data for comparison was subjected to a one-way analysis of variance followed by testing for pairwise differences by the New Multiple Range Test of Duncan (1955). The range of means tested in this way is represented by the standard error (\pm S.E.) derived from the residual mean square in the analysis of variance for $(n - k)$ degrees of freedom, where n = the number of samples and k = the number of treatments. The range of data not tested in this way is shown, where relevant, by the standard deviation (\pm S.D.).

The significance of the correlation coefficient calculated during the course of regression analysis was assessed for the hypothesis that r (the correlation coefficient) = 0, by comparison with tabulated values for Student's t distribution with $N - 2$ degrees of freedom, where N = the number of values (x, y) . Other statistics used in this thesis are described in the relevant chapters.

CHAPTER 3.

Chapter 3. Experiment 1: The Optimisation of Artemia Feeding Rate
for Carp Larvae.

3.1. Introduction.

Seale (1933) was one of the first authors to suggest the use of Artemia as a food for larval fishes. With the exploitation of natural stocks of Artemia in the USA and Canada, the use of Artemia for aquacultural purposes has grown exponentially in the last two decades (Sorgeloos et al, 1977b), such that Artemia has now become almost the only food used for commercial rearing of non-salmonid larval fish in the USA and Western Europe. This demand, which is largely due to the ease and convenience with which Artemia may be cultured, threatens to outpace supply and has sharply increased the price of cysts in the last few years (Sorgeloos, 1976; Sorgeloos et al, 1977a).

Nauplii of Artemia remain the most convenient first food for rearing of cyprinid fish larvae, and in view of the above facts concerning Artemia supply, it is surprising that no attempt has been made to quantify feeding rates which optimise utilisation of this expensive resource. Feeding rates have been determined for common carp larvae supplied with Daphnia carinata (Chiba, 1961) and the cladoceran Moina spp. (Baranova, 1974). Poorly controlled hatching and harvesting of Artemia nauplii can lead to substantial cyst wastage (Benijts et al, 1975), but probably greater wastage occurs if prolonged under- or over-feeding of fish larvae is practised. Minimisation of these losses would therefore be financially prudent.

Because it was intended to use Artemia as a control diet in subsequent experiments with artificial diets, it was considered important to optimise the growth rate of these fish in order to make realistic comparisons of growth performance between treatments.

Artemia have often been used as a control diet in feeding trials with artificial larval diets (Adron et al,1974; Appelbaum,1977), and under-feeding of such control fish can lead to erroneous conclusions about the efficacy of the experimental diets (Appelbaum and Dor,1978).

The following studies were therefore designed to evaluate the minimum quantity of Artemia nauplii concomitant with maximum growth rate of the carp larvae under the conditions of rearing used throughout the research, in order to both improve efficiency of cyst use and to provide a standard for subsequent growth experiments with artificial larval diets. It was also hoped that data on Artemia uptake and assimilation would provide some insight into the nutritional requirements of the larvae.

3.2. Materials and Methods.

The methods adopted for the production of carp larvae have been described in section 2.8, and the experimental facility and Artemia hatchery in sections 2.5 and 2.7.

The experiment was carried out in three separate trials, the larvae used in each trial being obtained from different parents. Experimental methodology was slightly altered in succeeding trials in order to evaluate potential sources of error identified in preceding trials and to improve the accuracy of the results. Eight rotatable tanks with detachable screens were used in each trial and were initially stocked with 200, two-day old carp larvae with absorbed yolk sacs.

Growth increments of the larvae given pre-determined amounts of Artemia calculated as a percentage of their body weight were monitored by sampling, the respective Artemia feeding rates being adjusted after each sampling to account for growth. In trial 1A, sampling was effected on alternate days, each sample of 20 fish

being batch weighed to an accuracy of 0.01 mg to obtain an average individual weight. This method of sampling and weighing resulted in considerable variation in feeding rate due to the rapid growth of the larvae and did not permit any estimation of growth differences within the experimental tanks. In order to rectify this failing in trials 1B and 1C, samples of ten larvae per tank were taken each morning and weighed in triplicate batches to provide a measure of growth dispersion. All sampled fish were weighed in pre-weighed aluminium foil trays and were oven dried at 105°C overnight for determination of dry weight.

The detachable tank screens used in trials 1A and 1B had a mesh aperture of 350 µm, which permitted passage of uneaten nauplii into the rearing trough. Because this could have caused over-estimation of feeding rate through poorer food utilisation, mesh aperture was reduced to 150 µm in trial 1C to remove this potential source of error.

Artemia nauplii were delivered to each of the experimental tanks via the time controlled peristaltic pump described in section 2.6, at three-minute intervals over 24 hours. Eight separate feeding reservoirs were allocated their required volumes of an aerated stock suspension of Artemia nauplii of known concentration, which were measured by a combination of pipettes. The reservoirs were then made up to a volume of 2 litres with saline of Specific Gravity 1.025. Passage through the peristaltic pump had no apparent effect on nauplii motility or survival.

After 48 hours incubation at 21°C and separation from hatching debris, the concentration of the stock suspension of Artemia nauplii was determined by sampling. Triplicate 1 - 2 ml samples of the aerated stock suspension were diluted to 100 ml with fresh saline; forty 200 µl subsamples of these aerated suspensions were taken

with a micropipette and the number of nauplii determined in each (subsamples being returned to the diluted sample before each subsequent subsample). The dilutions of the original stock suspension were chosen to yield between 10 and 20 nauplii in the 200 μ l subsamples, these being the most convenient numbers for counting. If the triplicate estimates of the concentration of the stock suspension did not agree to within 10%, further samples were taken. The volumes of stock suspension required for the different feeding rates were then calculated.

Feeding rates were initially chosen on the basis of a reported wet weight of Artemia of approximately 20 μ g (Benijts et al, 1975; Watanabe et al, 1978a). However, the dry weight and moisture content of the actual Artemia batch used were subsequently determined and feeding rate data altered accordingly.

The concentration of a stock suspension of Artemia nauplii derived from 10 g of cysts incubated in two litres of saline for 48 hours at 21^oC was determined by the method described above, but using 24 samples at three dilutions with 40 subsamples on each. Ten 5 ml aliquots were then suction filtered on oven-dried, pre-weighed sintered glass discs and washed three times with distilled water to remove external salt. The discs were oven-dried at 105^oC to constant weight. The remaining stock suspension was suction filtered and the nauplii transferred to 10 pre-weighed aluminium foil trays for moisture determination.

The trials were continued for a period of ten days, which was subdivided into two 5-day periods in trials 1B and 1C. The first feeding trial revealed a considerable reduction in growth rate over ten days, and feeding rates were therefore reduced after five days growth in trials 1B and 1C in an attempt to assess changes in feed requirement with age. Five days was considered a

practical minimum period over which to assess the average feeding requirement, and only larvae demonstrating similar weights after the first five days growth were treated in this manner. The order of magnitude of feeding rate was also reversed such that larvae initially fed maximally were fed minimally for days 5 - 10, and vice-versa.

Larvae and post-larvae sampled for weighing in trials 1B and 1C were pooled for carcass analysis in order to investigate possible changes in body composition with age and build up comparative data for later trials with artificial diets. Only fish exhibiting very similar body weights were pooled for analysis, which was carried out as detailed in Section 2.11. Due to the relatively small numbers of fish which could be held in the experimental system, even with pooling there was insufficient material for analysis of carcass lipid with the techniques available, and an approximation of carcass lipid was therefore obtained as $100 - (\text{carcass crude protein} + \text{carcass ash} + \text{carcass moisture})$. Since the NFE content of fish is always a small amount, this method of approximation was considered acceptable for comparative purposes.

3.3. Results.

Investigation of the weight and moisture content of Artemia nauplii yielded a value of $1.46 \pm 0.11 \mu\text{g}$ for dry matter content and $90.3 \pm 0.13\%$ for moisture, giving an overall value for the wet weight of Artemia of $15.05 \mu\text{g}$. This figure was used to quantify feeding rate in weight terms from the measured numbers of nauplii administered.

Mortality throughout all experiments was negligible. The data from the three trials are presented in Table 1, growth and feeding rate data for the first 5 days only of trial 1A being included. Because of the unsatisfactory method of sampling and

Table 1 : Growth and Feeding Rates of Artemia-fed Carp Larvae - Trial 1A.

Days 1 - 5 (Initial Weight 1.10 mg)		Nauplii fed/mg		Feeding Rate		Specific Growth	
Treatment Number	Live Weight after 5 days	live body wt.	% body wt./day	Mean	±S.D.	Mean	±S.D.
	Mean (mg)	Mean	Mean	Mean	Mean	Rate	(%/day)
1	1.53	12	18.8	6.3		6.6	
2	3.37	42	62.8	7.7		22.4	
3	5.67	69	104.0	6.3		32.8	
4	7.48	88	131.7	11.3		38.3	
5	9.30	114	170.3	25.4		42.7	
6	9.99	128	192.2	34.6		44.1	
7	10.62	149	223.8	52.6		45.4	
8	13.58	196	294.8	86.9		50.3	

Table 1 (continued): Growth and Feeding Rates of Artemia-fed Carp Larvae - Trial 1B.

(i) Days 1 - 5 (Initial Weight 1.24 ±0.01 mg)											
Treatment Number	Live Weight		Nauplii fed/mg		Feeding Rate		Specific Growth				
	after 5	after 10 days	live body wt.	live body wt.	% body wt./day	wt./day	Rate	Rate			(%/day)
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	
1	13.07	1.41	175	8	262.5	12.2	47.0 ^{a*}				
2	13.06	0.32	200	9	300.0	14.0	47.0 ^a				
3	13.12	1.77	225	10	337.6	15.7	47.0 ^a				
4	15.54	0.23	250	12	375.0	17.5	50.6 ^b				
5	15.43	0.30	275	13	412.5	19.2	50.4 ^b				
6	15.01	0.67	300	14	450.0	21.0	49.8 ^{ab}				
7	16.47	1.19	350	18	525.0	26.9	51.7 ^b				
8	15.00	0.18	400	19	600.0	28.0	49.9 ^{ab}				
											±S.E. 0.8**
(ii) Days 5 - 10											
1	67.43	6.13	175	14	262.5	21.3	32.8 ^a				
2	63.86	6.27	125	10	187.5	15.2	31.6 ^a				
3	64.93	6.73	100	8	150.0	12.2	32.1 ^a				
4	49.63	0.94	75	6	112.5	9.1	23.2 ^b				
5	37.71	4.38	50	4	75.0	6.1	17.7 ^c				
6	33.10	1.73	37	3	56.2	4.6	15.8 ^c				
7	24.90	2.76	25	2	37.5	3.0	8.2 ^d				
8	16.87	0.30	12	1	18.8	1.5	2.3 ^e				
											±S.E. 1.3

*Figures with the same superscript are not significantly different (P > 0.05)

**Standard error derived from the residual mean square in the analysis of variance

Table 1 (continued): Growth and Feeding Rates of Artemia-fed Carp Larvae - Trial 1C.

(i) Days 1 - 5 (Initial Weight 1.01 ± 0.07 mg)											
Treatment Number	Live Weight		Nauplii fed/mg		Feeding Rate		Specific Growth				
	after 5 or 10 days	±S.D.	live body wt.	±S.D.	% body wt./day	Mean	±S.D.	Rate			
	Mean(mg)		Mean		Mean			(%/day)			
1	5.90	0.13	87	5	130.1	7.4		35.3 ^a			
2	6.52	0.88	100	5	150.0	8.0		37.2 ^a			
3	8.39	0.04	112	6	168.8	9.0		42.4 ^b			
4	9.21	0.49	125	7	187.5	10.0		44.2 ^{bc}			
5	11.64	0.42	137	7	206.2	11.0		48.9 ^{de}			
6	9.27	0.07	150	8	225.0	12.0		44.4 ^{bc}			
7	10.04	0.73	162	9	243.8	13.0		45.9 ^{cd}			
8	12.98	0.02	175	9	262.5	14.1		51.1 ^e	±S.E. 1.1		
(ii) Days 5 - 10											
1	-	-	-	-	-	-	-	-			
2	-	-	-	-	-	-	-	-			
3	-	-	-	-	-	-	-	-			
4	50.66	1.77	110	11	165.2	16.8		34.1 ^{ab}			
5	55.45	3.48	101	9	151.4	13.4		31.2 ^{bc}			
6	52.78	3.75	90	9	135.2	13.7		34.7 ^a			
7	56.94	3.98	80	8	120.2	12.2		34.7 ^a			
8	61.08	7.38	70	7	105.1	10.7		30.8 ^c	±S.E. 1.0		

feeding, no quantitative conclusions could be drawn from trial 1A, but its partial inclusion was deemed worthwhile since it clearly demonstrated the effect of very low feeding rates. The effect of feeding rate on growth rate and food conversion is illustrated graphically in Figures 5 and 6, the data from trials 1A, 1B and 1C for the first five days after hatching being presented in Figure 5 and the data from trials 1B and 1C for the following five days being presented in Figure 6. Approximating curves were fitted to the data freehand, mathematically precise methods being considered misleading due to the dependence of the data on water temperature, larval genotype and Artemia strain.

3.3.1. Days 1 - 5 (Figure 5).

It can be seen from Figure 5 that the average specific growth rates of carp larvae fed Artemia in excess of 250% of their body weight per day plateau at a value of approximately 50% per day. In trial 1B, there was no significant difference between the specific growth rates of treatments 1, 2, 3, 6 and 8, corresponding to feeding rates of 262, 300, 337, 450 and 600% of body weight per day respectively, although treatments 1 - 3 were significantly ($P < 0.05$) different from treatments 4, 5 and 7 (Table 1). It is possible that Artemia loss through the 350 μ m tank screens caused the slightly lower growth rates in treatments 1 - 3, but it seems more likely that the significant differences were due to sampling error of larval weight. This conclusion is supported by the insignificant differences between treatments 1 - 3 and treatments 6 and 8, which represented excessive feeding rates.

In trial 1C, reduction of screen aperture to 150 μ m apparently produced a slight improvement in growth rate at lower feeding rates, treatment 5 (206% of body weight per day) not being significantly different from the highest feeding rate, treatment 8 (262% of body

KEY TO FIGURES 5 AND 6.

Trial 1A.

- Specific Growth Rate
- Food Conversion Ratio

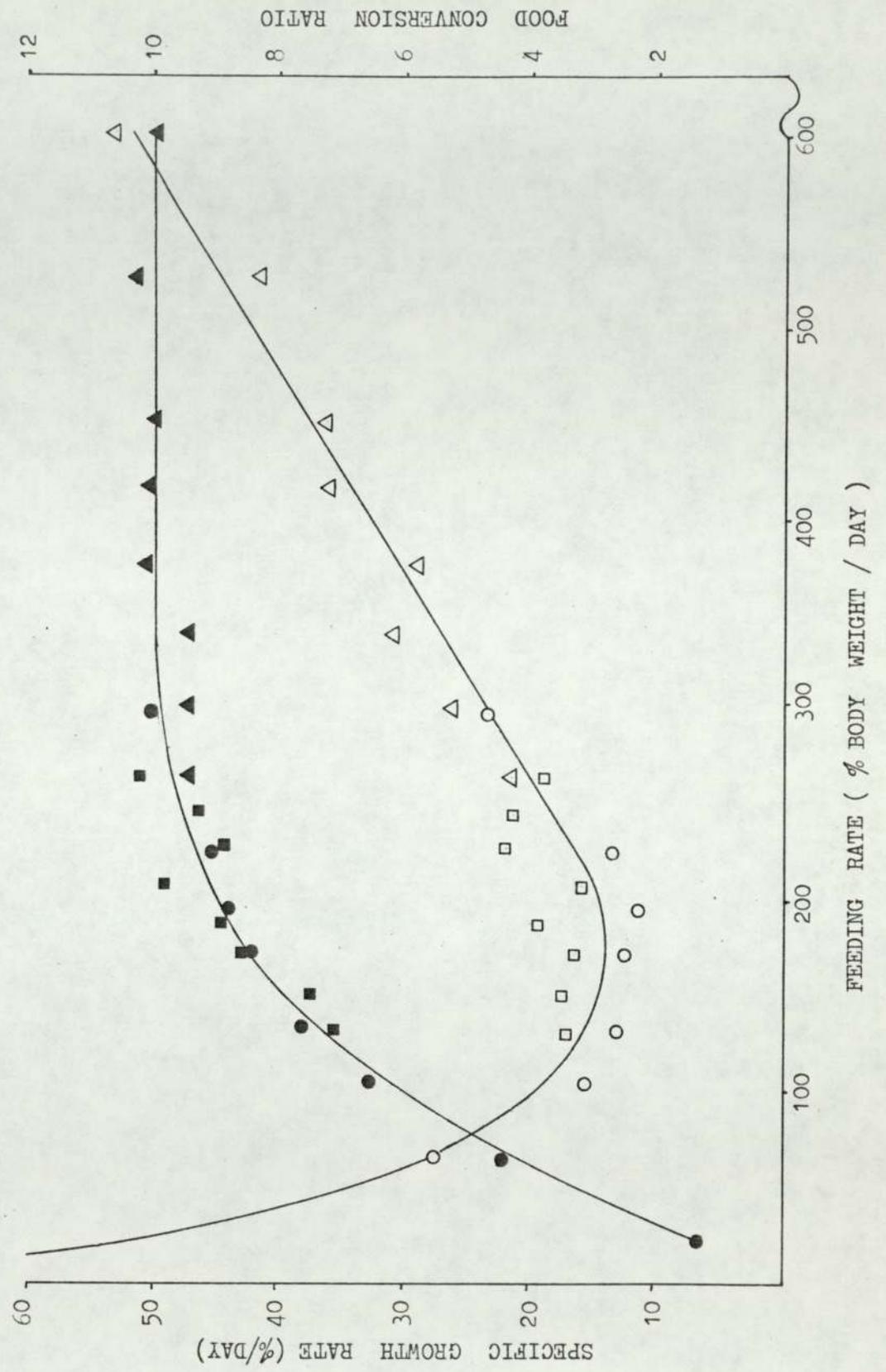
Trial 1B.

- ▲ Specific Growth Rate
- △ Food Conversion Ratio

Trial 1C.

- Specific Growth Rate
- Food Conversion Ratio

Figure 5. The Effect of Artemia Feeding Rate on Growth and Food Conversion - Days 1 - 5.



weight per day).

As anticipated, food conversion ratios in all three trials were better at feeding rates lower than those yielding maximum growth, the best occurring at a feeding rate of approximately 200% of body weight per day. It would thus appear that carp larvae show optimal growth during the first five days after commencement of feeding, under continuous light at a temperature of 24°C, when fed Artemia at a rate of between 200 and 250% of their body weight per day.

3.3.2. Days 5 - 10 (Figure 6).

In trial 1B, feeding rates of greater than 150% of body weight per day gave no significant increase in specific growth rate (treatments 1, 2 and 3, Table 1). However, the effect of gauze aperture was much more pronounced with these larger fish in trial 1C, a feeding rate of 105% of body weight per day (treatment 8, Table 1) producing a specific growth rate not differing significantly from one of 151% (treatment 5, Table 1), although intermediate feeding rates (treatments 6 and 7, Table 1) gave significantly ($P < 0.05$) higher growth rates. A comparable feeding rate of 112% of body weight per day in trial 1B (treatment 4, Table 1) gave much poorer growth and food conversion, probably due to Artemia loss through the tank screen.

The maximum specific growth rates achieved in trials 1B and 1C for this second five day period were between 30 and 35% per day, showing a reduction over the maximum specific growth rates achieved during the first five days after commencement of feeding. Optimal growth and food conversion occurred for a feeding rate of between 100 and 120% of body weight per day.

The pattern of the gradual diminution of specific growth rate is shown in Table 2, and it can be seen that a significant ($P < 0.05$)

Figure 6. The Effect of Artemia Feeding Rate on Growth and Food Conversion - Days 5 - 10.

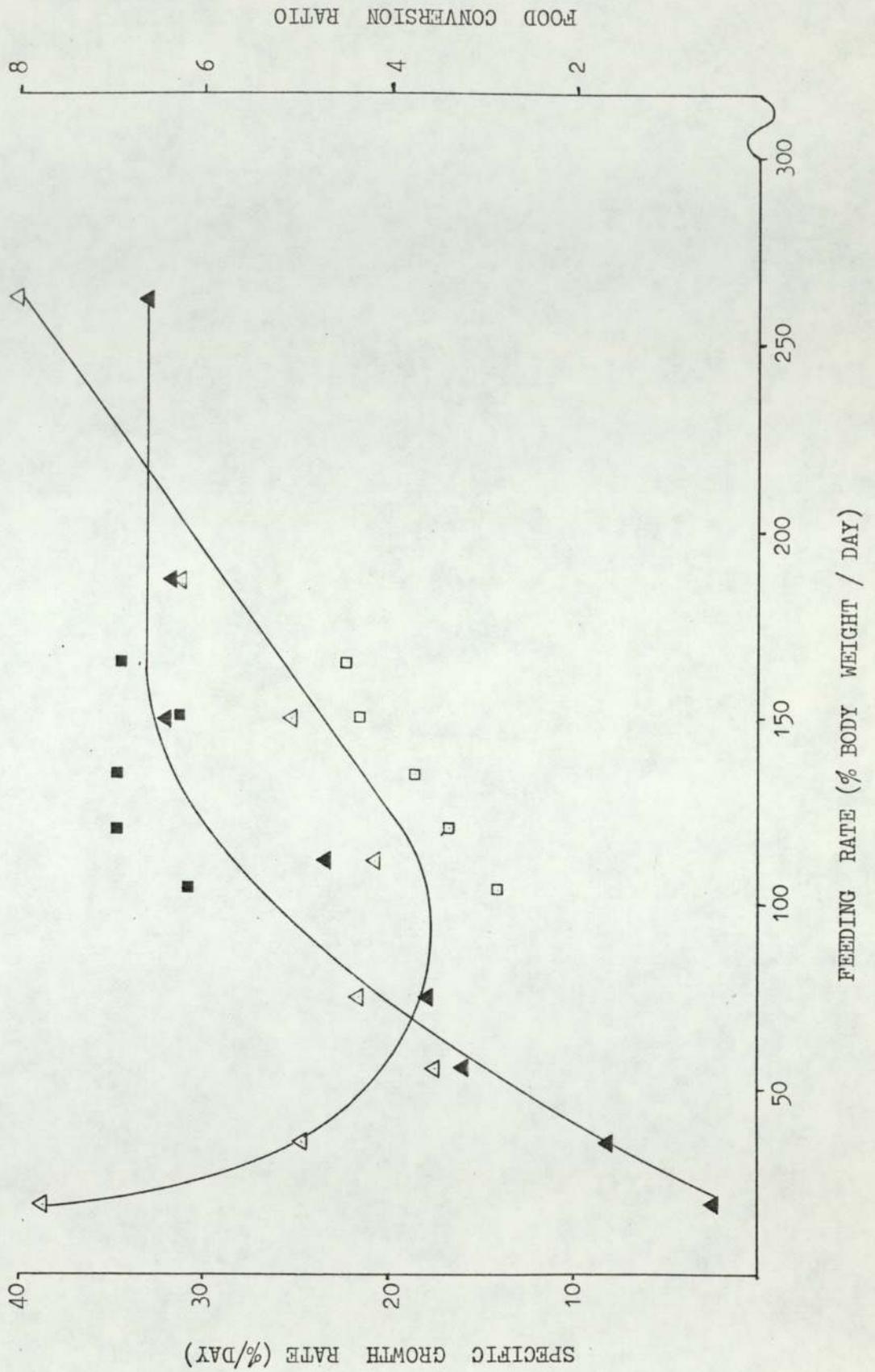


Table 2 : Maximum Growth Rates of Artemia-fed Carp Larvae.

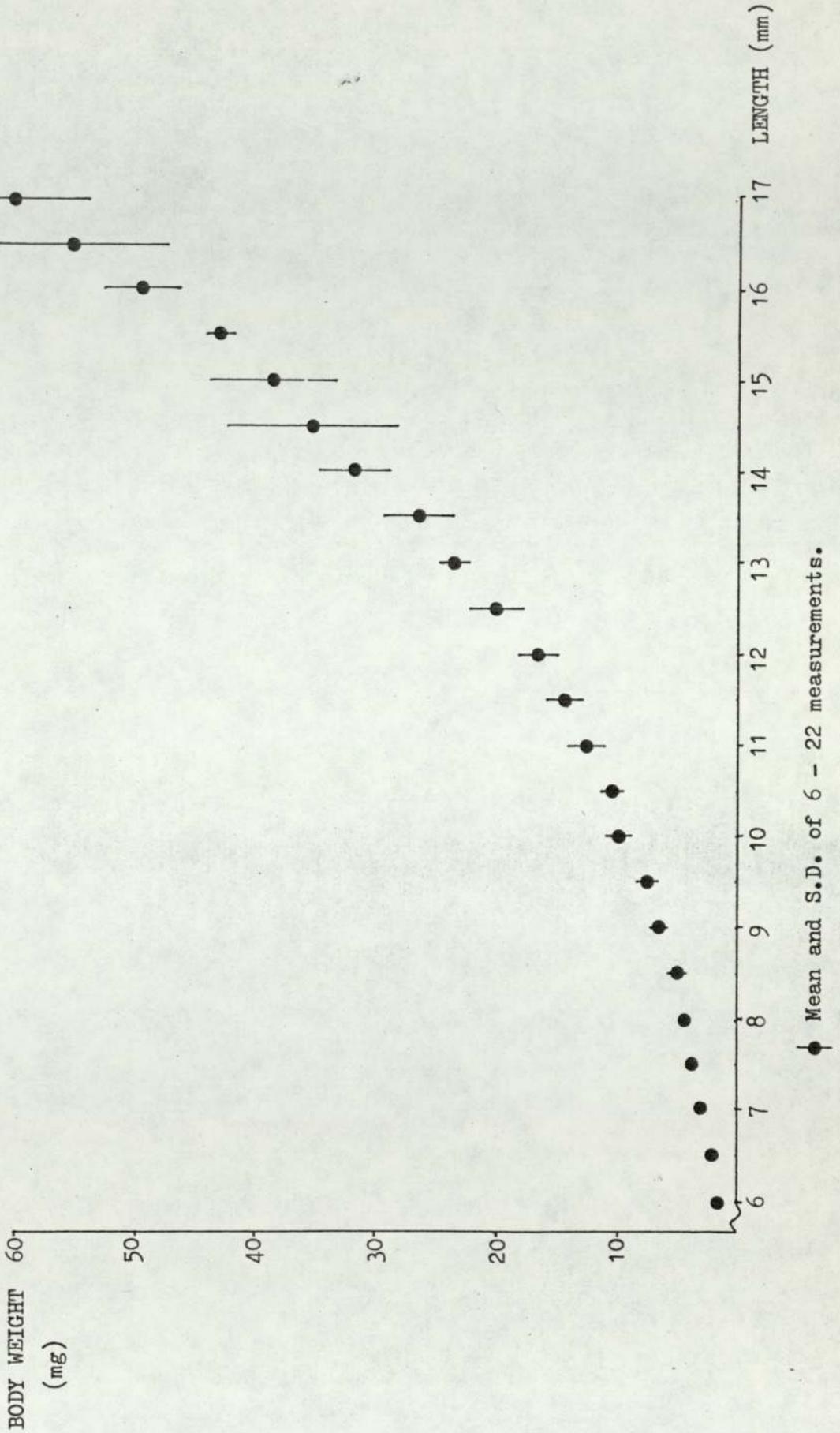
Days After 1st Feeding	Specific Growth Rate (%/day)	Average Weight	
		Mean(mg)	\pm S.D.
1	48.0 ^a	1.92	0.11
2	48.6 ^a	3.65	0.58
3	46.3 ^a	6.32	0.74
4	47.0 ^a	9.83	0.94
5	43.4 ^{ab}	14.37	1.61
6	41.4 ^b	20.05	2.35
7	37.0 ^b	26.33	2.84
8	34.4 ^{bc}	38.26	5.30
9	31.1 ^c	54.68	3.28
10	32.0 ^c	64.39	4.63

\pm S.E. 0.6

34% reduction in maximum specific growth rates occurred during the ten days after first feeding. Although no significant drop in growth rate was detected during the first five days after first feeding, it is probable that growth shows a gradual decrease over the first few weeks of development, implying that feeding rate should similarly be decreased continuously. This fact is of some importance in relation to economies of Artemia cyst use.

Figure 7 shows the relationship between body length and body wet weight. The two parameters are almost linearly related up to a body weight of approximately 5 mg, which would correspond to an age of 2 - 3 days for larvae fed optimally, thereafter increase in length falling behind increase in weight for the range of data collected. The body shape thus alters throughout early development, body depth gradually increasing. Condition factor, expressed as W / L^3 where W = body wet weight and L = body length, will therefore tend to increase during this stage of carp growth and would not give a satisfactory indication of body shape due to the latter's changing nature.

Figure 7. Relationship of Body Length to Body Weight for Artemia-fed Carp Larvae.



3.3.3. Carcass Analysis of Artemia-fed Carp Larvae.

The proximate carcass analyses of pooled samples from trials 1B and 1C are shown in Table 3. The results are expressed on a wet-weight basis, calculated from the analysed percentage moisture in the fish.

It can be seen from Table 3 that fish from trial 1B showed a significant ($P < 0.05$) decrease in carcass moisture with increasing body weight, from 85.83% in larvae of 1.93 mg live body weight to 83.96% in fry of 84.10 mg live body weight. This decrease in carcass moisture was accompanied by a significant ($P < 0.05$) increase in carcass ash content from 0.75% to 1.32%. A similar trend was evident in carcass fat content, although analysis of variance could not be performed on these data due to their derivation by difference from other analysed parameters. A significant increase in carcass protein was shown at the 95% confidence limit, but this was not significant at the 99% confidence limit and it is likely that carcass protein remained relatively constant.

A small but consistent difference in the values for carcass moisture, protein and ash was seen between fish from trial 1C and trial 1B, which were of different parental origin. These differences were probably due to genotypical factors. The fish from trial 1C demonstrated similar significant ($P < 0.05$) increases in carcass ash and decreases in carcass moisture to the fish from trial 1B. Analysis of variance of carcass protein content again showed significant increases at the 95% confidence limit but not at the 99% confidence limit, suggesting that observed variation was due to analytical error and individual variation among the sampled fish. The observed increases in carcass ash content were most probably due to skeletal development.

Table 3 : Carcass Analysis of Artemia-fed Carp Larvae, Post-larvae and Fry - Trials 1B and 1C.

Trial 1B.	1 - 8	1 - 8	1 - 8	1, 7 & 8	7	+S.E.*
Treatments pooled for analysis.						
Body Weight (mg)	1.93	6.10	14.59	29.23	84.10	-
+S.D.	0.12	0.76	1.41	1.17	0.84	-
Moisture content %	85.83 ^a	85.69 ^a	85.34 ^b	84.69 ^c	83.96 ^d	0.10
Protein content %	9.19 ^a	9.29 ^a	9.54 ^{ab}	9.69 ^b	9.70 ^b	0.13
Ash content %	0.75 ^a	0.78 ^{ab}	0.80 ^{ab}	0.90 ^{ab}	1.32 ^c	0.11
Fat content**%	4.23	4.24	4.32	4.72	5.02	-

Trial 1C.	4 - 8	4 - 8	4 - 8	4 - 8	4 - 8	4 - 8
Treatments pooled for analysis.						
Body Weight (mg)	1.62	4.03	10.63	24.18	55.38	-
+S.D.	0.07	0.18	1.50	2.31	4.77	-
Moisture content %	85.53 ^a	84.48 ^b	84.62 ^b	84.05 ^c	83.70 ^d	0.12
Protein content %	9.24 ^a	10.17 ^{ab}	9.88 ^{ab}	10.06 ^{ab}	10.37 ^b	0.31
Ash content %	0.98 ^a	1.08 ^a	1.32 ^b	1.46 ^{bc}	1.60 ^d	0.06
Fat content**%	4.25	4.27	4.18	4.43	4.63	-

*Standard Error derived from the residual mean square in the Analysis of Variance.

**Obtained by difference as 100 - (Moisture + Protein + Ash).

3.4. Discussion and Conclusions.

The data collected from the three trials demonstrated that carp larvae fed continually on Artemia nauplii, and held in continuous light at a temperature of $24 \pm 0.5^{\circ}\text{C}$, showed optimal growth and food conversion for feeding rates of between 200 and 250% of their body weight per day during the first five days after commencement of feeding. During the following five days, optimal growth and food conversion was exhibited at lower feeding rates of between 100 and 120% of body weight per day.

These results are in broad agreement with those of other researchers. Chiba (1961) reported that common carp larvae feeding on Daphnia carinata at a temperature of $18 - 23^{\circ}\text{C}$ took between two and three times their body weight daily when allowed to feed without restriction. Similarly, Baranova (1974) demonstrated that carp larvae showed maximum growth at a feeding rate of between 200 and 300% of their body weight per day when held at a temperature of $23 - 25^{\circ}\text{C}$ for ten days and fed on the cladoceran Moina spp.

However, neither of the above researchers investigated changes in growth rate over the course of their experiments, although these clearly have an important effect on the economics of feeding. The overall growth rate of larvae fed Moina spp. for ten days, as recorded by Baranova (1974), agrees with the average growth rate over ten days recorded in this study. Hamada et al (1975) recorded five and ten-day weight increments for wild carp fed to excess with Limnodrilus, and held at a temperature of 25°C . From an initial weight of 2 mg, larvae grew to 29 and 179 mg at successive five day intervals. Calculation of specific growth rate for this data gives values of 53.5 and 36.4% per day, which shows a 32% decrease over the ten day period, very similar to the 34% decrease in growth rates observed in this study. Huisman (1974) also fed carp larvae to ex-

cess with Artemia nauplii, the reduction in growth rate observable in his data corresponding closely with that observed in this study. Reduction in growth rate during the early stages of rearing has also been observed for other species of fish larvae (Huisman, 1979a; Hogendoorn, 1980).

The specific growth rates and food conversions recorded in trials 1B and 1C for the second five day period showed much greater variation than those for the first five day period, the effect of Artemia loss through the larger aperture tank screens also being more noticeable. This was probably due to two causes. Firstly, with the greater larval weights during days 5 - 10, increased error was involved in both determination of average larval weight by sampling and in administration of large numbers of nauplii. Secondly, Artemia nauplii are of an unsuitable size for larvae and post-larvae of greater than 10 - 15 mg live body weight, the preferred natural food at this time having a diameter of 1 - 3 mm and consisting of organisms such as larvae of Chironomidae, Culicidae and Copepoda (Matlak and Matlak, 1976).

In order to ingest sufficient quantities of the 450 - 500 μ m diameter Artemia nauplii, the carp larvae and post-larvae would have had to increase their preying activity in comparison with carp larvae of a smaller size. Because the automatic syphon arrangement effected relatively rapid water exchange in the experimental tanks, Artemia would have been lost before the fish had sufficient time to capture enough nauplii to satisfy their appetites. When Artemia were retained by the smaller aperture tank screens in trial 1C, the fish would have been able to capture nauplii at their leisure, resulting in improved growth rate and food conversion. It is possible that the unsuitable particle size of Artemia for older carp larvae and post-larvae was partially responsible for the reduced

growth rates shown by these fish. Wankowski and Thorpe (1979) investigated the effect of food particle size on the growth and food conversion of juvenile Atlantic salmon (Salmo salar) and found that for each size group of fish studied, classified by fork length, only one of six different grades of pelleted feed elicited maximum growth. They showed that the particle size required for maximum growth increased in direct proportion to fish length. Similarly, Nakamura and Kasahara (1956) obtained better growth from common carp post-larvae and fry fed on cladocerans and mosquito larvae retained by a 400 μm aperture sieve than from fish fed on cladocerans of <400 μm in size. They also showed that population growth amongst these fish was affected by the particle size of the food offered, those post-larvae and fry receiving the larger organisms demonstrating a marked positive skewness of body length distribution, while carp fed the cladocerans of <400 μm in diameter showed a more even population growth.

It is presumed that prey selectivity by fish represents an attempt to optimise foraging efficiency, to effect the greatest energy gain for minimum energy expenditure (Wankowski and Thorpe, 1979). Carp juveniles show prey selectivity in the wild (Matlak and Matlak, 1976) which would appear to be related mainly to the body size of the fish, although some gustatory selection may also occur. However, under artificial conditions where both predator and prey are maintained at high densities in limited water volumes, little energy expenditure will be required for prey capture. Thus the presentation of unsuitably sized food organisms to carp juveniles kept under such conditions is likely to have only a negligible effect on their growth rate at optimal and supra-optimal feeding rates, although its effect would be more pronounced at sub-optimal feeding rates.

Nakamura and Kasahara (1956) also investigated the effects of feeding rate on the population length distribution of common carp juveniles and observed that sub-optimal feeding rates increased the positive skewness of the population length distribution in comparison with the more normal distributions occurring among fish fed excessively. At the end of trials 1B and 1C, the remaining tank populations were weighed individually in order to estimate the relative dispersion of fish body weights about the mean. Although no quantitative data are presented here, because no data were collected for the body weight distributions at the beginning of the experiments, it was observed that at sub-optimal feeding rates the weight distribution showed varying degrees of positive skewness about the mean, in proportion to the severity of food deprivation with the exception of very low feeding rates. At feeding rates only slightly above a maintenance ration, nearly normal population weight distributions were obtained. These observations are therefore in agreement with those of Nakamura and Kasahara (1956), although the final population weight distributions could have been influenced to a substantial extent by the unknown degree of skewness of the initial body weight distribution. In a practical sense, the effect of feeding rate on the population growth distribution is of some importance, since it would be commercially useful to produce carp fingerlings of an even size. As well as representing the most efficient use of Artemia cysts, the optimal feeding rates determined in this study apparently produced relatively even population growth.

Larval stocking density can conceivably have a considerable influence on feeding behaviour as well as directly affecting water quality. No attempt was made in this study to assess the effect of stocking density on the optimum feeding rate, and stocking density was continually reduced throughout the experiment in terms of

numbers of fish per unit volume and increased in terms of weight per unit volume. Maximum stocking density amounted to approximately 7 g/litre by the tenth day of the experiment but did not appear to adversely affect the growth of the larvae, and water exchange was sufficient to maintain excellent water quality in all tanks throughout the experiment. The possibility that stocking density affected the growth of the experimental fish cannot be dismissed, but in terms of numbers of larvae per unit volume it remained a constant factor throughout the experiment. It was also intended to use identical stocking densities to those employed in the three Artemia trials in all future experiments with larvae and for this reason investigation of the effects of stocking density on growth was not considered worthwhile. Temperature also has a substantial effect on growth in the range 21 - 25°C (Baranova, 1974), which would largely account for the minor differences in optimum feeding rate determined in this study and by other researchers (Chiba, 1961; Baranova, 1974).

The changes in body composition of carp larvae and post-larvae with growth are of some interest. Few studies of the body compositions of larval fish during the early stages of their growth exist, comparable data to those reported here only being available for larval herring (Ehrlich, 1974a) and plaice (Ehrlich, 1974b). While the analytical techniques employed in this study were extremely crude, it was possible to demonstrate some significant changes in carcass composition with growth.

Most notable among these changes were the gradual increase in carcass ash content and decrease in carcass moisture. The increased ash content of the fish is similar to that observed by Ehrlich (1974a, b) for herring and plaice larvae, which can be ascribed to progressive ossification of the skeleton. Decreases in carcass moisture, which were also observed by the above author to occur in



herring and plaice larvae, may be explained by the large amounts of extracellular space associated with rapidly dividing tissues during the early stages of development, which decrease with increasing cell size as the fish grow. Ehrlich (1974a) also showed that herring larvae only began to accumulate neutral fat stores after reaching a length of 20 mm, and he suggested that it may be advantageous for the smallest larvae to convert food into growth rather than accumulating energy stores in order that the improved preying ability of larger individuals may be achieved as rapidly as possible. While unequivocal interpretation of the lipid content of the carcasses of carp juveniles is impossible in this study, due to their derivation by difference from other analysed parameters, it would appear that the carcass lipid content of carp larvae remains relatively static until they reach a body weight of approximately 15 mg. This point is associated with the end of larval development in the carp, when tissue differentiation is presumably complete, and this, taken with other evidence, may suggest a change in metabolism at approximately this point.

CHAPTER 4.

Chapter 4. Experiment 2: The Optimal Adaptation Weight for Carp

Larvae Offered a Commercial Artificial Fry Diet.

4.1. Introduction.

Intensive commercial production of carp larvae currently relies on the provision of natural or cultured live food, usually Artemia. Numerous attempts to rear carp larvae entirely on artificial diets have all shown to date that live food, either alone or in combination with artificial diets, enhances growth and survival during the early larval phase (Imam and Habashy, 1972; Jirasek, 1976; Anwand et al, 1976; Dabrowski et al, 1978; Dabrowska et al, 1979).

The same difficulty exists for other non-salmonid species of fish larvae. Barahona-Fernandes and Girin (1976) and Bromley (1979) have demonstrated that a period of live feeding and of weaning on mixed live and artificial diets is required for sea bass (Dicentrarchus labrax) and turbot (Scophthalmus maximus) larvae, before growth and survival on conventional artificial diets can be considered satisfactory. Huisman (1979a) has shown that an analogous technique is necessary for the rearing of grass carp (Ctenopharyngodon idella) larvae.

After several unsuccessful attempts to rear carp larvae directly on artificial diets immediately after hatching (Chapter 8), it was decided to adopt a different approach to the development of artificial diets for this species. Following the concept of a gradual weaning process propounded by Barahona-Fernandes and Girin (1976), and Bromley (1977), amongst others, it was decided to attempt to establish the lowest body weight at which carp larvae could be transferred directly from a diet of Artemia nauplii to a commercial dry compounded trout fry diet with acceptable growth and survival. While it was considered that such an "adaptation weight" was really an artefact derived from the nutritional inadequacies of present

artificial larval diets, it was nevertheless thought to provide a useful practical datum from which to assess improvements in diet performance.

By direct transfer from Artemia to an artificial diet, rather than a gradual weaning process, it was hoped to investigate whether an improved ability to utilise the diet was an abrupt or gradual development, and thus whether it was associated with development of a physiological mechanism such as the ability to secrete a particular enzyme. After defining a particular weight from which successful rearing on a conventional artificial diet was possible, it was intended to feed semi-purified diets of known composition and gain some idea of the nutritional requirements of post-larvae and fry. Development of a nutritionally optimal diet for these older fish should theoretically have permitted transfer to the diet at an earlier age with acceptable growth and survival, whereupon the whole process of nutritional optimisation of defined diets could have been begun again. In this way, it was hoped to be able to gradually develop a successful artificial diet for larvae to be fed immediately after hatching, on a much more rational basis than the trial-and-error approach adopted in earlier experiments.

As the initial step in this program of research therefore, the following study was designed to establish the lowest body weight at which carp larvae could be transferred directly from Artemia nauplii to a commercial trout fry diet, which was chosen to be the control diet in all further experiments comparing the growth response of fish fed semi-purified experimental diets with a control.

4.2. Materials and Methods.

All experimental fish were held in a single row of eight experimental tanks (Section 2.5) and were continuously illumin-

ated for the duration of the experiment. A commercial trout fry food (Edward Baker Ltd, "Omega" brand; grades "Fine Starter and No.0) was dispensed at two-hourly intervals over 24 hours via the automatic feeders described in section 2.6, and was sieved into four grades (120 - 250 μm , 250 - 355 μm , 355 - 500 μm and 500 - 850 μm) in order to feed carp juveniles ranging in weight from 2.5 - 500 mg.

Over a period of eighteen days after hatching, seven batches of between 100 and 250 carp larvae and post-larvae weighing approximately 2.5, 5.0, 7.5, 10.0, 15.0, 25.0 and 35.0 mg were transferred to the experimental facility. Up until the time of transfer, these fish were fed exclusively on a diet of Artemia nauplii, and were presented with the commercial trout fry diet immediately after transfer to the experimental facility. At the time of each transfer, a batch of 50 larvae or post-larvae of identical weight to the experimental fish were placed in the disused experimental tank lattice (section 2.5) held in the lower rearing trough (section 2.5) and starved for the experimental duration, daily mortality being recorded. Each experimental weight class was fed to excess with appropriately sized trout fry food for a period of eighteen days after stocking, with the exception of the 25.0 and 35.0 mg weight classes. Due to the rapid growth of these fish, high stocking density led to water quality deterioration and forced premature termination of the experiment after 15 and 12 days respectively.

Excess feeding was achieved by distribution of approximately 75% of body weight per day for larvae up to 15 mg, 50% of body weight per day for post-larvae of between 15 and 50 mg, and 25% of body weight per day for fish weighing more than 50 mg, after the suggested feeding rates of Anwand et al (1976) for carp larvae offered dry artificial diets. Food quantity was adjusted after each weighing, thus the amount of excess feeding was roughly equal

within each experimental treatment.

Live and dry weight increments of the experimental fish were determined by sampling at three-day intervals, daily mortalities also being noted. Groups of 6 - 10 fish per sample, depending on individual size, were subdivided and weighed in triplicate batches in the manner described in section 2.10 to obtain an average individual weight. In order to facilitate comparison of mortalities between weight classes, it was arranged that the total numbers of fish sampled comprised 20% of the original experimental population. It was thus possible to exclude sampled fish from calculations of cumulative mortality, since they represented a constant proportion of each population.

4.3. Results.

Body weight and growth rate data for the experiment are presented in Table 5, and mortality data (including those for starved controls) in Fig. 8. Mortality of the starved control fish corresponded closely with that reported for carp larvae and fry held at a similar water temperature by Horoszewicz (1974). It can be seen from Fig. 8 that without exception, initial mortality of fed larvae preceded the onset of mortality amongst starved control larvae.

Table 4. Proximate Analyses of the Experimental Diets.

Edward Baker Ltd.	<u>Parameter</u>	<u>Mean %</u>	<u>+S.D.</u>
"Fine Starter"	Moisture	9.26	0.12
	Crude Protein	55.79	0.67
	Crude Lipid	7.12	0.16
	Ash	11.52	0
	NFE	16.31	-
Edward Baker Ltd. "No.0"	Moisture	9.49	0.03
	Crude Protein	52.13	0.16
	Crude Lipid	8.25	0.25
	Ash	11.47	0.08
	NFE	18.66	-

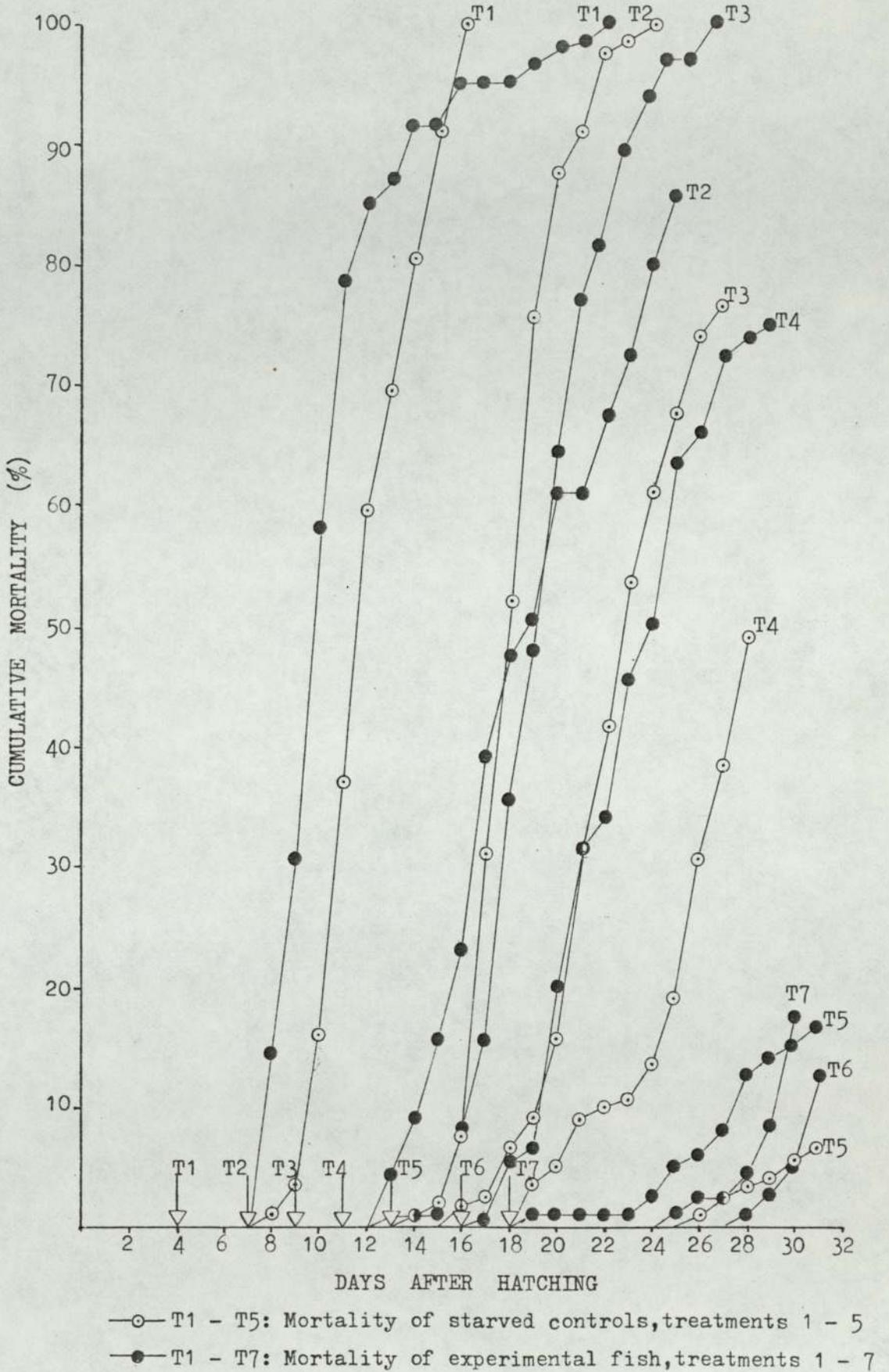
Table 5. Experiment 2: The Effect of Adaptation Weight on Growth of Carp Juveniles.

Treatment Number	Initial live weight		Final live weight after 18 days.		Specific Growth Rate (%/day)	
	Initial fish numbers	Mean(mg) ±S.D.	Mean(mg) ±S.D.	Mean(mg) ±S.D.	Rate	±S.E.
1	250	2.44	0.10	6.70	2.86	5.1 ^{a*}
2	250	5.60	0.45	15.49	0.54	5.7 ^a
3	250	8.13	1.10	24.73	1.65	6.2 ^a
4	250	9.49	0.71	56.42	4.64	9.9 ^b
5	150	14.29	1.87	92.22	6.02	10.4 ^b
6	100	24.48	4.01	180.08**	2.47	13.4 ^c
7	100	36.03	5.54	209.92**	14.95	14.8 ^c ±S.E. 0.7

* Numbers with the same superscript are not significantly different (P > 0.05)

** Experiment terminated prematurely due to deterioration in water quality

Figure 8. Cumulative Mortalities of Experimental and Starved Control Fish.

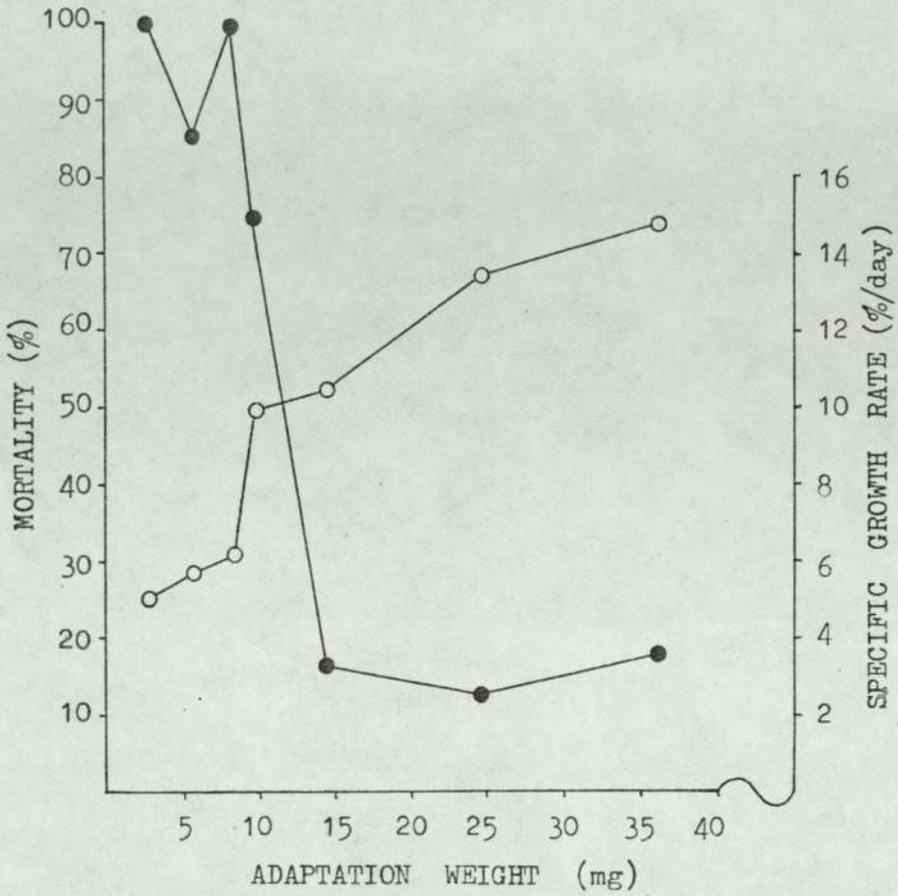


This implies that mortality in the experimental fish was a direct result of feed intake and was not due to an inability to assimilate the experimental diet. It is however difficult to explain why treatment T2 showed marginally improved survival compared with treatments T1 and T3, where mortality was total by the end of the experiment. Unfortunately, limited experimental facilities precluded duplication of treatments and it is impossible to say whether this result would be replicable. In treatments T4 - T7, survival showed gradual improvement with increasing initial fish weight, the increases in mortality in treatments T5, T6 and T7 from days 24, 28 and 25 respectively almost certainly being due to worsening water quality as a result of overstocking.

Although samples of moribund fish were preserved in Bouin's fixative for later sectioning and histological examination, no explanation can be offered for the cause of death amongst fish of a low initial body weight given the experimental diet. No obvious differences were evident between gross external and histological appearance of moribund experimental fish and healthy Artemia-fed carp of a similar body weight. It was observed that considerable oedema of the gut occurred in larvae of less than 5 mg body weight given the experimental diet, but due to lack of foresight, no samples of fish displaying this characteristic were taken for histological examination. At the time of the experiment, this symptom was put down to the ingestion of air by larvae feeding at the water surface.

Fig.9 compares the total mortality recorded for each treatment with the average specific growth rate attained over the experimental period. A decrease in mortality was accompanied by an improvement in specific growth rate for treatments 4 and 5, mortalities for treatments 5, 6 and 7 exhibiting a general

Figure 9. Effect of Adaptation Weight on Growth and Survival.



- Mortality of experimental fish
- Specific Growth Rate of experimental fish

stabilisation. A marked improvement in survival and growth is noticeable for treatment 5, corresponding to larvae with an initial weight of 14.29 mg (Table 5), a similar improvement in growth but not in survival occurring in treatment 4.

A statistical comparison of average specific growth rates (in order to circumvent the differences in initial weight) is shown in Table 5. There was no significant difference between the specific growth rates achieved in treatments 1, 2 and 3, but a significant ($P < 0.05$) increase in growth rate was evident between these treatments and treatments 4, 5, 6 and 7. Treatments 4 and 5 did

not differ significantly from each other, but showed significantly ($P < 0.05$) lower specific growth rates than treatments 6 and 7. It would thus appear that carp larvae and post-larvae with an initial body weight of 9.5 mg and above exhibited an improved ability to utilise and survive upon the experimental diet, compared with larvae of a lower initial body weight.

The specific growth rates achieved in treatments 6 and 7 did not differ significantly from each other, but were both significantly ($P < 0.05$) higher than previous treatments, suggesting that these were the maximum attainable growth rates for the particular size of post-larvae and type of diet used in the experiment. Similar specific growth rates were later recorded for comparably sized fish (Chapters 5, 6 and 7), although some difficulty in comparing growth rates is inevitable because of the continuously decreasing growth rates with time (Chapters 3 and 5).

4.4. Discussion and Conclusions.

While this study demonstrated that carp post-larvae could be reared on a commercial trout fry diet from an initial body weight of approximately 15 mg with better than 80% survival, the dependence of this "adaptation weight" on diet composition is amply illustrated by the results of other researchers. Jirasek (1976) reported that adaptation of carp fry to a commercial diet could only be accomplished from an initial body weight of 30 mg, although Anwand et al (1976) were able to wean carp larvae onto a dry compounded feedstuff with approximately 50% survival after only three days feeding on live food and one day of mixed administration. If the optimal feeding rates determined for Artemia (Chapter 3) were to be used, carp larvae could have been transferred directly from Artemia to the Edward Baker Ltd. trout fry diet some 5 - 6 days after first feeding.

No simple explanation of the inability of the experimental diet to support growth and survival at lower initial body weights is available. Dabrowski et al (1978) have postulated that exogenous proteolytic enzymes may play a significant role in diet assimilation and demonstrated a slight increase in survival of carp larvae fed diets containing bovine trypsin. Nose et al (1974) have shown that diet pH can affect absorption of nutrients in juvenile carp. Dabrowska et al (1979) have discussed the probable importance of the protein component of larval diets for carp and amino acid interactions which may affect its absorption and assimilation. All of these factors could have contributed to the inadequacies of the experimental diet.

Appelbaum (1977,1979) has succeeded in rearing larval carp and grass carp entirely on an artificial diet, composed largely of the yeast Candida lipolytica, without additional live food. Such an achievement clearly demonstrates the feasibility of rearing larvae entirely on artificial diets, even though growth was poor in comparison to that achieved with Artemia-fed larvae.

That a sharply improved ability to utilise the commercial diet should become manifest at a weight of approximately 15 mg, which corresponds with the generally accepted end of larval development, is probably coincidental. However, the ability develops for a doubling in weight from approximately 8 to 16 mg, a relatively small change in terms of the rest of development, therefore it must reflect a change in the physiology of the larvae, if only with respect to the particular diet formulation in question. An explanation of this change would be of great interest.

Recently, Fluchter (1980) has reported that whitefish (Coregonus) larvae, although beginning feeding on copepods on the fifth day after hatching, only begin to digest food on the twelfth day and

will die if copepods are the only available food. Survival and growth are only assured by the presence of Daphnia or Artemia. This observation Fluchter (1980) ascribes to the unsuitable biochemical composition of copepods, suggesting that whitefish larvae have very precise nutritional requirements during the first few days after hatching. It is of interest to note that a larval fish should show such (presumably) specific requirements, which might imply that carp larvae are similarly particular during their early stages.

CHAPTER 5.

Chapter 5. Experiment 3: The Optimisation of Feeding Rate for Carp

Post-larvae and Fry Adapted to a Commercial Trout Fry Diet.

5.1. Introduction.

Following the definition of a suitable adaptation weight from which carp post-larvae could be reared on a commercial trout fry diet (Chapter 4), it was decided to use a similar diet as control in all subsequent experiments attempting to develop a nutritionally optimal diet for post-larvae. It was therefore decided to determine the correct feeding rates for such a commercial diet, as a preliminary step in this course of research.

Huisman (1979b) has recently drawn attention to the importance of feeding rate in presentation of diets to juvenile fishes, and has criticized the somewhat arbitrary feeding rates adopted by some other researchers (Anwand et al, 1976; Schlumpberger et al, 1976; Appelbaum, 1977), poor growth rates being attributable to gross underfeeding. Not only must food be available in sufficient quantity to ensure adequate growth, it must also be available in quantities which minimise wastage. Overfeeding of young fishes, apart from representing a waste of food, can rapidly cause water pollution with conventional artificial diets and predispose towards disease, eventually increasing mortality and reducing the growth rate of the fish.

Feeding to excess, as was done in some earlier experiments (Chapters 4 and 8), was considered to prohibit meaningful comparisons of growth rate between different dietary treatments and to be more reflective of palatability than diet utilisation. It was felt that feeding to a predetermined percentage of body weight represented a more satisfactory method of feeding. It was observed on several occasions that underfeeding of carp post-larvae and fry increased the incidence of cannibalistic behaviour and abnormal

fish, therefore it was thought important to feed all experimental diets at a level which would yield close to maximum growth for the control diet, necessitating the following study of the correct feeding rate for the commercial trout fry diet.

5.2. Materials and Methods.

In order to investigate possible changes in feed requirement with age, and to obtain information for later experiments with carp fry, two age/weight classes of post-larvae and fry (of different parents) were offered graded quantities of trout fry food (Edward Baker Ltd., "Omega" brand) expressed as a percentage of their live body weight.

All experimental fish were held in a single row of the swivelling experimental tanks (Section 2.5) in continuous artificial light, and were fed at two-hourly intervals over 24 hours via the automatic feeding equipment described in Section 2.6. The allotted daily rations were divided into twelve approximately equal portions on the automatic feeder discs.

5.2.1. Experiment 3A. Optimum Feeding Rate for Post-larvae.

The first weight class of post-larvae initially weighing approximately 15 mg was chosen on the basis of experiment 2 (Chapter 4) as the lowest weight which could be successfully reared on the commercial diet. Lots of 100 post-larvae per treatment were allocated to the experimental tanks and fed at rates of 10, 12.5, 15, 17.5, 20, 25 and 30% of their body weight per day, measured as the direct weight of the experimental diet (ie. without correction for dietary water content). Growth increments were monitored by sampling at three-day intervals, feeding rate being altered after each sampling so that the actual feeding rates were only nominally as stated above. Samples consisted of 10 - 15 fish which were weighed in triplicate in the manner described in Section

2.10. Daily mortalities were noted and the experiment continued for twelve days.

5.2.2. Experiment 3B. Optimum Feeding Rate for Fry.

The second weight class of experimental fish consisted of fry initially weighing approximately 100 mg. Lots of 15 fry per treatment were allocated to the experimental tanks, and these were batch weighed as described in Section 2.10 at three-day intervals to an accuracy of one mg to obtain a total tank weight and average individual weight. The fry were fed at nominal rates of 2, 5, 10, 15, 17.5, 20, 25 and 30% of their body weight per day, adjusted at each weighing, and the experiment continued for a period of twelve days.

5.3. Results.

5.3.1. Experiment 3A. Optimum Feeding Rate for Post-larvae.

Growth and mortality data for the first weight class of post-larvae are presented in Table 6, and growth increments of each experimental treatment in Fig. 10. Statistical comparison of average specific growth rates (Table 6) revealed no significant increase in growth for feeding rates in excess of a nominal 15% of body weight per day, treatments 3 - 7 not being significantly different. Fig. 11 compares growth and food utilisation for the different treatments and it can be seen that food utilisation, calculated from the true dry weight of food fed, was best at feeding rates lower than those yielding maximum growth. Acceptable growth and food conversion occurred for feeding rates of between 15 and 17.5% of body weight per day.

With the exception of treatment 3, mortalities for each treatment (Table 6) followed increases in feeding rate. It can be seen from Fig. 10 that for the highest feeding rate of 30% of body weight per day, some decline in growth was evident during the last three

Table 6. Experiment 3A: Growth Performance of Post-larvae.

Treatment Number	Final live weight after 12 days Mean(mg) <u>±S.D.</u>	Specific Growth Rate (%/day)	Mortality (%)
Initial live weight 17.02 <u>±</u> 3.52 mg (100 fish per treatment)			
1	47.02 1.80	8.6 ^{a*}	0
2	81.04 12.42	13.1 ^b	1
3	126.60 8.83	16.9 ^c	14
4	134.60 6.47	17.4 ^c	3
5	148.53 8.01	18.2 ^c	15
6	153.91 5.00	18.5 ^c	20
7	152.22 6.86	18.4 ^c	18
		<u>±S.E.</u> 1.0	

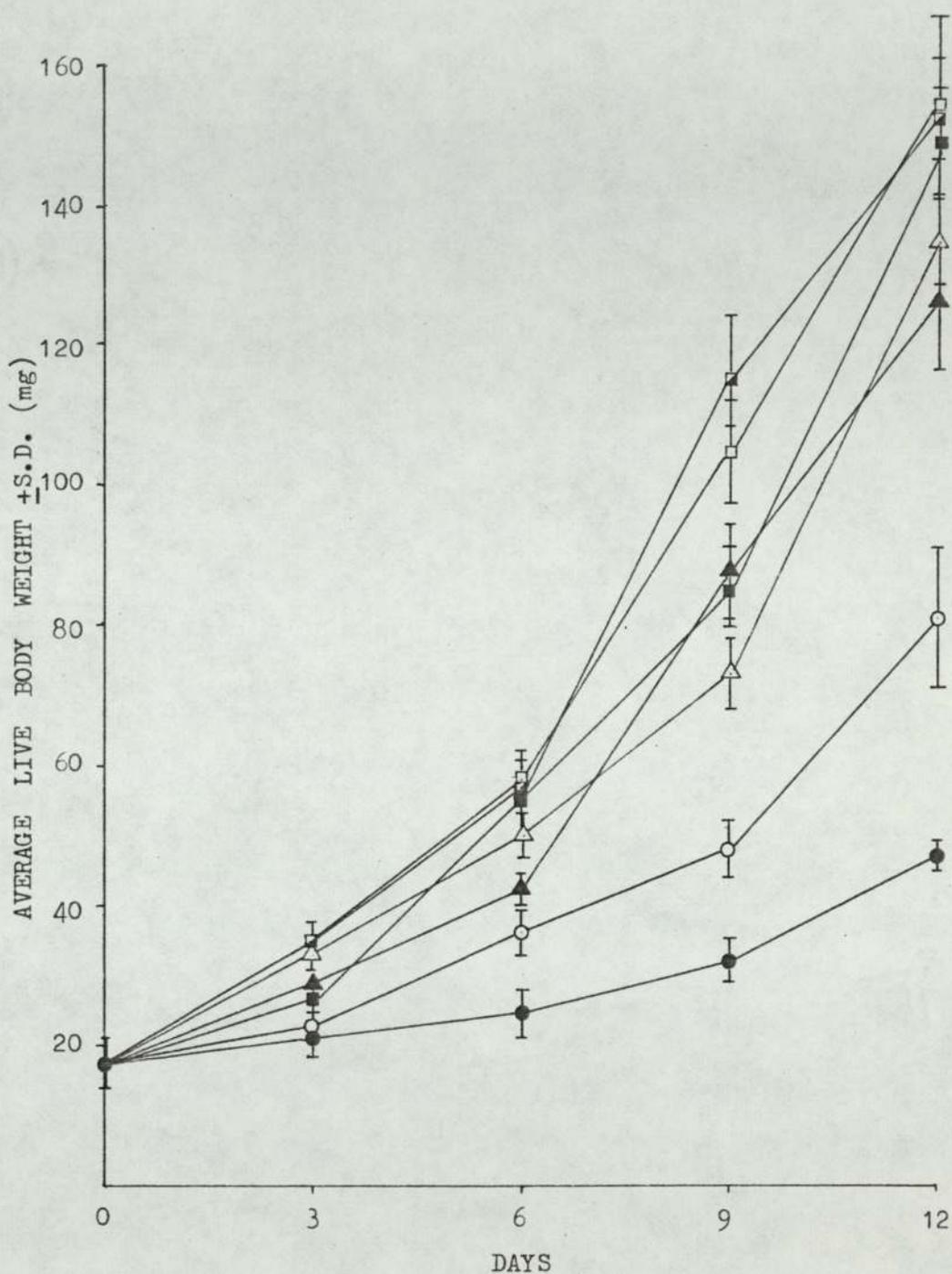
Table 7. Experiment 3B: Growth Performance of Fry.

Treatment Number	Average initial live weight (mg)	Average final live weight after 12 days (mg)	Specific Growth Rate (%/day)
1	130	145	0.9 ^a
2	105	170	4.0 ^a
3	117	405	10.4 ^b
4	108	500	12.8 ^b
5	123	437	10.6 ^b
6	92	381	11.8 ^b
7	96	383	11.5 ^b
8	102	461	12.6 ^b
		<u>±S.E.</u> 1.3	

*Numbers with the same superscript are not significantly different

(P > 0.05)

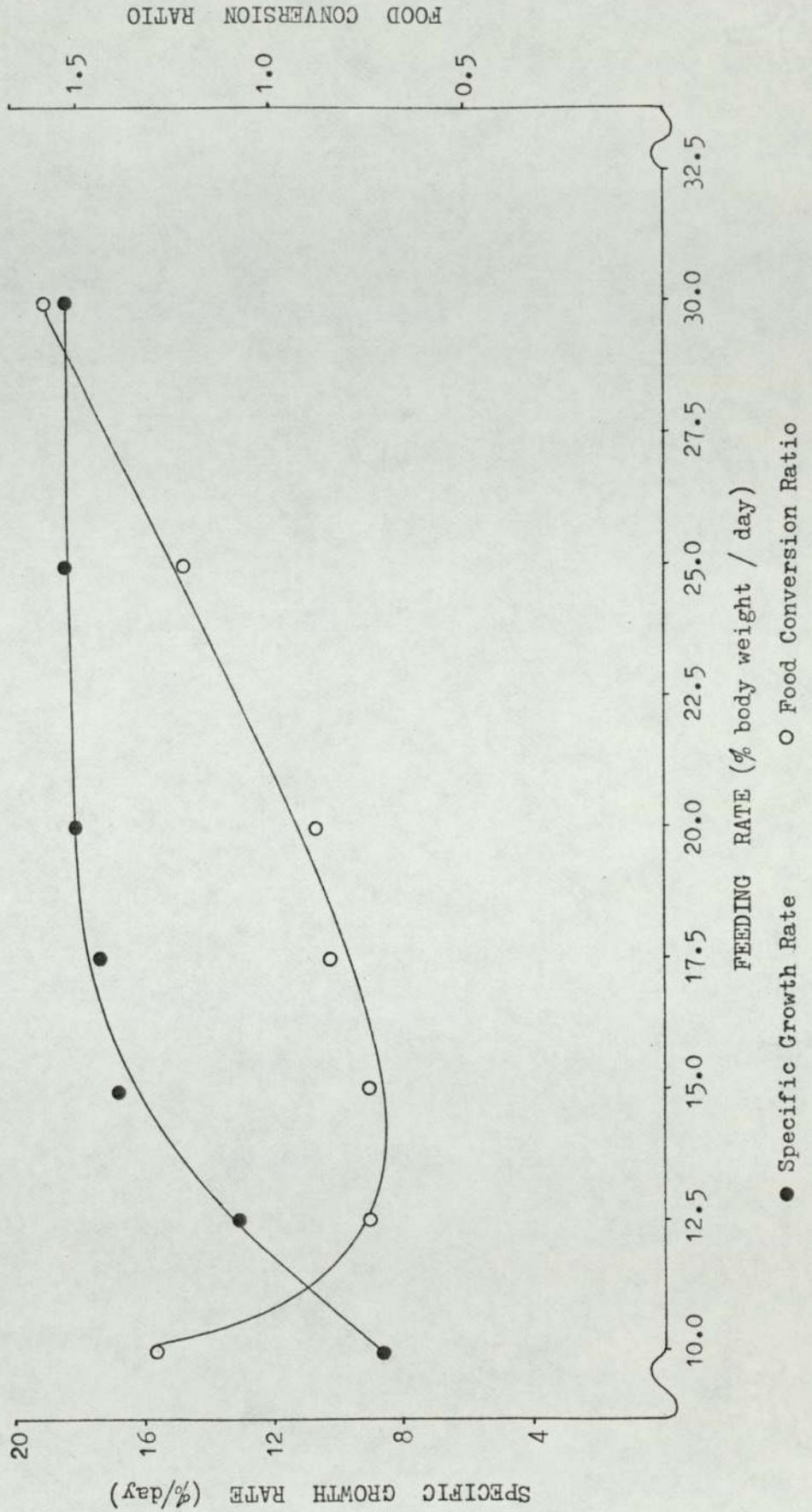
Figure 10. Live Weight Increments of Carp Post-larvae Fed at Different Rates.



Key to Feeding Rates (nominal % body weight/day)

- | | |
|---------|-------|
| ● 10% | ■ 20% |
| ○ 12.5% | □ 25% |
| ▲ 15% | ▣ 30% |
| △ 17.5% | |

Figure 11. The Effect of Feeding Rate on Growth and Food Conversion of Post-larvae.



days of the experiment. This may have partly been due to mortality upsetting the collection of representative samples for weighing, but it also seems likely that some deterioration in water quality occurred for this grossly excessive feeding rate. It is of interest to note that the levels of mortality in treatments 6 and 7, in which food administration was excessive, compared well with the mortalities recorded in Experiment 2 (Chapter 4) for post-larvae of similar initial weight correspondingly fed to excess. However, it is difficult to explain the high level of mortality in treatment 3 (Table 6), which represented an approximately optimal feeding rate.

5.3.2. Experiment 3B. Optimum Feeding Rate for Fry.

Growth data for the second weight class of fry are presented in Table 7. No mortalities occurred during this experiment. A statistical comparison of average specific growth rates (Table 7) between treatments showed that feeding rates in excess of a nominal 10% of body weight per day did not elicit any significant increase in specific growth rate amongst these fish. The maximum specific growth rates obtained were considerably lower than those obtained with the first weight class of post-larvae, indicating a reduction in growth with age. Fig. 13 compares growth and food utilisation for the different feeding rates, and it can be seen that optimal growth and food conversion occurred for nominal feeding rates of between 10 and 15% of body weight per day.

Fig. 12 shows the growth increments of each experimental treatment over the experimental period, and it can be seen that a nominal feeding rate of 2% of body weight per day represented an approximately maintenance level of feeding for this size of carp. No explanation can be offered for the gradual decrease in growth over the last six days of the experiment for the group receiving a nominal 17.5% of their body weight per day. This represented

Figure 12. Live Weight Increments of Carp Fry Fed at Different Rates.

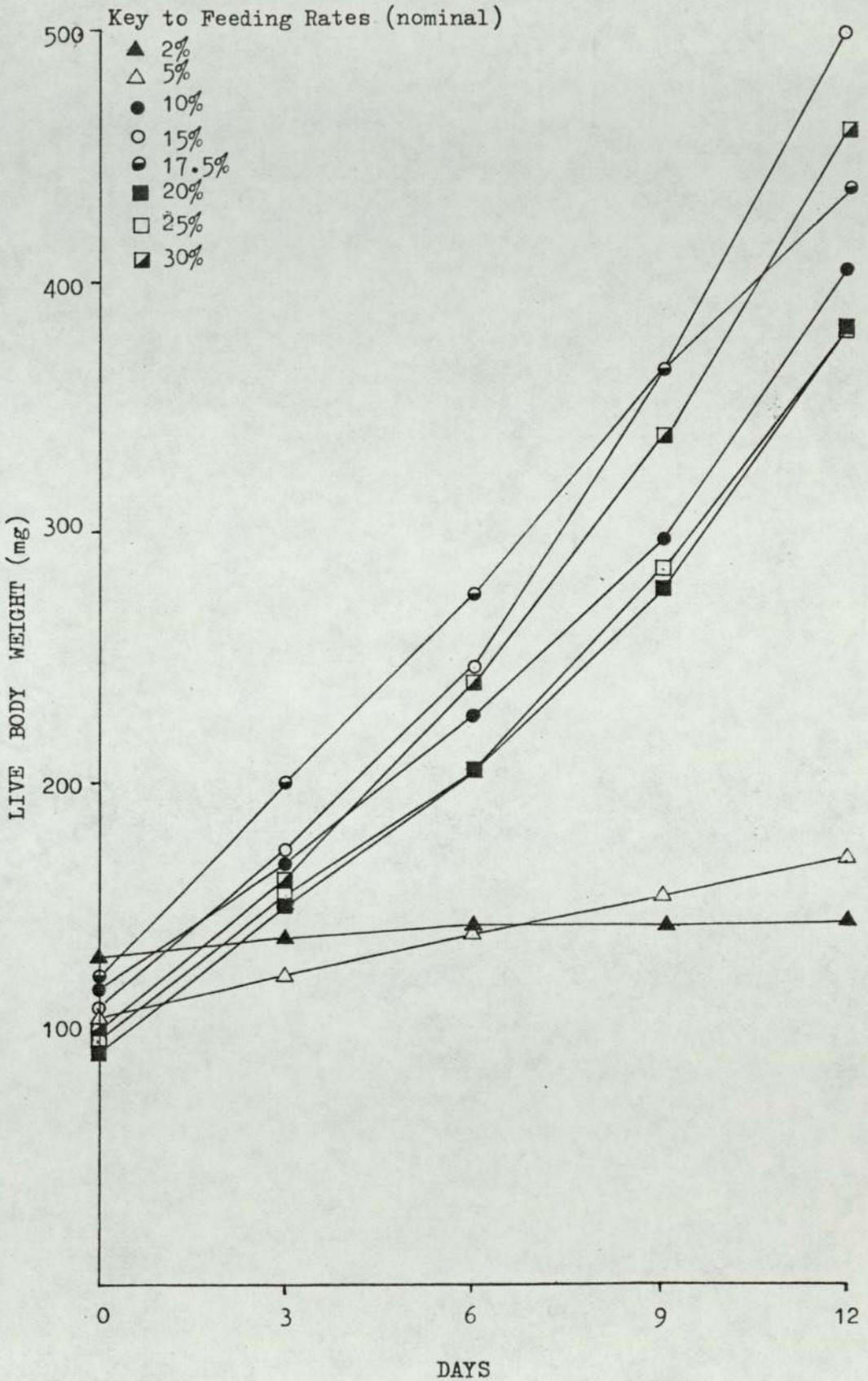
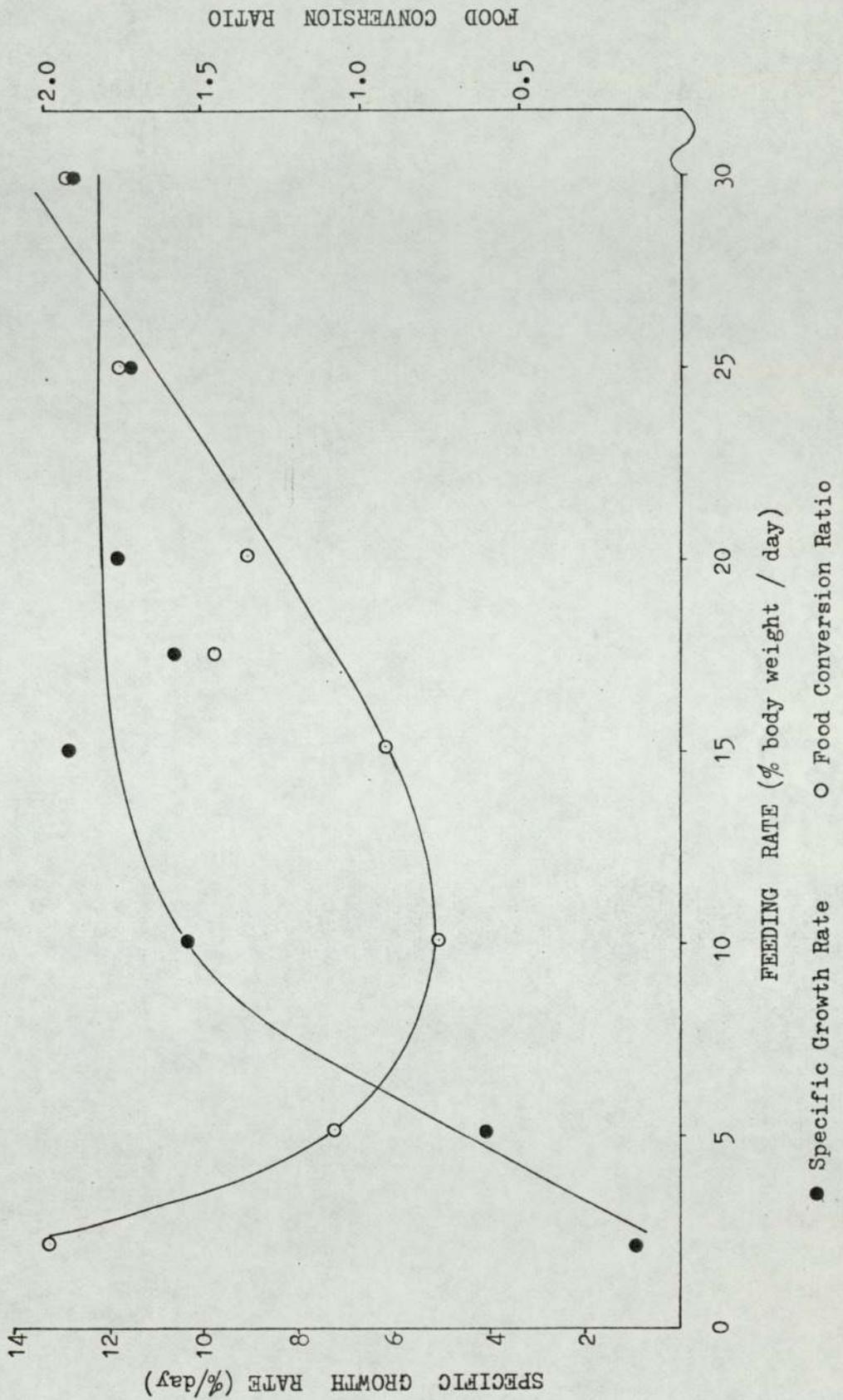


Figure 13. The Effect of Feeding Rate on Growth and Food Conversion of Fry.



only a slightly supra-optimal feeding rate, and the decrease cannot be explained by any deterioration in water quality. Because of the low stocking densities employed in the experiment, this remained excellent in all tanks for its duration. It may have been that one or two of the experimental fish were not growing as well as the others, as can occasionally happen, but this possibility could not be verified due to an accidental upset of the tanks at the end of the experiment, when the individual weights of the experimental fish would normally have been measured.

5.4. Discussion and Conclusions.

The study showed that carp post-larvae fed on the commercial diet used in the experiment exhibited optimal growth and food conversion for nominal feeding rates of between 15 and 17.5% of body weight per day. Carp fry showed a slight reduction in feed intake and growth compared with post-larvae, optimal growth and food conversion for the weight class of fry used in the experiment occurring for nominal feeding rates of between 10 and 15% of body weight per day.

Determination of correct feeding rates for an artificial diet for carp is only made possible by the suitable feeding behaviour of the young fish. Several commercially important species of fish cannot be reared on artificial diets during their larval stages because they do not easily learn to recognise inert particles as food (Bromley, 1977; Girin, 1979). Carp larvae, however, will take inert diet particles immediately after hatching, and quickly learn to feed solely on artificial diets (Anwand et al, 1976; Appelbaum, 1976; Grudniewski et al, 1979). Carp post-larvae and fry will feed throughout the whole water column and search actively for food, therefore little administered food will be wasted, enabling realistic measurements of food utilisation to be

made.

The importance of feeding rate in larval diet presentation would appear to have been largely overlooked, as has been pointed out by Huisman (1979b). Feeding rates of 50% of body weight per day have been suggested for common carp post-larvae of a similar size to the experimental fish by Anwand et al (1976), which was shown to be excessive in a previous experiment (Chapter 4). Huisman (1979a) has proposed feeding rates of 400% of body weight per day for larval grass carp, but it is unclear whether this figure refers to live or dry foods. Huisman (1979a) also recorded food conversion ratios for grass carp fry of 0.2 to 1.3 g body weight fed with a commercial trout fry food, the quoted values of 1.1 - 1.4 being much worse than those obtained here for carp fry of 0.1 to 0.5 g body weight fed optimally. However, similar values to these were obtained for carp fry fed considerably to excess (Fig.13).

Weighing of the experimental fish was carried out at three-day intervals for practical reasons, since a compromise had to be struck between a weighing frequency which would minimise the risk of mortality from weighing stress and one which would allow a reasonably constant feeding rate to be achieved. The actual feeding rates were therefore only nominally as chosen because of the rapid growth of the fish. An approximation of the true feeding rates was made by estimating intermediate body weights from the recorded values, and calculating an overall feeding rate average for each treatment from the daily feeding rates thus estimated. A correction was also made for dietary water content in the quantity of food administered.

The true feeding rates calculated in this way approximated to 9.8, 12.3, 12.9, 15.7, 16.9, 21.4 and 25.4% of body weight per day

for the post-larvae, and 2, 4.8, 9.0, 13.1, 15.7, 17.7, 22.2 and 26.3% of body weight per day for the fry. Optimal growth and food conversion was therefore exhibited at feeding rates of between approximately 12.9 and 15.7% of body weight for the post-larvae, and between 9.0 and 13.1% of body weight for the fry.

It is of interest to compare the above optimal feeding rate for post-larvae with that obtained in Experiment 1 (Chapter 3) for similarly sized fish fed on Artemia. Artemia-fed post-larvae weighing between 15 and 150 mg showed optimal growth for feeding rates ranging from 100 to 150% of their body weight per day, which corresponds to a dry matter intake of approximately 10 - 15% per day. The feed intake for post-larvae fed the commercial diet is within the same range, so that it would appear that both Artemia-fed and artificial diet-fed fish take in the same nutrient density. However, growth rate for the Artemia-fed fish was almost double that achieved with the commercial diet, due to superior conversion of Artemia on a dry weight basis. This would imply that the artificial diet is of very much poorer nutritional value than Artemia, or that the carp post-larvae are unable to assimilate the nutrients in the form in which they occur in the artificial diet. Poor growth on the artificial diet is definitely not due to disparity in food intake.

CHAPTER 6.

Chapter 6. Experiment 4: The Effects of Feeding Frequency and Diet
Colour on Diet Utilisation by Carp Fry.

6.1. Introduction.

It has been reported on numerous occasions that feeding frequency can have a substantial affect upon the growth rate and food conversion efficiency of fish (Rozin and Mayer, 1961; Andrews and Page, 1975; Grayton and Beamish, 1977; Chua Thia-Eng and Teng Seng-Keh, 1978). Moreover, optimal feeding frequencies change with the body size of the particular species under study, in relation to changing quantitative food requirements with age (Ishiwata, 1968a; Murai and Andrews, 1976), as well as with the composition of the food (Rozin and Mayer, 1961; Ishiwata, 1968b; Grove et al, 1978).

In experiment 3 (Chapter 5) the optimal feeding rates for carp post-larvae and fry were assessed for an arbitrarily chosen feeding frequency of 12 feeds per day, dictated by the design of the automatic feeding equipment. It was therefore considered of some importance to determine whether this feeding frequency was suitable for the post-larvae and fry, giving close to optimal growth and food conversion, or whether an increased feeding frequency would improve the utilisation of the diet. Twenty-four hour feeding was chosen because it has been reported by several authors that carp larvae continue to feed in the dark (Alikunhi, 1952; Appelbaum, 1976), so it seemed sensible to exploit this behaviour and keep the juvenile fish in continuous light in order to maximise feed intake. Thus no attempt was made to investigate the effect of feeding frequency on food intake, firstly because the approximate satiation feeding rate had already been determined for the commercial trout fry diet in experiment 3, and secondly because it was impossible to feed such small fish to satiation by hand throughout the day and night.

Behavioural studies with a wide variety of fish species have established beyond doubt that fish possess colour vision (Burkamp, 1923; Hurst, 1953; Muntz and Cronly-Dillon, 1966), and there is strong circumstantial evidence to suggest that this is in many cases trichromatic (Muntz, 1974). This therefore raises the question of whether fish have particular food colour preferences, and specifically whether colour plays any part in the acceptance of artificial diets by carp juveniles.

All artificial diets offered to carp post-larvae and fry in earlier experiments (Chapters 7 and 8) were coloured with an orange dye to resemble the colour of Artemia, since the colour of these invertebrates could not have been particularly unattractive to carp larvae and should not have exerted a negative effect on the acceptability of the artificial diets. However, it was not known whether another colour could enhance the attractiveness of artificial diets for carp juveniles, and it was thought worthwhile to study this possibility. Although very little work would appear to have been done on the colour preferences of fish, some studies have convincingly demonstrated colour preferences (Wolf, 1953; Ginetz and Larkin, 1973). Cyprinids certainly possess colour vision, and which is almost certainly trichromatic (Muntz and Cronly-Dillon, 1966; Tomita et al, 1967; Muntz, 1974), thus it was decided to investigate the effects of diet and background colour on feed utilisation by juvenile carp. Assessment of the effect of background colour was thought necessary because Ginetz and Larkin (1973) demonstrated that alteration of background colour affected the choice of coloured food by rainbow trout.

Although for the sake of completeness it would have been desirable to investigate the effects of feeding frequency and diet colour on feed utilisation of both post-larvae and fry, this was

unfortunately not possible, and the experiments were carried out with carp fry only. In the case of the diet colour experiment, this was due to the need for a light basal diet which would minimise the interference of the natural diet colour with that of the added dyes, because of the very dark colour of the commercial control diet. It was therefore decided to carry out the diet colour experiment after an attempt to develop a nutritionally optimal diet (Chapter 7), but it proved impossible to develop a semi-purified diet which would support growth and survival of post-larvae as well as the commercial trout fry diet, and it was thus not possible to investigate the effects of diet colour on post-larvae.

6.2. Materials and Methods.

6.2.1. Experiment 4A: Feeding Frequency.

Five groups of 15 carp fry initially weighing approximately 100 mg were allocated to each of five swivelling experimental tanks (section 2.5), and were fed at a nominal feeding rate of 15% of their body weight per day on a commercial trout fry diet (Edward Baker "Omega" brand). The experimental diet was dispensed via automatic feeders (section 2.6) which were arranged so as to deliver food to the respective tanks at intervals of 2, 4, 6 and 8 hours, and continuously, during a 24 hour period. The feeding discs were perforated with 12 equi-distant slots and rotated once every 24 hours, therefore feeding intervals were determined by the omission of some feeding slots; continuous feeding was achieved by placing two feeders over one tank, such that each delivered food alternately. The respective quantities of food were divided into approximately equal portions, corresponding with the desired number of feeds per day.

The fry were weighed in the manner described in section 2.10 at three-day intervals, the feeding rate being altered after each

weighing in order to account for growth, and the experiment continued for 12 days. All the experimental fry were weighed individually at the end of the experiment so as to assess the growth dispersion within each treatment.

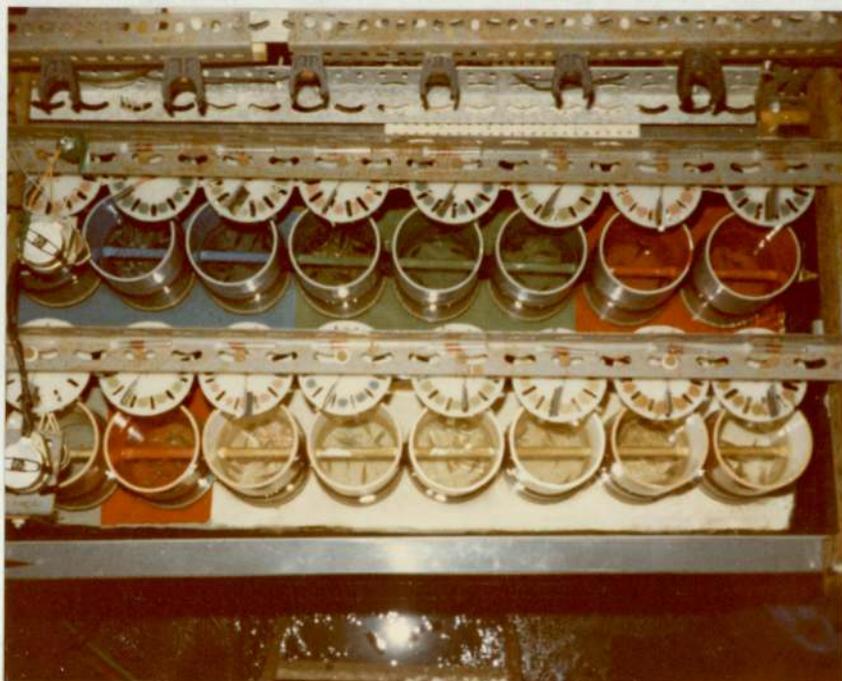
6.2.2. Experiment 4B: Diet Colour.

Fourteen groups of 15 carp fry initially weighing approximately 150 mg were allocated to each of fourteen specially prepared swivelling experimental tanks (section 2.5) and were fed at a nominal feeding rate of 15% of their body weight per day on an experimental basal diet coloured with three different commercial food dyes. Twelve combinations of diet and tank background colour were tested for their effects on the experimental fry, in addition to two control groups consisting of the undyed basal diet and a commercial trout fry food (Edward Baker "Omega" brand).

The experimental tanks were prepared by painting them with four different colours of a non-toxic waterproof paint ("Japlac" lacquer; International Paint Co., Ltd.) designated by the manufacturer as "signal red", "saxe blue", "emerald green" and white. The tanks were painted in adjacent groups of three with the colours, and in a single row of five with the white paint. In addition, the base of the rearing trough containing the experimental tanks (section 2.5) immediately underneath the experimental tanks was painted with the corresponding colour in order to obtain an homogeneous coloured environment (Plate 1).

The experimental basal diet was formulated to contain 50% crude protein and 10% crude lipid on the basis of the results of experiment 5 (Chapter 7). A white fishmeal (Caledonian Fishmeal Co., Ltd) was used as the sole protein source in the diets, and the lipid content of the diets, derived from equal proportions of cod liver oil and corn oil, was balanced to the desired level by these

Plate 1. Experiment 4B: Arrangement of Experimental System.



lipid sources after account had been taken of the proximate lipid content of the fishmeal. Similarly, the ash content of the diets was balanced to approximately 14% of the dry matter content of the diet by addition of a mineral pre-mix. The composition of the basal diet is shown in Table 8. All of the ingredients were thoroughly mixed in the dry state before addition of the mineral mix in solution/suspension, followed by water containing one of three commercial food dyes. The diet was then mixed to form a smooth dough which was extruded through a syringe to form thin strings, these being placed on aluminium foil sheets and dried for 48 hours at room temperature in a stream of cold air from a household fan. The coloured, dried diets were then ground in a pestle and mortar and sieved to obtain a particle size of 500 - 850 μm .

Table 8. Composition of Basal Diet: Experiment 4B.

Ingredient	% of dry diet	Proximate Composition %		
		Dry Matter	Mean	+S.D.
White fishmeal	66.4	Moisture	9.46	0.02
Corn oil	3.4	Protein	50.36	0.80
Cod liver oil	3.4	Lipid	10.84	0.02
Corn starch	15.4	Ash	14.30	0.41
Alpha cellulose	5.0			
Vitamin pre-mix*	2.0			
Mineral pre-mix**	2.4			
Binder (CMC)***	2.0			

*To supply per 100 g of dry diet: Thiamine hydrochloride 5 mg; riboflavin 5 mg; Ca pantothenate 10 mg; niacin 20 mg; biotin 0.6 mg; pyridoxine hydrochloride 4 mg; folic acid 1.5 mg; cyanocobalamin 0.01 mg; inositol 200 mg; ascorbic acid 100 mg; choline chloride 400 mg; menadione 4 mg; PABA 5 mg; α -tocopherol acetate 40 mg; vit. A acetate 200 IU; bulking agent α -cellulose.

** $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ 13.58%; Ca lactate 32.70%; Fe citrate 2.97%; MgSO_4 13.20%; K_2PO_3 23.98%; $\text{Na}_2(\text{PO}_3)_2$ 8.70%; NaCl 4.35%; $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 0.02%; KI 0.02%; CuCl 0.01%; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.08%; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.10%; ZnSO_4 0.30%.

***Carboxy methyl cellulose, Na salt, high viscosity.

As far as was possible, dye was added to each diet in amounts sufficient to approximate the colour of the diets to the painted tanks. The blue dye (Pearce Duff and Co.Ltd) contained Brilliant blue FCF and Ponceau 4R (E124), stabilised by acetic acid (quantitative concentrations of ingredients unknown), and gave the closest approximation to the background colour. The green dye (Pearce Duff and Co.Ltd) contained Tartrazine (E102) and Yellow 2G green S (E142) stabilised by isopropanol and acetic acid, and failed to give a close approximation to the background colour. The red dye contained carmine stabilised by glycerine, isopropanol and potassium hydroxide (E.F. Langdale Ltd) and again did not produce a very close match between the diet and the tank background. Approximately 12 ml of each dye

were added to 100 g portions of the basal diet, a quantity insufficient to detectably affect the proximate composition of the basal diet.

The coloured and control diets were delivered to the experimental fish at two-hour intervals over 24 hours via automatic feeders, each diet colour being offered to fry held in tanks of the four different background colours (Plate 1). The control diets were only offered to fish held on a white background, due to a numerical limitation on the available experimental tanks. The experimental system was continuously illuminated for the duration of the experiment, light intensity at the water surface having an average value of 1200 lux, although considerable variation in light intensity within the experimental tanks was inevitable because of the differently coloured backgrounds.

The fry were weighed at three-day intervals in the manner described in section 2.10, the distributed ration being altered after each weighing to account for growth, and the experiment was continued for twelve days. All the experimental fish were weighed individually at the end of the experiment in order to assess the growth dispersion within each experimental treatment.

6.3. Results.

6.3.1. Experiment 4A: Feeding Frequency.

All the experimental fry grew well on the commercial trout fry diet, and there was negligible mortality throughout the experiment. Growth data for the experimental fry are summarised in Table 9 and Figure 14. In order to obtain a measure of the effect of feeding frequency on the population weight distribution, the moment coefficient of skewness (Spiegel, 1972) of each tank population was calculated from the individual fish weights at the end of the experiment. This was defined as:

Table 9 . Growth of Experimental Fry: Experiment 4A.

Feeding Frequency Feeds/day	Average Initial Wt.(mg)	Final Live Wt. after 12 days Mean(mg)	+S.D.	Specific Growth Rate %/day	Moment coeff- icient of skewness a_3
24	69.1	372.4	77.6	14.0 ^{a*}	-0.79
12	73.3	374.3	90.3	13.6 ^a	0.04
6	74.0	354.4	82.1	13.0 ^a	0.19
4	67.9	273.8	57.9	11.6 ^b	0.26
3	66.0	230.5	58.9	10.4 ^b	0.53
				+S.E. 0.8	

*Numbers with the same superscript are not significantly different (P > 0.05).

$$\text{Moment coefficient of skewness } a_3 = \frac{m_3}{\sqrt{m_2^3}}$$

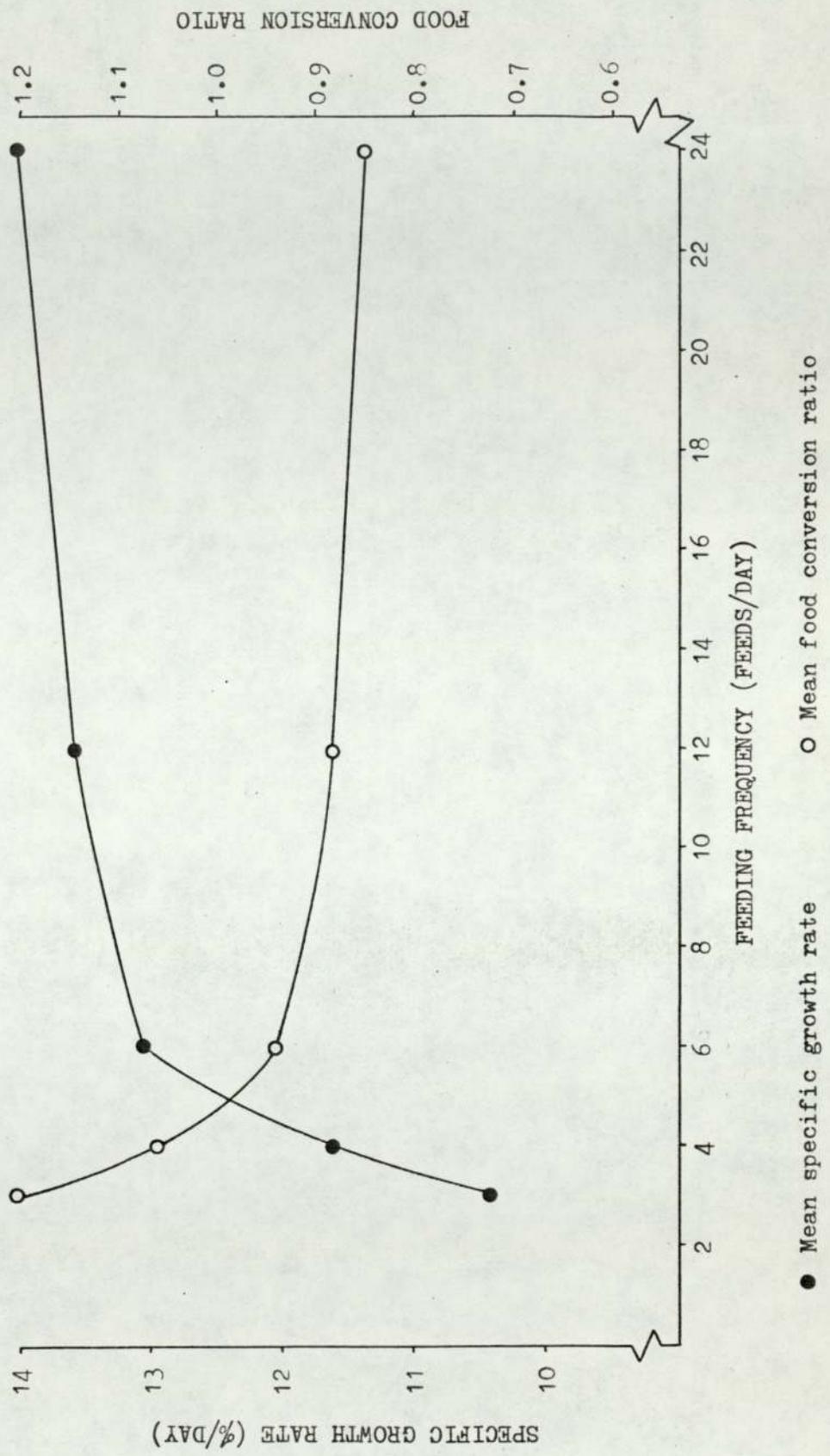
where m_2 and m_3 are the second and third moments about the mean \bar{X} respectively, defined as:

$$\text{The } r\text{th moment about the mean } m_r = \frac{\sum (X - \bar{X})^r}{N}$$

where the value X assumes N values. The numerical value of m_2 is equivalent to the variance s^2 . The value of a_3 is zero for perfectly symmetrical distributions such as the normal distribution, the distribution being positively or negatively skewed according to the absolute positive or negative value of a_3 . This statistic therefore provides a comparative measure of the degree of departure of a population distribution from the normal distribution.

The computed values of a_3 for the experimental populations are tabulated in Table 9 above. It can be seen from Table 9 that there was no significant difference among the specific growth rates of fry fed a nominal 15% of their body weight per day at frequencies

Figure 14. The Effect of Feeding Frequency on Growth and Diet Utilisation of Carp Fry: Experiment 4A.



of 6, 12 and 24 feeds per day, although these growth rates were significantly different ($P < 0.05$) from those exhibited by fry fed at frequencies of 3 and 4 feeds per day. In addition, the difference between the growth rates of these latter groups closely approached statistical significance. Thus it would appear that distribution of a ration amounting to a nominal 15% of body weight per day to carp fry in less than eight portions during a 24 hour period would result in inferior growth and food conversion in comparison with a more frequent feeding regime. (Fig. 14).

Examination of the values computed for the moment coefficient of skewness of each population (Table 9) showed that a positive skewness of body weight developed in each population for a decrease in the feeding frequency. However, continuous feeding resulted in the development of a strongly negatively skewed population weight distribution, the closest approach to normality being achieved by fry fed at a frequency of 12 feeds per day. The initial moment coefficient of skewness of the experimental populations had a value close to zero (0.02), thus the development of population skewness during the course of the experiment was not a result of elaboration of an initially skewed population weight distribution, and must have been elicited by the differing experimental feeding frequencies.

On the basis of this observation, and the recorded effect of feeding frequency on the growth rate of the fry, it would appear that distribution of a ration comprising a nominal 15% of the body weight per day to carp fry is best accomplished in 12 feeds over a 24 hour period. Since it is generally desirable to maintain as even a population growth as possible, this would appear to be close to the optimal feeding frequency for the size of carp under study, striking a reasonable compromise between maximum growth and food conversion efficiency and evenness of population growth. The results

would seem to indicate that a feeding frequency intermediate between continuous and two-hourly feeding would be optimal, but they also provide some justification for the arbitrary feeding frequency used in experiments 2 and 3 (Chapters 4 and 5).

6.3.2. Experiment 4B: Diet Colour.

Mortalities were negligible throughout the experiment with the exception of treatment 11 (Table 10), where six fry died during the course of the experiment. These deaths all occurred after weighing, therefore feeding rates were corrected without loss of food, but some error was involved in determination of intermediate average body weight and thus growth rate in this treatment, due to slight differences in individual weight of the experimental fry. In addition, all the experimental fry fed on the experimental basal diet showed poorer growth than the control fish fed on the commercial trout fry diet, and a decrease in growth rate over the experimental period. No such decrease was evident among the control fish fed the commercial diet, therefore it must have been caused by the experimental basal diet and was thought to be due to a decrease in palatability of the diets as the experiment progressed. However, the decrease in growth rate observed among the experimental fish fed the basal diet was approximately constant for all treatments, and it was thus possible to make meaningful comparisons between treatments.

The growth data from the experiment are presented in Table 10 in ranked order of specific growth rate so as to demonstrate the apparent colour preferences of the fish. The large value of the standard error derived from the residual mean square in the analysis of variance, and caused by the decrease in growth rate amongst fry fed the basal diets, prevented the demonstration of any significant difference in specific growth rate among the individual treatments,

Table 10. Growth and Food Utilisation of Experimental Fry: Experiment 4B.

Treatment Number	Diet Colour	Tank Colour	Initial Wt. (mg)	Final live Wt. after 12 days Mean(mg)	Specific Growth Rate (%/day)		Food Conversion Ratio	
					Mean	±S.D.	Mean	±S.D.
14	Control	white	177.5	783.1	12.4	0.5	1.0	0
6	green	green	191.3	542.0	8.7	1.8	1.6	0.3
12	green	white	170.0	470.5	8.5	1.6	1.6	0.3
9	green	red	178.8	490.3	8.4	1.8	1.6	0.4
7	red	red	166.8	454.8	8.3	2.5	1.8	0.7
2	blue	blue	188.9	510.2	8.2	1.3	1.6	0.3
3	green	blue	172.7	447.6	7.9	1.2	1.7	0.3
13	undyed	white	171.2	432.3	7.7	2.4	2.0	0.9
8	blue	red	184.4	462.0	7.6	2.0	1.9	0.6
11	blue	white	174.6	426.3	7.4	2.3	2.0	0.6
5	blue	green	167.6	403.8	7.3	2.6	2.1	0.7
1	red	blue	171.5	391.0	6.9	1.6	2.1	0.5
4	red	green	185.1	410.7	6.6	1.4	2.1	0.4
10	red	white	179.9	363.4	5.9	2.6	3.2	2.1

although it was possible to show significant colour preferences among the experimental fish by using grouped data.

It can be seen from Table 10 that the control fish fed the commercial trout fry diet (treatment 14) achieved an approximately 30% faster rate of growth than the best achieved by fry fed the experimental basal diet (treatment 6), food conversion among the control group being correspondingly superior. Among the fry fed the coloured basal diets, a clear trend in specific growth rate in response to the colour of the diet was evident. Treatment 6 (green diet on green tank background) surprisingly gave the best specific growth rate, suggesting that contrast between diet and background did not play a major role in determining the attractiveness of the diet to the fry.

The green diets consistently gave better specific growth rates than any other diet colour, irrespective of background colour, and similarly blue diets generally performed better than red. However, both blue and red diets fed to fry held in tanks of a similar colour to the diets gave a better performance than blue or red diets on any other background. Thus it would appear that matching of diet colour to background colour improved the performance of all the diet colours tested.

A statistical comparison of the grouped means of treatments 3,9 and 12; 5,8 and 11; and 1,4 and 10 (Table 10), after exclusion of the specific growth rate data pertaining to coloured diets on matching backgrounds, showed a significant ($P < 0.05$) improvement in specific growth rate in response to diet colour in the order green > blue > red. It would thus appear that the experimental carp fry showed a positive response towards coloured diets in this descending order, improvement in growth rate presumably being attributable to decreased wastage of the experimental diets. There was no sig-

nificant difference among the specific growth rates of fry fed diets matching the colour of the tank background.

Fry fed the undyed basal diet (treatment 13, Table 10) showed a specific growth rate intermediate between those evoked by the coloured basal diets. The undyed diet (a light tan in colour - see Plate 1) was only fed to fry held on a white tank background due to a shortage of experimental tank space, its main purpose being to assess whether the commercial dyes used to colour the experimental diets exerted a detrimental effect on the performance of the diets. Since some diets containing each of the three different food dyes elicited superior growth compared with the growth of fry fed the undyed basal diet, it was concluded that the dyes did not affect the performance of the basal diet.

Examination of the population weight distribution in each of the experimental treatments at the end of the experiment revealed no tendencies correlatable with diet colour. All of the experimental fry populations exhibited considerable skewness about the mean weight at the end of the experiment, probably as a result of the initially positively skewed population weight distribution of the fry at the beginning of the experiment (moment coefficient of skewness 0.49). All of the experimental populations were positively skewed with the exceptions of treatments 2 and 6 (Table 10), which corresponded to fry fed blue and green diets on matching backgrounds. However, fry fed a red diet on a matching background (treatment 7) possessed a positively skewed population weight distribution, thus tending to exclude the possibility that proffering of diets matching the background colour had any real effect on the population weight distribution.

6.4. Discussion and Conclusions.

When offered the commercial trout fry diet in quantities amounting to a set percentage of their body weight per day, the specific growth rates and food conversion ratios of the carp fry in experiment 4A approached maximum and minimum values respectively when the diet was presented in six or more approximately equal portions during a 24 hour period. Based upon this result and the observation that other feeding frequencies appeared to increase growth disparity amongst the experimental fry, it was concluded that distribution of a ration comprising a nominal 15% of body weight per day to carp fry ranging in weight from 100 to 500 mg, and held at a temperature of $24 \pm 0.5^{\circ}\text{C}$, was best accomplished in 12 approximately equal feeds over 24 hours.

Several studies have demonstrated that food intake and food conversion efficiency of fish approach a maximum value with increasing feeding frequency, thereafter remaining constant or slightly decreasing (Palmer et al, 1951; Ishiwata, 1969a,b; Kono and Nose, 1971; Andrews and Page, 1975). The optimal values of the feeding frequency and the satiation food quantity will largely depend on the species and size of fish under consideration, water temperature and the composition of the diet. Since for practical reasons no attempt was made to investigate the effect of feeding frequency on food intake in experiment 4A, the deterioration of growth and food conversion with more infrequent feeding, and the growth disparity within treatments observed in the experiment were a result of the chosen method of feeding. Several authors have observed that feeding frequency generally has little affect on food conversion efficiency, but on the amount of food ingested at each meal when the fish are fed to satiation (Grayton and Beamish, 1977; Chua Thia-Eng and Teng Seng-Keh, 1978; Grove et al, 1978).

At the lower feeding frequencies employed in experiment 4A, the amount of food available to the fry at each feed must have been in excess of their appetitive requirement. Due to the design of the experimental tanks (section 2.5), any food not immediately consumed by the fry was rapidly removed from the tanks by the action of the automatic syphon. This arrangement was evidently responsible for the poor growth rates and food conversion ratios of the infrequently fed experimental fish, as well as the growth disparity within each treatment, due to food wastage. Infrequent feeding caused a positive population weight skewness to develop (Table 10), which was progressively reduced with more frequent feeding, and reversed with very frequent feeding, fry fed continually demonstrating a negatively skewed population weight distribution.

Development of a positively skewed population weight distribution is a result of a few fish growing more than the bulk of the population, and is best explained in terms of intraspecific competition for food (Nakamura and Kasahara, 1956). In a situation where food was only available for a limited period, those fry which began feeding at the beginning of food distribution would gain a growth advantage over other fry which either began to feed at a later stage, or were at a competitive disadvantage to the more aggressive feeders. Thus a few fry would benefit to a greater extent during a feeding period and would grow to a greater extent than the remainder of the population. Increased frequency of feeding would therefore tend to reduce the occurrence of this phenomenon.

However, in the reverse situation where food is available continually, the majority of fry would obtain sufficient food, only those at the greatest competitive disadvantage being unable to effectively compete for their share, which would be likely to result in the development of a negatively skewed population weight

distribution. There would thus appear to be an intermediate feeding frequency which would yield the most even population growth, at which the most aggressive feeders would be fed to satiation in time to allow the least aggressive to obtain adequate food.

The interval between periods of maximum feed intake has been correlated with gastric evacuation time in Salmo gairdneri (Adron et al,1973; Grove et al,1978) and the dab,Limanda limanda (Jobling et al,1977),resulting in discrete bouts of feeding activity in these species interspersed by relatively long periods of inaction. While this appears to be characteristic behaviour in stomached species,cyprinids do not possess a recognisable stomach and tend to feed continuously (Rozin and Mayer,1961). Carp larvae and juveniles have been observed to continue feeding throughout the day and night (Alikunhi,1952; Appelbaum,1976),therefore these fish tend to show poorer growth when fed infrequently than do species which possess a stomach (Kono and Nose,1971). However,the fact that carp fry fed continually and at four hour intervals in experiment 4A showed insignificantly different growth rates does suggest that continuous feeding was not absolutely necessary for close to maximum growth for this size of fish. It is thus probable that ingestion of a sufficient quantity of food would be followed by a temporary decline in feeding activity by the fry,which would provide support for the suggestion that development of a negatively skewed population weight distribution among fry fed continually was caused by the inability of fish at the bottom of a feeding hierarchy to obtain sufficient food. The continuous distribution of a limited quantity of food would probably not have allowed the most aggressive feeders to feed to satiation,and they would thus feed continuously,to the detriment of the less aggressive feeders. Slightly more infrequent feeding would allow the most aggressive

feeders to feed to satiation, whereupon their decline in feeding activity would allow the less aggressive fry to feed, resulting in a more even population growth rate. This situation would probably not arise if uneaten food was not removed from the rearing tank.

The problem of uneven population growth becomes less acute as the fish grow, since decreasing frequency and quantity of food intake occurs with increasing fish size. Murai and Andrews (1976) found that channel catfish (Ictalurus punctatus) fry of less than 1.5 g showed their best growth rate when fed 8 times per day (once every three hours) while larger fry fed four times daily between 8.00 a.m. and 5.00 p.m. showed growth rates insignificantly different from other treatments. Feed intake also decreased from approximately 10 to 5% of body weight per day as the fish grew from 0.25 to 4 g. Ishiwata (1968a) showed that the ratio of stomach weight/body weight increased with decreasing fish weight, and maintained that smaller fish had larger stomachs in comparison with larger fish, but the increased food intake of smaller fish must also be due to an increase in their frequency of feeding.

Lee and Putnam (1973) showed that the voluntary food intake of rainbow trout increased in response to a decreased dietary energy content. Similar observations were made for Carassius auratus by Rozin and Mayer (1961) and for trout by Grove et al (1978) when the diet was diluted by kaolin, and subsequent studies have largely confirmed that fish feed to meet their energy requirement. An increased food intake in trout was accomplished by an increased frequency of feeding, accompanied by a decrease in gastric evacuation time (Grove et al, 1978). Increasing dietary energy content would therefore result in a decreased feeding frequency for most species, thus the optimum feeding frequency determined in this study for carp fry applies only under the

experimental conditions used, namely a water temperature of 24°C, continuous illumination, a particular size range of fish and a particular diet. Alteration of any of these parameters would almost certainly change the response of the fry to feeding frequency.

The results of experiment 4B, in which differently coloured diets were fed to carp fry, suggested that diet colour had a significant affect on the acceptability of diets to carp fry. A preference for coloured diets in the order green > blue > red was demonstrated, and in addition it appeared that presentation of a coloured diet to fry held on a similarly coloured background tended to override colour preferences to some extent and improved the performance of the most favoured diet colour, green.

In view of the fact that well-developed colour vision is present in the majority of commercially important fish species (Muntz, 1974), it is surprising that an apparent paucity of published information on diet colour preferences in fish exists, especially since at least one study (Wolf, 1953) with Salmo gairdneri has demonstrated the potential commercial usefulness of coloured diets. In that study, it was shown that a red-dyed food pellet elicited a more vigorous feeding response from trout than pellets coloured green, blue or yellow, and that red pellets were retrieved from the bottom of the experimental raceways whereas green, blue and yellow pellets were ignored.

The only reasonably detailed study of colour preference in trout would appear to have been made by Ginetz and Larkin (1973), who fed all possible combinations of dyed trout eggs to trout held on several differently coloured backgrounds, at high and low light intensities, and observed the food intake of the fish. However, it was reported by these authors that the trout eggs were

dyed with proprietary fabric dyes, and it would appear that no control of undyed eggs was included, therefore it is difficult to exclude the possibility that the apparent colour preferences observed were in some measure due to the effect of the dyes on the palatability of the eggs.

Ginetz and Larkin (1973) found that trout held on a greenish-blue background in daylight showed significant ($P < 0.05$) consumption preferences for coloured eggs in the order blue > red > black > orange > brown > yellow > green. In addition, some combinations of coloured eggs significantly ($P < 0.05$) increased total consumption. At low light intensities, the order of preference of different colours became yellow > red > blue > black. In general, matching of egg colour to background reduced consumption with the exception of blue eggs on a blue background, which increased consumption. The authors therefore concluded that the primary factor influencing preference for a particular colour was contrast between the egg colour and the background, especially at low light intensities, but that blue held a special attractiveness for the trout at higher light intensities.

These results are considerably at variance with those obtained for carp fry in experiment 4B, as well as with the results obtained by Wolf (1953). The colour preferences of the carp fry cannot be explained in terms of contrast between diet and background, because matching of diet colour to background consistently improved the performance of the coloured diets. Even though the match between the diets and the backgrounds was poor, thus providing some contrast, much greater contrast was present in other treatments. Poor experimental design meant that light intensities were not constant between the experimental treatments, nor was the physical brightness of the differently coloured diets the

same, therefore the possibility that the observed colour preferences were due to the different light intensities within the experimental tanks cannot be dismissed, but it does seem unlikely.

One moderately plausible explanation for the observed overriding of colour preference by background colour may be that continual exposure to a normally less favoured colour diminishes a possibly innate avoidance response to that colour. However, it is difficult to envisage the purpose of instinctive colour avoidance, other than for avoidance of predation. Red "shyness" has been observed in a number of fish species (Bauer, 1910; Reeves, 1919), thus the poor performance of the red diets in experiment 4B may have been a manifestation of this phenomenon.

Within the limitations of the experimental design, a definite colour preference among carp fry was demonstrated. The experiment would greatly have benefitted from duplication of the experimental treatments as well as the use of a nutritionally better basal diet, but it nevertheless served to indicate that further investigation of colour preferences among carp juveniles would be worthwhile. It is possible to speculate endlessly on possible causes for the colour preferences observed in this experiment, but this will remain speculation until more rigorous experiments are carried out with a wider range of colours under controlled lighting conditions.

CHAPTER 7.

Chapter 7. Experiment 5: An Investigation of the Dietary Protein and Lipid Requirements of Carp Post-larvae and Fry.

7.1. Introduction.

Following upon the determination of the correct adaptation weight, feeding rate and feeding frequency for optimal utilisation of the commercial trout fry control diet (Chapters 4, 5 and 6), it was decided to begin an investigation of the nutrition of carp juveniles which were capable of using conventional artificial diets. It was hoped that carp post-larvae and fry would be able to grow and survive equally as well on semi-purified experimental diets as upon the commercial trout fry diet.

That nutritional requirements alter with fish size has been demonstrated on several occasions (Gerking, 1952; Woodall and la Roche, 1964; Kitamikado et al, 1964), and Huisman (1979b) has said "...it would be even naive to assume that a thousandfold increase in weight during the initial growth phases should not change these (nutritional) requirements". Not only may quantitative requirements alter during infancy, qualitative requirements may change as well. For example, the young of rats, fowl and man require amino acids considered inessential in adults of these species (Passmore and Robson, 1973).

Determination of the optimal protein content of artificial diets for juvenile carp was considered to be the most important requisite in a process of nutritional optimisation of such diets, since protein is the basic building nutrient of the growing animal and is required in disproportionate amounts to other nutrients. It has been demonstrated that quantitative protein requirements alter with size for channel catfish (Page and Andrews, 1973). It was also considered important to simultaneously investigate the overall energy content of diets complementary to optimal utilisation of

dietary protein, since protein utilisation will only reflect the quantity and quality of the dietary protein when an adequate energy intake occurs (Cowey, 1979).

The minimum dietary protein level giving optimal weight gain has now been studied for a large number of fish species, and has been found to be uniformly high. Ogino and Saito (1970) were among the first to investigate the dietary protein requirement for carp, and found it to be approximately 38% of the dry diet for young carp weighing between approximately 2 and 10 g. Subsequent work has shown that this value may be reduced to around 31% by inclusion of suitable energy levels in the diet (Takeuchi et al, 1979a), since dietary energy can "spare" protein for growth by minimising protein catabolism to supply energy. It would appear that dietary energy in the form of carbohydrate or lipid is equally acceptable to young carp (Ogino et al, 1976; Takeuchi et al, 1979b), in marked contrast to some other fish species such as rainbow trout (Ogino et al, 1976).

Because carbohydrate does not form a significant part of the natural diet of carp larvae and post-larvae, it was decided to provide non-protein dietary calories in the form of lipid when increasing the total energy content of the experimental diets. Lipid was thought to be an acceptable source of calories because of the very high lipid content (up to 30% of the dry matter) of live foods like Artemia (Watanabe et al, 1978a).

In all, three separate attempts were made to determine the optimum protein to lipid ratio in diets for carp post-larvae and fry, but the third experiment had to be abandoned due to a system failure which caused large mortality amongst the experimental fish, and is not included here. In addition, the first experiment was beset by practical difficulties and is only partially included, some discussion being necessary because of its influence on the

design of the second experiment, which is reported in full.

7.2. Materials and Methods.

In order to investigate possible changes in nutritional requirements between the post-larval and fry stages, as well as to compare data with those published for older individuals of the same species, all experimental diets were offered to two age/weight groups of carp post-larvae and fry. These groups were chosen on the basis of previous experiments (Chapters 4 and 5) and consisted of post-larvae initially weighing approximately 15 mg (ie. of a suitable weight to be adapted to the commercial trout fry control diet), and of fry initially weighing approximately 100 mg.

All the experimental fish were housed in two ranks of eight swivelling experimental tanks (Section 2.5) giving 16 experimental treatments in all, and were continuously illuminated and fed at two-hourly intervals over 24 hours via automatic feeders (Section 2.6) for the duration of each trial. All fish were fed at a nominal feeding rate of 15% of their body weight per day, adjusted at two-day and three-day intervals after weighing for the post-larvae and fry respectively, all administered food being weighed directly without correction for dietary water content.

Batches of 200 post-larvae and 15 fry per experimental treatment were allocated to the experimental tanks, and were weighed either by sampling (10 post-larvae per sample) or by batch weighing in the manner described in Section 2.10. Where possible, the trials were continued for a period of 12 days. In both experiments 5A and 5B, post-larvae and fry were fed on diets containing a single complex protein source as the only source of amino-acid nitrogen in the diets.

7.2.1. Experiment 5A.

Fifteen different diets were formulated, containing five protein

levels (40,45,50,55 and 60% crude protein) with three lipid levels at each protein level (10,15 and 20% crude lipid). These were chosen on the basis of the proximate composition of live food similar to that normally forming the diet of carp juveniles in ponds (Watanabe et al,1978a).Artemia,Daphnia and Acartia spp. composition investigated by these researchers showed a crude protein content ranging from 55 - 70% of the dry matter content, and a crude lipid content ranging from 10 - 25%. It was reasoned that the requirements of carp post-larvae for protein and lipid would fall within these ranges.

Partly in order to facilitate comparison of results with those of other researchers, vitamin-free casein (Sigma Chemical Co., Ltd) was chosen as the sole protein source in the experimental diets, as has been used by other workers on many occasions (Ogino and Saito,1970; Ogino et al,1976; Takeuchi et al,1979b). Casein has repeatedly exhibited a relatively high Biological Value for fish (Ogino and Chen,1973; Cowey and Sargent,1979) and was therefore considered a logical choice of protein source, despite some limitations in its amino acid composition.

Prior to mixing, all dietary ingredients were ground and sieved to a particle size of less than 50 μm to ensure reasonable homogeneity within the fine particle size feeds used; casein was also chosen because of its powdery consistency, which saved much time during this preparatory stage.

The composition of the experimental diets is shown in Table 11. The desired lipid content of each diet was obtained by the addition of a 1:1 mixture of corn and cod liver oils in order to satisfy the known essential fatty acid requirements of carp (Watanabe et al,1975a,b; Takeuchi and Watanabe,1977; Castell,1979), and a binder (carboxymethyl cellulose, sodium salt, high viscosity) was added at

Table 11. Composition of Experimental Diets (g/100 g of dry diet) : Experiment 5A.

Ingredient	Diet Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Casein	41.2	41.2	41.2	46.3	46.3	46.3	51.5	51.5	51.5	56.6	56.6	56.6	61.8	61.8	61.8
Corn oil	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0
Cod liver oil	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0
Dextrin	30.8	25.8	20.8	25.7	20.7	15.7	20.5	15.5	10.5	15.4	10.4	5.4	10.2	5.2	0.2
Alpha-cellulose	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Vitamin pre-mix*	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix*	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Carboxy methyl-cellulose	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
<u>Dry Matter</u>	<u>Proximate Analysis of Diets.</u>														
Moisture	9.7	7.5	7.3	7.9	7.1	7.2	7.9	7.8	7.2	8.0	7.4	7.6	8.2	7.0	7.5
Crude Protein	39.4	40.8	39.2	44.0	45.6	45.1	50.9	51.6	49.9	54.8	56.0	55.3	62.0	58.9	60.1
Crude Lipid	9.8	15.6	21.2	10.4	15.2	20.0	10.6	15.8	22.0	11.0	15.3	20.9	10.6	15.8	20.6
Ash	3.2	3.8	3.8	4.0	3.8	3.8	3.8	3.9	4.0	4.0	4.0	3.6	3.8	4.0	4.1
NFE	37.9	32.3	28.5	33.7	28.3	23.9	26.8	20.9	16.9	22.2	17.3	12.6	15.4	14.3	7.7

* See Table 12.

a level of 2% to all diets to improve water stability. This binder was chosen because it has been widely used by other researchers with good results (Nose, 1963; Wilson et al, 1977), although Forster (1972) has shown that this is not an ideal binder for use in prawn diets. Alpha-cellulose was added both as a bulking agent and as a fibre source to encourage the fish to produce solid faeces, which aided the self-cleaning action of the experimental tanks. It was maintained at a constant level in all diets because it was suspected that variable fibre levels could exert a significant effect on digestibility of the experimental diets. That this is so has recently been shown by Takeuchi et al (1979a), who found that the digestibility of energy in diets for carp decreased with an increase in dietary cellulose levels.

All dietary ingredients were thoroughly mixed in the dry state before pelletisation, with the exception of the vitamin and mineral components. The required amount of mineral pre-mix in solution/suspension was pipetted into the diets and mixed in before addition of the vitamin mix, in order to avoid as far as possible degradation of vitamins by free cations. Water containing 0.1% of an orange dye (Sunset yellow FCF; ICI Ltd) in order to approximate the colour of the diets to that of Artemia was added to the diets to produce a smooth paste, which was then extruded through a syringe. The thin strings of diet were air dried at room temperature for 48 hours, then ground in a pestle and mortar and sieved to obtain the appropriate particle sizes (120 - 250 μm , 250 - 355 μm , 355 - 500 μm and 500 - 850 μm).

Where relevant, all dietary ingredients were analysed for proximate composition (Section 2.13) to permit diet formulation, the protein source also being subjected to an amino acid analysis to permit calculation of the amino acid profile of the experimental

diets. Completed diets were also analysed before use in order to check whether the proximate composition agreed with the dietary formulation, and whether dietary water contents were sufficiently similar to minimise errors in direct weighing.

7.2.2. Experiment 5B.

Due to the failure of the casein-based diets of experiment 5A to support growth and survival of carp post-larvae and fry, which was largely ascribable to palatability difficulties, it was decided to repeat the experiment using fishmeal as a protein source in all diets. A white fishmeal (Caledonian Fishmeal Co. Ltd) normally manufactured for use as a milk-replacer in diets for calves was chosen for this purpose because of its low fat and high protein content, as well as its finely divided nature (average particle size $< 75 \mu\text{m}$).

Fifteen diets were again formulated, ranging in protein content from 30 - 50% in increments of 5%, and containing lipid levels of 5, 10 and 15% at each protein level. These levels of lipid and protein were chosen on the basis of experiment 5A. The composition and nutrient content of the experimental diets is shown in Table 12.

The methods used in formulation and manufacture of the diets were identical to those described in the previous section, with the exception that soyabean oil was used as an alternative to corn oil because of its availability. In addition to an amino acid analysis of the fishmeal protein source, an amino acid analysis was also carried out upon the commercial trout fry control diet for comparative purposes. The calculated amino acid profile of the experimental diets and the analysed amino acid composition of the fishmeal and control diet are presented in Table 13.

Upon completion of the experiment with fry, all experimental fish were killed and pooled for carcass analysis. Due to the small

Table 12. Composition of Experimental Diets (g/100 g of dry diet) : Experiment 5B.

Ingredient	Diet Number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
White fishmeal	39.5	39.5	39.5	46.1	46.1	46.1	52.7	52.7	52.7	59.3	59.3	59.3	65.8	65.8	65.8
Soybean oil	1.6	4.1	6.6	1.4	3.9	6.4	1.3	3.8	6.3	1.1	3.6	6.1	1.0	3.5	5.9
Cod liver oil	1.6	4.1	6.6	1.4	3.9	6.4	1.3	3.8	6.3	1.1	3.6	6.1	1.0	3.5	5.9
Dextrin	43.4	38.4	33.4	38.4	33.4	28.4	33.2	28.2	23.2	28.2	23.2	18.2	23.1	18.1	13.3
Alpha-cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin pre-mix*	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix**	4.9	4.9	4.9	3.7	3.7	3.7	2.5	2.5	2.5	1.3	1.3	1.3	0.1	0.1	0.1
Carboxy methyl-cellulose	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
<u>Proximate Analysis of Diets</u>															
Dry Matter	9.0	8.7	7.7	8.6	8.3	7.4	8.5	7.8	7.3	8.4	7.8	7.1	8.5	7.8	6.9
Moisture	31.9	31.2	29.6	34.9	34.9	35.8	40.5	40.6	40.6	45.0	44.6	44.8	51.2	49.4	50.9
Crude Protein	4.4	9.7	15.2	4.9	10.0	16.0	5.3	10.2	16.3	5.6	11.4	16.6	6.0	11.6	17.1
Crude Lipid	11.3	11.3	11.8	12.0	12.1	12.2	12.8	12.1	12.7	13.2	13.3	13.6	13.8	13.9	14.2
Ash	43.4	39.1	35.7	39.6	34.7	28.6	32.9	29.3	23.1	27.8	22.9	17.9	20.5	17.3	10.9
NFE***	4.00	4.25	4.48	4.01	4.29	4.70	4.15	4.41	4.75	4.23	4.55	4.85	4.41	4.63	5.04
Estimated energy value (Kcal/g)***	45.1	41.5	37.3	49.2	46.0	43.0	55.1	52.0	48.3	60.1	55.4	52.2	65.6	60.3	57.1
% calories as Protein															

*PTO.

Table 12 (continued).

*Vitamin pre-mix to supply per 100 g of dry diet: Thiamin hydrochloride 5 mg; riboflavin 5 mg; calcium pantothenate 10 mg; nicotinic acid 20 mg; biotin 0.6 mg; pyridoxine hydrochloride 4 mg; folic acid 1.5 mg; cyanocobalamin 0.01 mg; inositol 200 mg; ascorbic acid 100 mg; choline chloride 400 mg; menadione 4 mg; alpha-tocopherol acetate 40 mg; para-amino benzoic acid 5 mg; vitamin A acetate 200 IU. All ingredients were diluted with alpha-cellulose of particle size < 40 µm.

**Mineral pre-mix (National Research Council, 1973) No. 5, containing: calcium biphosphate 13.58%; calcium lactate 32.70%; ferric citrate 2.97%; magnesium sulphate 13.20%; potassium phosphate 23.98%; sodium biphosphate 8.70%; sodium chloride 4.35%; aluminium chloride 0.015%; potassium iodide 0.015%; cuprous chloride 0.010%; manganese sulphate 0.080%; cobalt chloride 0.100%; zinc sulphate 0.300%. A solution/suspension containing 1 g/ml of the mineral mix was pipetted in the required volumes into the experimental diets during mixing, prior to the addition of the vitamin pre-mix.

***Based on an estimated 5.65 Kcal/g protein, 9.45 Kcal/g lipid and 4.1 Kcal/g carbohydrate. Dietary lipid and protein calories were calculated from the proximate analyses of the diets, carbohydrate calories being calculated from added dextrin, other dietary carbohydrates being considered indigestible.

Table 13. Calculated Amino Acid Profile of Experimental Diets.

Amino- acid	White fishmeal	Number of experimental diet															AA Req.*	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		CONTROL
Asp.	8.8	2.8	2.8	2.6	3.1	3.1	3.6	3.6	3.6	4.0	3.9	4.0	4.0	4.5	4.4	4.5	4.2	1.5
Thr.	4.1	1.3	1.2	1.4	1.4	1.5	1.7	1.7	1.7	1.8	1.8	1.8	1.8	2.1	2.0	2.1	1.9	
Ser.	4.2	1.3	1.2	1.5	1.5	1.7	1.7	1.7	1.7	1.9	1.9	1.9	1.9	2.2	2.1	2.1	2.0	
Glu.	14.1	4.5	4.2	4.9	4.9	5.7	5.7	5.7	5.7	6.3	6.3	6.3	6.3	7.2	7.0	7.2	7.4	
Pro.	5.0	1.6	1.5	1.8	1.8	2.0	2.0	2.0	2.0	2.3	2.2	2.2	2.2	2.6	2.5	2.6	2.7	
Gly.	7.1	2.3	2.1	2.5	2.5	2.6	2.9	2.9	2.9	3.2	3.2	3.2	3.2	3.6	3.5	3.6	3.7	
Ala.	6.2	2.0	1.8	2.2	2.2	2.2	2.5	2.5	2.5	2.8	2.8	2.8	2.8	3.2	3.1	3.2	3.1	
Cys.	1.3	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.7	0.6	0.7	0.5	
Val.	5.8	1.8	1.7	2.0	2.0	2.1	2.4	2.4	2.4	2.6	2.6	2.6	2.6	3.0	2.9	3.0	2.3	1.4
Met.	3.2	1.0	1.0	1.1	1.1	1.1	1.3	1.3	1.3	1.4	1.4	1.4	1.4	1.6	1.6	1.6	1.4	1.2
Isol.	4.0	1.3	1.2	1.4	1.4	1.4	1.6	1.6	1.6	1.8	1.8	1.8	1.8	2.1	2.0	2.1	2.0	0.9
Leu.	7.0	2.2	2.1	2.4	2.4	2.5	2.8	2.8	2.8	3.2	3.1	3.1	3.1	3.6	3.5	3.6	3.2	1.3
Tyr.	3.3	1.0	1.0	1.1	1.1	1.2	1.3	1.3	1.3	1.5	1.5	1.5	1.5	1.7	1.6	1.7	1.4	
Ph.Ala.	6.7	2.1	2.0	2.3	2.3	2.4	2.7	2.7	2.7	3.0	3.0	3.0	3.0	3.4	3.3	3.4	1.8	1.3
His.	2.1	0.7	0.6	0.7	0.7	0.7	0.8	0.8	0.8	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.1	0.8
Lys.	7.4	2.4	2.2	2.6	2.6	2.6	3.0	3.0	3.0	3.3	3.3	3.3	3.3	3.8	3.7	3.7	3.6	2.2
Arg.	10.4	3.3	3.1	3.6	3.6	3.7	4.2	4.2	4.2	4.7	4.6	4.6	4.6	5.3	5.1	5.3	2.9	1.6

*The essential amino acid requirements of carp, after Nose (1979). All values are expressed as a percentage of the dry diet, with the exception of white fishmeal, which is expressed as a percentage of total protein.

quantities of material available for proximate analysis, it was impossible to measure carcass lipid directly by the methods available and an approximation of carcass lipid was therefore obtained as $100 - (\% \text{carcass protein} + \% \text{carcass ash} + \% \text{carcass moisture})$. Analysed values were obtained by the methods described in section 2.11.

7.3. Results.

7.3.1. Experiment 5A.

Beginning on the sixth day after the commencement of the experiment, all post-larvae (initial weight 15.16 ± 0.13 mg) offered the experimental diets began to exhibit mortality, amounting to between 30 and 50% of each population by the evening of the eighth day, when the experiment was abandoned. The pattern of these mortalities was similar to that observed amongst larvae of initial weights 5.6 and 9.5 mg fed on the commercial trout fry diet in experiment 2 (Chapter 4). Mortality amongst the control fish fed on the commercial trout fry diet amounted to only 4% of the experimental population in this time, in accordance with that expected from the results of experiment 3A (Chapter 5). Growth of the fish fed the experimental diets was very poor and similar among all treatments, the approximate weight of the experimental fish being 20 - 25 mg on the sixth day of the experiment, in contrast to the 44.62 mg weight achieved by the control fish in the same time. Abandonment of the experiment was considered necessary because it would have been impossible to gain any interpretable results.

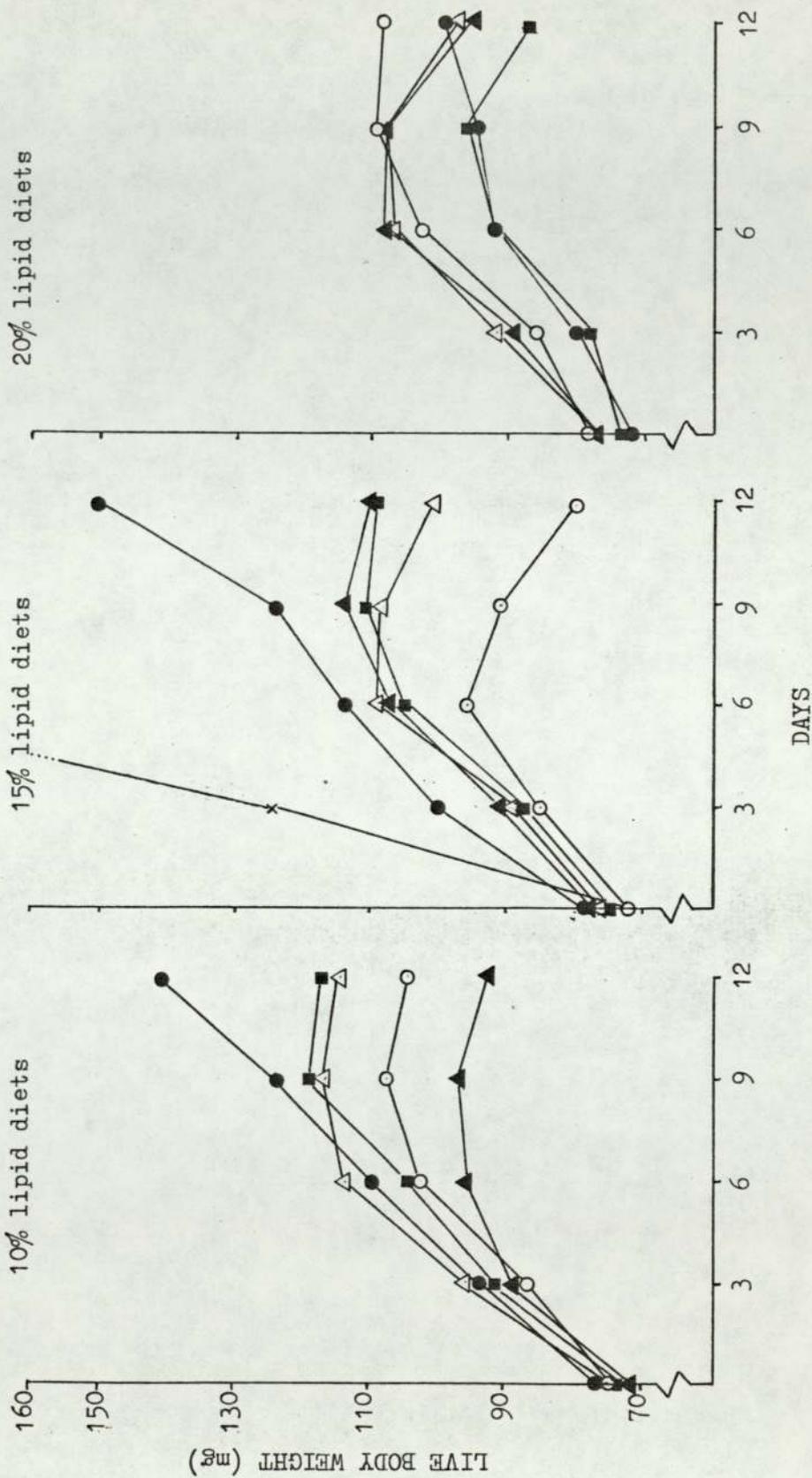
The experimental diets were then offered to carp fry initially weighing between 72 - 79 mg for a period of 12 days. These larger fish showed considerable reluctance to consume the experimental diets at first, and it proved necessary to substitute a finer grade (250 - 355 μm) of diet than would normally have been used in order to encourage the fish to eat. However, substantial food wastage

KEY TO FIGURE 15.

- 40% protein diets
- 45% protein diets
- ▲ 50% protein diets
- △ 55% protein diets
- 60% protein diets
- x Control diet (Edward Baker Ltd.;
trout fry diet No."0")

All protein and lipid contents of the diets are expressed as the calculated amounts. For the proximate composition of the experimental diets, see Table 11.

Figure 15. Experiment 5A: Growth of Experimental Fry.



occurred both because of the unsuitable particle size of the feed and the continuing reluctance of the fish to eat the diets. This palatability problem was almost certainly due to the extremely hard consistency of the diets, which was a result of the natural binding properties of casein.

The growth of the experimental fish is shown in Fig. 15. Due to the problem described above, growth was poor on all the experimental diets, the maximum weight increase being achieved by fry fed on diet 2, which contained 40.8% crude protein and 15.6% crude lipid (Table 11). These fish grew from 78.5 to 149.4 mg over the course of the experiment, in comparison with the control fish fed the commercial trout fry diet which grew from 71.9 to 375.2 mg in the same time. The specific growth rate of the control fish (13.8%/day) was within the normal range obtained on other occasions with this diet.

Although it was obviously impossible to draw any quantitative observations from this experiment, the results obtained did to some extent influence the design of experiment 5B. It can be seen from Fig. 15 that slightly better growth was obtained on diets containing 40% protein and 10 and 15% lipid than in any other treatments, and it was therefore decided to lower the overall protein and lipid contents of subsequent diets in case the poor results obtained were due in part to a deleterious effect of too high a protein and fat intake. While the actual loss in body weight occurring after six days in treatments other than 1 and 2 (Table 11) was almost certainly due to the total rejection of the diets by the fry, it had to be considered that the poor palatability of the diets was partially due to their nutrient content as well as to their unsuitable physical consistency.

7.3.2. Experiment 5B.

Post-larvae of average initial weight 13.33 ± 1.35 mg offered the experimental diets began to exhibit rapid cumulative mortality on the sixth day after commencement of the experiment, as was observed in experiment 5A, with the exception of treatments 1 - 6. Mortality for treatments 7 - 15 ranged from 10 - 20% of the original population by the eighth day of the experiment, when it was abandoned due to the impossibility of accurately administering a measured food quantity. By contrast, mortality in treatments 1 - 6 and in the control fish ranged from 0 - 2.5% in the same time.

Growth rates of the experimental fish in treatments 7 - 15 generally exceeded that of the control fish, the greatest occurring in treatment 15 (50.9% protein, 17.1% lipid). However, no significance can be attached to these data because the increased growth rates observed could simply have been due to an increased food quantity being available to surviving fish because of the death of their companions. Growth rates in treatments 1 - 6, where mortality was comparable with that occurring in the control, were generally less than that of the control fish. The growth and mortality data collected for the post-larvae are summarised in Table 14, and it can be seen that no definite trends in either can be identified. Increased mortality was possibly correlatable with an increased fishmeal content of the experimental diets, perhaps suggesting the presence of some inhibitory factor in the white fishmeal.

When the experimental diets were offered to fry of initial weights ranging from 112 - 129 mg, mortality was negligible amongst the experimental fish and the best growth rates approximated to that achieved by the control fish fed the commercial trout fry diet. Growth and diet utilisation information for the experimental fry are presented in Table 15. Although no palatability difficulties

Table 14. Growth and Mortality of Experimental Post-larvae. Experiment 5B.

Average initial weight 13.33 ± 1.35 mg.

	Treatment Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16*
Average weight after 6 days	26.55	25.30	24.48	36.10	25.42	30.02	32.66	30.30	35.82	32.04	31.20	32.30	33.52	36.07	37.81	31.56
+S.D. (mg)	1.53	1.22	0.81	2.67	2.53	0.10	0.30	0.86	2.51	1.91	2.21	2.72	1.31	2.33	0.79	1.28
Specific Growth Rate (%/day)	11.5	10.7	10.2	16.6	10.7	13.5	14.9	13.7	16.5	14.6	14.2	14.8	15.4	16.6	17.4	14.4
Total Mortality (%)	1	1.5	0	2.5	0	0	10	10	10	8	8.5	12	20	20	9	2.5

*Control

comparable to those experienced in experiment 5A were encountered in this experiment, a continuous decrease in growth rate, amounting to approximately 40% in all treatments, was observed over the experimental period. This was not a natural reduction in growth rate with size as was observed for Artemia-fed fish in experiment 1 (Chapter 3) because no similar reduction in growth rate occurred amongst the control group fed on the commercial trout fry diet. This reduction in growth rate gave rise to considerable variation about the mean specific growth rate computed from the three-day weighing periods and used for comparative purposes, therefore it was impossible to demonstrate any significant differences in growth rate among the experimental treatments (Table 15). However, since the reduction in growth rate was approximately constant in all treatments bar the control, some clear trends in growth in response to diet composition were evident, which permitted at least some tentative interpretation of the data. No satisfactory explanation of the fall in growth rate of the experimental fish could be devised, although it seems most likely that a decrease in palatability of the diets occurred as the experiment progressed, leading to increased diet wastage.

It can be seen from Table 15 that the maximum specific growth rate among fry given the experimental diets was demonstrated by treatment 13, corresponding to a diet containing 51.2% crude protein and 6% crude lipid. The addition of extra lipid to diets containing in excess of 40% crude protein would appear to have resulted in a depression of growth rate proportional to the quantity of added lipid, as can be seen from Fig. 16 which shows the growth rate of the experimental fry in relation to the protein calorie / total calorie content of the diets. Even at low dietary protein content, there was little evidence to suggest a marked protein sparing action of increased dietary calories, only marginal improvement in

Table 15. Experiment 5B: Growth and Diet Utilisation Data for the Experimental Fry.

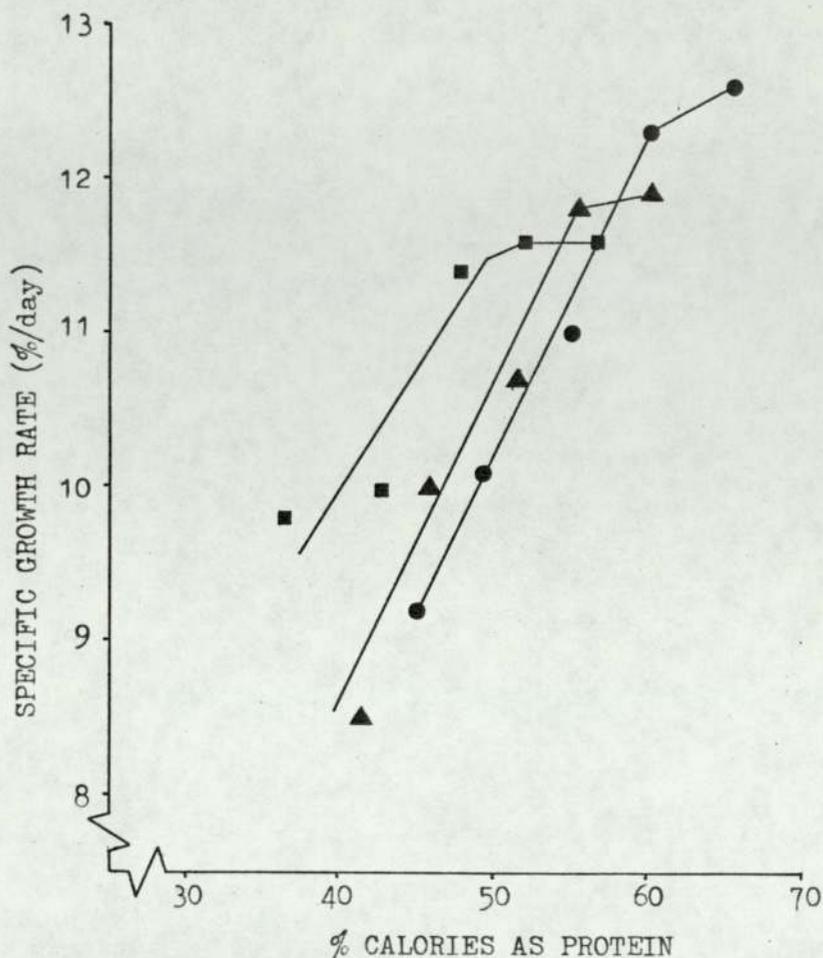
Parameter	Treatment Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Control
Average initial weight (mg)	119	129	113	122	122	112	121	117	114	115	112	125	113	120	107	119
Average final weight (mg)	359	357	365	409	406	374	453	422	449	503	460	504	514	504	432	616
Specific Growth Rate (%/day)	9.2 ^{a*}	8.5 ^a	9.8 ^{ab}	10.1 ^{ab}	10.0 ^{ab}	10.0 ^{ab}	11.0 ^{ab}	10.7 ^{ab}	11.4 ^{ab}	12.3 ^{ab}	11.8 ^{ab}	11.6 ^{ab}	12.6 ^{ab}	11.9 ^{ab}	11.6 ^{ab}	13.7 ^b
Food conversion ratio	1.4	1.4	1.3	1.2	1.2	1.2	1.2	1.1	1.1	1.0	1.1	1.1	1.0	1.0	1.2	0.8
Protein efficiency ratio	2.3	2.2	2.7	2.3	2.3	2.2	2.1	2.2	2.3	2.3	2.1	2.1	1.9	2.0	1.6	2.3
Protein retention efficiency (NPU app.%)**	27.2	26.1	32.5	28.1	28.7	28.8	25.7	28.2	29.2	30.2	26.9	28.6	25.3	24.6	20.2	29.0
Lipid retention efficiency (%)***	176.1	88.2	71.0	129.7	83.2	57.7	143.1	82.8	70.4	130.5	83.5	67.4	130.7	87.9	55.0	147.8
Energy retention efficiency (%)***	33.1	32.0	37.3	34.2	34.4	33.7	34.8	36.1	40.0	39.0	38.8	40.7	37.8	40.4	32.8	-

*Numbers with the same superscript are not significantly different (P < 0.05).

**Defined as protein, lipid or energy retained / protein, lipid or energy administered.

***Carcass lipid calculated from carcass analysis as 100 - (%moisture + %protein + %ash).

Figure 16. Experiment 5B: Growth of Experimental Fry in Relation to the Percentage of Dietary Calories as Protein.



- 5% lipid diets $SGR = -0.18 + 0.20(\% \text{ calcs})$; $r = 0.99$ ($P < 0.05$)
- ▲ 10% lipid diets $SGR = -0.41 + 0.22(\% \text{ calcs})$; $r = 0.98$ ($P < 0.05$)
- 15% lipid diets $SGR = 4.22 + 0.14(\% \text{ calcs})$; $r = 0.91$ ($P < 0.05$)

growth and food conversion occurring in treatments 3 and 9, which corresponded to diets containing 15.2 and 16.3% crude lipid and 29.6 and 40.6% crude protein respectively. In fact, the growth rates obtained in treatments 1, 4 and 7 (diets containing approximately 30, 35 and 40% crude protein and 5% crude lipid respectively) were intermediate between those of treatments 2, 5 and 8 and treatments 3, 6 and 9, which diets contained approximately 10 and 15% crude lipid respectively. This result would tend to exclude any possibility of lipid sparing having occurred. For crude protein levels of 45 and 50% of the dry diet, growth rates appear to have

been reduced by the addition of lipid levels of 10 and 15% to the diet, and this depression was most marked among treatments 13, 14 and 15; the difference in growth among these treatments closely approached statistical significance.

For all diets, growth rate was strongly correlated with increasing dietary protein, irrespective of lipid level, up to a protein level of approximately 45% of the dry diet. Thereafter, growth rate showed some tendency to decline for further enrichment of protein to 50% of the dry diets. It is possible that further increases in dietary protein would not have resulted in any further growth, particularly at the 5% lipid level, and that a dietary protein content of approximately 45% represented the optimal value for fry of this size, but this possibility could not be verified.

Protein efficiency ratio (PER) showed no consistent tendency to increase with increasing lipid supplementation of the diets, and was surprisingly similar for all diets with the exception of treatment 15, where a sharp decrease in PER was elicited. These results tend to support the view that protein sparing did not occur within the ranges of protein and lipid concentrations tested. PER was significantly ($P < 0.05$) negatively correlated with dietary protein content for diets having lipid contents of approximately 10 and 15% (PER = 2.8 - 0.02P; $r = -0.89$; and PER = 4.0 - 0.04P; $r = -0.97$, respectively, and where P = dietary protein content), but was not significantly correlated with dietary protein for diets containing 5% crude lipid.

For protein levels ranging from 30 - 40% of the dry diet, the apparent efficiency of protein retention (NPU app.) was slightly improved for increasing dietary lipid levels, indicating some reduction in protein catabolism, but diets containing protein levels of 45 and 50% demonstrated a reversal of this trend.

The results of carcass analysis of the experimental fish are presented in Tables 16 - 18. Treatments 4 - 15 demonstrated a significant ($P < 0.05$) decrease in carcass moisture for increasing dietary lipid at each protein level; there was no significant change in carcass moisture for increasing dietary protein level at a fixed lipid level (Table 16).

Carcass protein content of the experimental fish (Table 17) showed no significant alteration for increasing dietary lipid at a fixed dietary protein level in treatments 1 - 12, but a significant ($P < 0.05$) decrease was evident between treatment 13 (51.2% dietary crude protein, 6.0% lipid) and treatments 14 and 15. There was also a significant ($P < 0.05$) increase in carcass protein at all lipid levels with increasing dietary protein, with the exception of treatment 15, which showed a significant ($P < 0.05$) decrease in carcass protein in relation to treatment 12, which contained a similar dietary lipid content but lower dietary protein content.

There was no significant difference amongst any of the experimental treatments in carcass ash content, with the notable exception of treatment 15 (Table 18) which showed greater than 50% reduction in ash content.

The calculated lipid contents of the experimental fish are shown graphically in Fig. 17. It can be seen that increasing the calorie/protein ratio at each dietary protein level by addition of lipid to the diets caused an increase in the deposition of lipid in the fish carcass. Increasing the protein/calorie ratio at each lipid level in the diet initially caused a fall in the accumulation of carcass lipid, followed by another rise as the protein/calorie ratio increased further, this rise being accelerated by an increased dietary lipid level. Protein retention efficiency, on the other hand, remained relatively constant at each

Table 16. Carcass Composition of Experimental Fish - Moisture Content (%).

Lipid level in diet %	Protein level in diet %					CONTROL	±S.E.
	30	35	40	45	50		
5	(1)79.0 ^{a*} _{p**}	(4)79.7 ^a _p	(7)79.3 ^a _p	(10)79.7 ^a _p	(13)78.9 ^a _p	77.5 ^a	0.5
10	(2)78.0 ^a _p	(5)78.6 ^a _{pq}	(8)78.3 ^a _p	(11)77.4 ^a _q	(14)77.5 ^a _q	77.5 ^a	0.4
15	(3)77.6 ^a _p	(6)77.3 ^a _q	(9)76.1 ^a _q	(12)75.6 ^a _r	(15)78.0 ^a _q	77.5 ^a	0.7
CONTROL	77.5 _p	77.5 _q	77.5 _{pq}	77.5 _q	77.5 _q		
±S.E.	0.9	0.6	0.5	0.5	0.2		

Initial moisture content of fish 83.5%

*Numbers with the same superscript in rows are not significantly different ($P > 0.05$)

**Numbers with the same subscript in columns are not significantly different ($P > 0.05$)

Table 17. Carcass Composition of Experimental Fish - Crude Protein Content.

Lipid level in diet%	Protein level in diet %					CONTROL	+S.E.
	30	35	40	45	50		
5	(1)11.2 ^a _p	(4)11.5 ^a _p	(7)11.6 ^{ab} _p	(10)12.4 ^{cd} _p	(13)12.7 ^d _p	12.0 ^{bc}	0.2
10	(2)11.2 ^a _p	(5)11.6 ^{ab} _p	(8)12.2 ^{bc} _p	(11)12.3 ^c _p	(14)12.0 ^{bc} _q	12.0 ^{bc}	0.2
15	(3)11.5 ^a _p	(6)12.0 ^{ab} _p	(9)12.2 ^b _p	(12)12.9 ^c _p	(15)11.8 ^{ab} _q	12.0 ^{ab}	0.2
CONTROL	12.0 _p	12.0 _p	12.0 _p	12.0 _q	12.0 _q		
+S.E.	0.2	0.2	0.2	0.2	0.1		

Initial crude protein content of fish 10.0%

Table 18. Carcass Composition of Experimental Fish - Ash Content.

Lipid level in diet %	Protein level in diet %				CONTROL	+S.E.
	30	35	40	45		
5	(1)1.1 ^a _p	(4)1.0 ^a _p	(7)1.3 ^a _p	(10)1.3 ^a _p	(13)1.1 ^a _p	1.2 ^a 0.1
10	(2)1.1 ^a _p	(5)1.1 ^a _p	(8)1.1 ^a _p	(11)1.2 ^a _p	(14)1.3 ^a _p	1.2 ^a 0.1
15	(3)1.1 ^a _p	(6)1.2 ^a _p	(9)1.2 ^a _p	(12)1.1 ^a _p	(15)0.5 ^b _q	1.2 ^a 0.1
CONTROL	1.2 _p	1.2 _p	1.2 _p	1.2 _p	1.2 _p	
+S.E.	0.1	0.1	0.1	0.1	0.1	0.1

Initial ash content of fish 1.4%

Figure 17. Experiment 5B: Carcass Lipid Content of Experimental Fry.

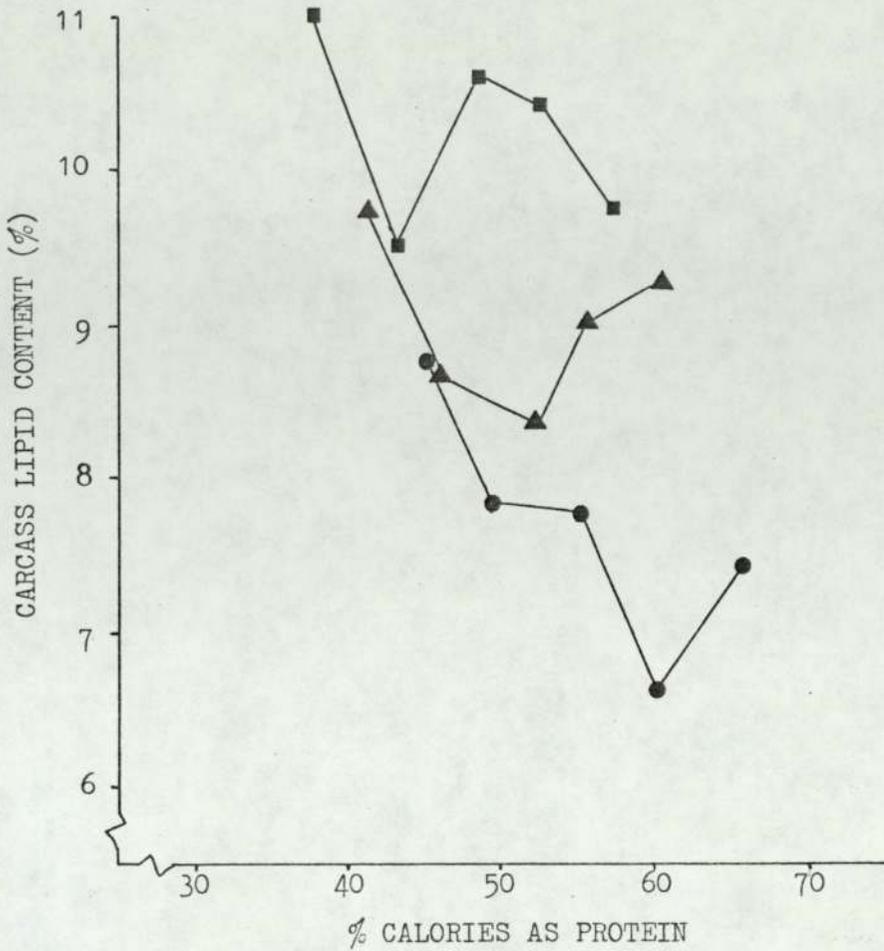
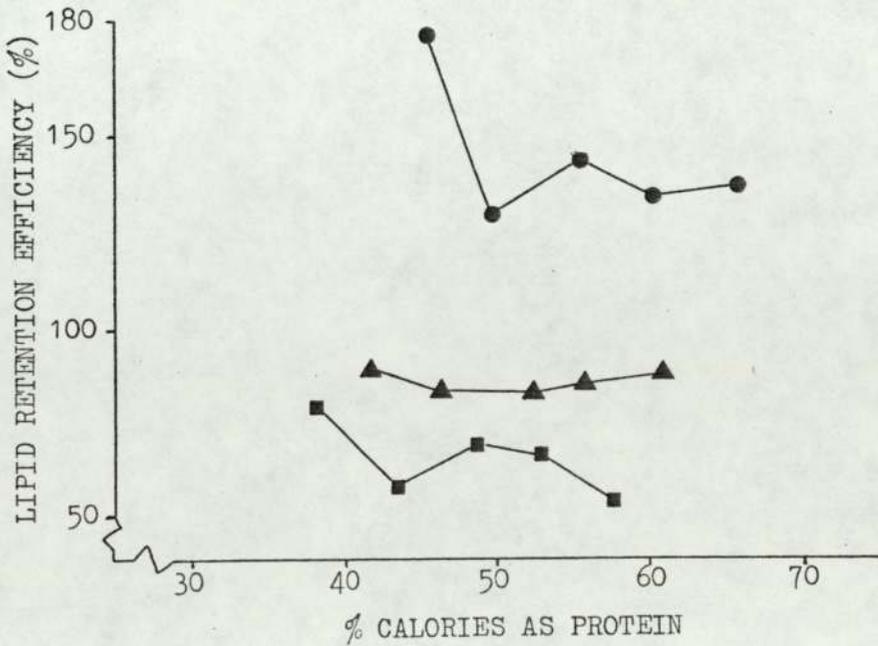


Figure 18. Experiment 5B: Lipid Retention Efficiency of Fry.



● 5% lipid diets ▲ 10% lipid diets ■ 15% lipid diets

dietary lipid level, fish fed on the lowest lipid diets accumulating lipid in excess of their intake. This would indicate the synthesis of lipid from carbohydrate or protein.

7.4. Discussion and Conclusions.

All the experimental diets used in experiments 5A and 5B performed poorly in comparison with the commercial trout fry diet employed as a control, particularly with the post-larvae. Since the precise composition of the trout fry diet was not known, it was difficult to identify any specific factors which may have contributed to its comparative success. Although the reason for the death of the post-larvae offered the casein based diets in experiment 5A could largely be attributed to the unsuitable physical consistency of these diets, no such explanation applied to the fishmeal based diets employed in experiment 5B. These diets were generally well-accepted by the post-larvae, which would suggest that their poor performance was due to a nutritional imbalance. A specific physical cause of death could not be established.

Examination of the amino acid profile of the experimental diets used in experiment 5B revealed no great dissimilarities between the experimental and the control diets. In comparison with the known amino acid requirements of carp (Nose, 1979), all experimental diets containing in excess of 40% crude protein, together with the control diet, exceeded requirement. It is impossible to explain in terms of amino acid content why diets containing 30 and 35% crude protein, and with dietary contents of threonine, histidine and methionine marginally below requirement, should have shown mortalities similar to the control post-larvae. It would have been of interest to have continued experiment 5B with these diets to investigate whether mortalities similar to those occurring in the other treatments developed at a later stage. However, this would not

have contributed to the initial aims of the experiment.

No information on the tryptophan content of the diets was available due to its destruction by acid hydrolysis during the preparation of the fishmeal and trout fry diet samples for amino acid analysis, but tryptophan content was unlikely to have been limiting because of the relatively low requirement of carp for this essential amino acid (0.3% of the dry diet at a dietary crude protein level of 38.5%; Nose, 1979). The normal tryptophan content of most fishmeals ranges from 0.7 - 1.0% of the protein content (National Research Council, 1977), thus the tryptophan content of the lowest protein experimental diets would have been in the region of 0.2 - 0.3% of the dry diet.

On the basis of the present knowledge of the essential amino acid requirements of carp therefore, the unsatisfactory performance of the experimental diets used in experiment 5B with post-larvae cannot be ascribed to any quantitative amino acid failing, although the possibility of poor amino acid availability cannot be dismissed (Dabrowska et al, 1979). Such a statement also begs the question of the quantitative amino acid requirements of such small fish, for which no data are available.

In relation to most branches of fish nutrition, the vitamin requirements of fish are comparatively well-understood, and the vitamin requirements of young carp have been elucidated. In relation to the vitamin requirements of carp (reviewed by Halver, 1979), the quantities of vitamins supplied by the pre-mix added to all the experimental diets was more than adequate to satisfy the requirement. Although the enormous surface area/weight ratio of the very small particle size feeds used would have permitted rapid leaching of water-soluble vitamin components from the diets, as has been shown by several researchers (Murai, 1966, cited by

Murai and Andrews,1975; Goldblatt et al,1979),the same problem would presumably have affected the control diet. In an ancillary experiment,the supplementation of a diet containing 50% crude protein (from white fishmeal) and 10% crude lipid with levels of 2,3,4 and 6% of the vitamin pre-mix had no effect on the survival or growth of post-larvae offered these diets. While such a total elevation of all dietary vitamins was obviously a crude and unsatisfactory means of assessing the correct inclusion level of vitamins in the diet,not least because it poses the problem of fat-soluble vitamin toxicity,it did suggest that poor growth and mortality among post-larvae were not connected with significant loss of vitamins from the diets through leaching. As a source of mortality,the vitamin content of the experimental diets can probably be discounted.

Possible deleterious effects of the mineral component of the diets cannot be dismissed in the same way. Mineral nutrition in fish is poorly understood,since determination of requirements is complicated by the fact that minerals are not only available to the fish from dietary sources,but from those dissolved in the water as well. It is suggested (Cowey and Sargent,1979) that as there is a continual net outward flux of ions from freshwater fish,a dietary supply of minerals must be essential. Ogino and Kamizono (1975) on the other hand found no marked improvement in growth of carp fed a casein/gelatin diet supplemented with up to 8% of a mineral pre-mix in comparison with fish given a mineral-free diet. Growth of carp has been improved by administration of dietary phosphorous (Ogino and Takeda,1976) and it was demonstrated by these authors that carp possess an ability to absorb calcium from the environment. It was suggested that diets containing 0.6 - 0.7% phosphorous promote optimal growth. The magnesium requirements of carp

have been estimated to be 0.04 - 0.05% of the dry diet (Ogino and Chiou, 1976), and other mineral requirements are gradually being quantified. Because of its high mineral (ash) content, the use of fishmeal in diets is likely to satisfy most, if not all, of the mineral requirements of fish (Andrews et al, 1973), although whether these minerals are present in balanced amounts and an available form is questionable.

The inclusion of 4% of the mineral pre-mix in the casein-based diets of experiment 5A was intended to satisfy all of the dietary mineral requirements of the fish, and was chosen on the basis of the results of Ogino and Kamizono (1975) who found that inclusion of a mineral mix at levels above 4% of the dry diet slightly (but not significantly) depressed the growth of carp. In experiment 5B, the use of fishmeal with a high ash content (18.4% of the dry matter) should have supplied the major element requirements of the fish. It was therefore decided to balance the ash contents of the diets by addition of varying amounts of mineral pre-mix (0.1 - 4.9% of the dry diet) in order to meet trace element needs. This method is open to criticism, although it has been used by many researchers in the past, and it is possible that the mineral component of the experimental diets, whether from the fishmeal or the added mix, was responsible for the recorded mortalities among the post-larvae in experiment 5B. Certainly differing proportions of minerals were present at each dietary protein level, but this was an unavoidable consequence of using fishmeal as a protein source.

The possibility that other added ingredients in the diets were responsible for the observed mortalities was tested to some extent. The orange dye (Sunset yellow FCF), common to all the experimental diets but not the control came under suspicion, but the addition of this dye to the control diet had no significant affect

on diet acceptability or growth of fry offered the diets. The recorded specific growth rates of these fish (12.37 ± 0.48 and $11.84 \pm 1.47\%$ /day for dyed and undyed diets respectively) were within the normal range expected. Unfortunately, this test was not carried out with post-larvae, but it seems unlikely that the dye was responsible for the adverse effects of the experimental diets. J. Adron (pers. comm. 1980) has suggested that the binder used in all the experimental diets (carboxy methyl cellulose, Na salt, high viscosity) may have been at fault, since he has observed a deleterious effect of this binder on growth of plaice and turbot larvae. This possibility was not examined.

That the diets used in experiment 5B proved adequate for fry suggests that these slightly larger fish are more tolerant of dietary nutrient variation than are larvae and post-larvae, and it may ultimately prove necessary to formulate diets for the smallest fish within very narrow limits.

The relationship between energy content and protein content of diets has now been examined for several species of fish. Certain results have been common to most studies. Increasing the energy content of diets at a constant dietary protein level has generally resulted in an increased deposition of carcass lipid and an improved food utilisation. Protein efficiency ratio (PER) has been found to improve up to a maximum with increasing dietary energy at each protein level, and is negatively correlated with increasing protein calorie/total calorie ratio. Similarly, net protein utilisation (NPU) is improved up to a maximum at each dietary protein level with increased dietary calories (Cowey and Sargent, 1979). These phenomena are associated with reduced catabolism of protein for energy production as other dietary calorie sources adequately meet the energy requirements of the fish.

The inconclusive results obtained in experiment 5B when the experimental diets were fed to fry were largely a result of the intractable problems associated with quantitative studies of nutrition in small fish. For the method of feeding chosen, it had to be assumed that the palatability of all the experimental diets was equal and that little, or constant, food wastage occurred among the different treatments. The second assumption was probably not justified.

It was impossible to assess the level of food wastage within the experimental system used. Some researchers (eg. Bromley, 1980) have attempted to correct for uneaten food by collection and subsequent weighing, but this is prone to gross errors because of diet fragmentation and contamination with faeces, as has been pointed out by Cowey et al (1972). In order to ensure that tiny fish completely consume all of an offered diet, it would be necessary to hand-feed every grain, an obvious impracticality, especially where a large number of experimental treatments are involved. The feeding levels employed in experiment 5B probably approached a satiation feeding rate for the size of fish used, therefore some level of wastage had to be accepted. Previous experiments had shown that hand feeding was unsatisfactory because of the low growth rates obtained, as well as the sensitivity of the fish to disturbance. Automatic feeding was therefore chosen as a means of obtaining satisfactory growth rates, but sub-optimal feeding might have reduced food wastage and improved the precision and reliability of the results. The automatic feeders themselves were also a potential source of error, since they were not so perfect as to deliver every scrap of food to the experimental tanks. In terms of the actual quantities of food involved, a loss of a few mg of feed during loading of the feeders and delivery could have made a

substantial difference to the growth of the experimental carp. However, these losses should have been relatively constant for all treatments.

In the feeding trial with fry in experiment 5B, there was little evidence of a sparing of dietary protein by increased dietary calories. The growth rates of fish fed diets containing 30 - 40% crude protein and 5% lipid were intermediate between those of fish fed diets containing 10 and 15% lipid, and the food conversion ratios and values for PER and NPU_{app} were very similar for all treatments. Although there was some evidence to suggest that the requirement for dietary protein for maximum growth was reduced by increasing dietary energy as lipid (Fig. 16), fastest growth was achieved on a diet rich in protein and low in lipid, maximum growth being depressed proportional to the inclusion level of lipid in the diet.

Studies of the effect of increasing the calorie/protein ratio in fish diets (Ringrose, 1971; Lee and Putnam, 1973; Adron et al, 1976; Ogino et al, 1976; Watanabe et al, 1979) have clearly demonstrated the protein sparing effect of increased dietary calories at a constant protein level in many fish species. Studies with carp (Sin, 1973a,b; Jauncey, 1979; Takeuchi et al, 1979a) have not demonstrated any growth depressing or pathological effects of very high levels of dietary lipid of up to 18% of the dry diet (Jauncey, 1979), although carcass lipid was correlated with intake. However, growth depression with excessive dietary energy intakes has been observed for other fish species on several occasions (Ringrose, 1971; Page and Andrews, 1973; Lee and Putnam, 1973; Bromley, 1980).

Lee and Putnam (1973) reported that rainbow trout adjust their total food intake to a set energy level and thus diets with low protein to energy ratios depress growth due to reduced protein in-

take.

Bromley (1980) fed diets containing four different combinations of protein and energy to turbot at four feeding rates corresponding to daily energy intakes calculated as 20,50,100 and approximately 150 (energy satiation) calories per g of fish per day. At the highest feeding rate of 150 cal/g/day, the turbot fed to a satiation level ranging from 135 - 138 cal/g/day, and increase in weight for all diets fed at intermediate and satiation rates was greatest for the highest protein diets. High lipid levels in the diet restricted growth and led to a drop in the amount of protein deposited in the fish, in contrast to the results of Adron et al (1976). However, energy retention efficiency was not significantly affected by the protein/lipid level of the diets.

The possibility that the minimal protein sparing and apparent growth depression associated with increasing levels of lipid in the diets fed to carp fry in experiment 5B (Fig.16) could have been due to differences in palatability amongst the experimental diets cannot be completely rejected, but the food conversion ratios obtained do not support this conclusion very well. If high levels of lipid had decreased the palatability of the diets, it would have been reasonable to expect poorer food conversion ratios for the high lipid diets. In fact, food conversion ratios were very similar between treatments (Table 15) and showed, if anything, a slight tendency to improve with increased dietary lipid levels for the diets containing protein levels of 30, 35 and 40%. For dietary protein levels of 45 and 50%, the reverse was true, food conversion ratios tending to worsen with increasing dietary lipid.

If the feeding rates employed in experiment 5B represented an energy satiation level for the fry, it is possible to place a different interpretation on the results. Certainly the feeding

rates used represented a satiation level in terms of growth for the commercial trout fry control diet (experiment 3B, Chapter 5). At dietary protein levels of 30, 35 and 40% crude protein, feeding to a set energy level would have resulted in a lower intake of protein for those fish fed diets containing 10 and 15% lipid, but protein sparing by lipid would improve the efficiency of use of this protein. It is therefore possible that protein sparing to some extent compensated for the reduced protein intake and produced similar growth rates amongst the treatments. Bromley (1980) observed that evidence of protein sparing was marginal at the satiation feeding rate, but all his dietary protein levels were similarly high. This could to some extent explain the results obtained at higher dietary protein levels.

Because growth rates began to fall off at protein levels of 50% for diets containing 5 and 10% lipid, and convincingly plateaued for diets containing 45 and 50% protein and 15% lipid, it is probable that a protein level of 45% represented the optimal protein inclusion level for diets containing Caledonian white fishmeal as a sole protein source. Dietary protein in excess of this amount would presumably be increasingly catabolised for energy production, necessitating an increased metabolic rate. This supposition is supported by the carcass analysis of the fish (Table 17). The carcass protein content of the fish given the lowest lipid diets did not significantly increase for an increase in dietary protein from 45 to 50%, while for the same protein levels, fry fed the 10% lipid diets showed a slight decrease in carcass protein and fry fed the 15% lipid diets demonstrated a significant ($P < 0.05$) decrease in carcass protein content. While remaining elevated, carcass lipid content of the fry fed the 15% lipid diets at protein levels of 45 and 50% decreased (Fig. 17), but the carcass

lipid content of the fry fed the 5 and 10% lipid diets increased. Maximal energy retention was observed for all diets containing 45% protein irrespective of lipid content. These results are compatible with the suggestion that optimal growth occurred at a dietary protein level of 45%.

That increased dietary lipid content caused a reduction in growth at high dietary protein levels may be a misleading observation. Bromley (1980) has pointed out that a unit of energy deposited in the form of protein is accompanied by a weight increase of approximately eight times that associated with the deposition of a similar quantity of energy as lipid, since lipid can be accumulated in concentrated form while protein is diluted with water in the cytoplasm. At a dietary protein level of 50%, carcass protein was significantly ($P < 0.05$) reduced by an increase in dietary lipid to 15%, while carcass lipid was elevated (Fig. 17). It is therefore possible that the differences in weight obtained were a result of the differing carcass compositions of the experimental fry. The energy retention efficiencies of fry fed diets containing 45 and 50% protein were very similar, with the exception of treatment 15 (highest protein, highest lipid diet), thus the energy content of the carcasses reflected the energy content of the diets.

The high dietary protein requirement of fry would suggest a similarly high essential amino acid requirement, which may provide an explanation of the failure of the experimental diets to support growth and survival of post-larvae. Several authors have suggested that the amino acid requirements of fish larvae must be reflected by the amino acid composition of their bodies (Albrecht and Wunsche, 1972; Anwand et al, 1976; Appelbaum, 1979). Such an idea is nonsense; the protein composition of fish need bear

no resemblance whatever to their dietary requirement for amino acids, since these compounds can be used for synthesis of a wide variety of metabolic intermediates such as hormones which would not be included in a tissue amino acid analysis (Dabrowski,1979).

Several anomalies appeared in the results of the carcass analysis of carp fry fed the highest protein/lipid diet. PER,NPU app, and energy retention efficiency of fish fed this diet showed a marked reduction in comparison to the values obtained for all the other experimental diets. In addition,carcass moisture increased in direct opposition to what would have been expected,and carcass ash showed a significant ($P < 0.05$) and marked reduction. These data would suggest a metabolic disturbance among these fish,especially as the only recorded mortalities of the experiment occurred in this treatment. The high protein requirements of fry indicated by this study may imply that such small fish preferentially use protein for energy,and a luxus of both protein and lipid in the diet may overload their ability to cope with dietary lipid.

In conclusion,it is necessary to state that a considerable amount of additional research is required to investigate the nutritional requirements of fish larvae and fry. The results obtained here are to some extent contradictory,and require verification,but the study has served to demonstrate the possibility of carrying out controlled feeding experiments with small fish. Some considerable improvement in experimental technique is necessary, and this study has at least indicated how these improvements may be brought about. Quantitative feeding studies with larvae and post-larvae would benefit from an increased number of experimental animals than was used here,because of the difficulties involved in obtaining representative samples of each experimental population for weight determination. A larger number of experimental fish

would also have minimised the effects of mortality on the accuracy of food administration, since food uneaten by moribund larvae would then constitute a much less significant proportion of the remaining population. Similarly, a larger number of experimental fry would have improved the accuracy of the results obtained with these larger fish. As was found in experiment 2 (Chapter 4), the total number of fish that could be held in the experimental system was severely limited by the size of the experimental tanks and the available water supply, thus all experiments had to be designed within the limitations of the experimental system. Duplication of treatments would have been valuable, but the initial purpose of the experiments was to screen as wide a range of protein and lipid combinations as possible in the event that only one combination would be suitable for the post-larvae. The failure to produce an experimental diet which performed as well as the commercial trout fry diet unfortunately precluded later, more detailed studies of nutritional requirements as originally intended, but there is no reason to believe that this cannot be achieved. Perhaps the use of lyophilised protein sources would provide better results.

While the duration of the experimental trials was short in comparison with the normal length of nutritional trials with older fish, which was partially due to the limitations of the experimental system preventing longer trials because of water quality deterioration, it should be borne in mind that the body weight increases obtained in most of the experiments reported here compare favourably in percentage terms with those from much longer trials with adult fish. They were generally sufficient to permit the demonstration of significant differences in growth response between treatments.

CHAPTER 8.

Chapter 8. Experiment 6: Screening of Variously Processed Experimental and Commercial Diets for the Direct Rearing of Carp Larvae.

8.1. Introduction.

The success of complete, compounded artificial diets for commercial rearing of post-juvenile fish has recently stimulated much interest in the practicalities of rearing larval and juvenile fish on similar artificial diets. While extension of existing fishfeed technology to the preparation of larval diets has proved reasonably successful for salmonid species, this cannot be said of other freshwater and marine species. The inability to formulate successful artificial diets for non-salmonid fish species has frequently been ascribed to the difference in post-hatching size between salmonid and other larval species (van Limborgh, 1979) and the consequent differences in feed particle size requirement and feeding behaviour.

The practical difficulties associated with manufacture and presentation of very fine particle size feeds has been a subject of some discussion (Meyers and Butler, 1971; Meyers, 1979; van Limborgh, 1979), the most important of which are the maintenance of nutritional homogeneity within the feed particles, water stability and prevention of nutrient leaching, and physical attractiveness and palatability of particles to fish larvae. In comparison with young salmonids most other, smaller fish larvae are inefficient feeders, and a recurrent problem in rearing trials has frequently been mortality due to water pollution by wasted food (Adron et al, 1974; Anwand et al, 1976; Kainz, 1976; Appelbaum, 1977). Thus, presentation of an inert particle will require development of suitable delivery systems just as much as suitable particles. Considerations during presentation must include such parameters as optimal particle concentration, larval stocking density, rearing temperature, feeding

frequency, water quality and circulation, tank size, shape, depth and colour, photoperiod and illumination intensity, and so on (Houde, 1973). Apart from a nutritionally adequate content, the diet itself must have suitable properties in water, such as stability, low susceptibility to bacterial or fungal attack, physical and possibly chemical attractiveness, and adequate digestibility in order to ensure that optimal particle acceptance is achieved. Failure to fully meet these criteria may mean that nutritionally excellent diets perform poorly.

The majority of attempts to develop artificial diets for fish larvae have revolved around dry formulations, because these are relatively simple to manufacture, store and handle. However, more recent attempts (Metailler et al, 1979; Jones et al, 1979) have begun to investigate the feasibility of using moist preparations more nearly resembling the natural food of fish larvae, and a dichotomy of preparatory techniques has become evident. Diets can either be conventionally made by manufacture of a moist, stabilised mix of dry ingredients followed by drying, grinding and sieving to obtain the desired particle size, or by immediate preparation of correctly sized particles from such a mix by means of extrusion, emulsification or micro-encapsulation techniques.

The possibility of utilising micro-encapsulation techniques to confer suitable physical properties on artificial larval diets, particularly water stability and resistance to bio-degradation, was first suggested by Meyers and Butler (1971), and has subsequently excited much interest among aquaculturists. The idea is attractive because it is theoretically possible to manufacture a very wide range of diets with differing physical properties which could be tailored to meet the requirements of individual species of fish larvae. However, in practice relatively few micro-encapsulation

techniques hold promise for application in the field of aquaculture despite the large number of diverse manufacturing methods available, partly because the simplest techniques have been developed with different goals in mind.

The experiments described in this chapter on the following pages were initiated at an early stage in the research, and were an attempt to optimise the physical acceptability of inert feed particles and rear carp larvae immediately after hatching on artificial diets. Various techniques of diet preparation were investigated, including micro-encapsulation, and their effect on the acceptability of diet particles to carp larvae assessed. In addition, several commercial larval diets were tested. These trials were somewhat premature, since the selection of dietary ingredients was a purely arbitrary process without knowledge of the nutritional requirements of the larvae, and it was presumed that one of the major factors contributing to the difficulties experienced by other researchers attempting to rear carp larvae on artificial diets was the unsuitable physical properties of the diets. Although to a greater or lesser extent, negative results were obtained from these experiments, they influenced the design of ensuing experiments and contributed towards the more logical approach to development of artificial diets for carp juveniles eventually adopted, and described in Chapters 3 - 7.

In the course of a study of micro-encapsulation techniques which might have proved of use in manufacture of larval diets, it was found that appropriate techniques may be conveniently divided into two general categories, chemical and physical, of which the chemical methods of micro-encapsulation of particular interest may be further classed as physico-chemical and chemical.

The physico-chemical methods of micro-encapsulation include

the phenomenon of coacervation, which was one of the first methods of micro-encapsulation to be developed. It exploits the fact that a colloid such as gelatin can be caused to concentrate from aqueous solution to form a two-phase mixture, one phase consisting largely of water and the other a colloid-rich fraction, the coacervate. The coacervate can then coalesce about suitable hydrophobic nuclei in the system and thus encapsulate them, the capsules so formed being recovered from the aqueous medium by one of several separation techniques. This method of micro-encapsulation has found wide application in numerous industries (Sirine, 1967; Luzzi, 1970). Two variations of coacervation are commonly used, termed simple and complex coacervation respectively.

Simple coacervation essentially consists of removal of the aqueous solvation layer around the colloid, which is a function of its charge, usually by the addition of a salt or alcohol with a greater affinity for water than the colloid. Complex coacervation is effected by mixing solutions of two colloids which carry opposite charges. A combination of gelatin and gum acacia will fulfill this condition. At the correct pH, gelatin carries a net positive charge because of the protonation of its basic amine groups, while acacia has a negative charge of approximately equal magnitude due to the ionisation of its glucuronic acid groups. The two colloids attract each other and separate into a distinct liquid phase, the coacervate, which begins with the formation of sub-microscopic clusters of the colloid molecules. These coalesce to form microscopic droplets, which then form a thin film around the particles of the substance to be coated as a consequence of the very low interfacial tensions of the colloid mixture. Due to the inhibition of coacervation by free ions (Phares and Sperandio, 1964), this elegant technique is generally unsuited to the encapsulation of

dietary (hydrophilic) materials.

More familiar among the purely chemical methods of micro-encapsulation are those relying on interfacial polymerisation. Here, a capsule wall can be formed of a polymer such as nylon deposited by reaction at the interface of two immiscible liquids, the reactants being contained in the separate phases. Where one of the phases exists as an emulsion in the other, polymer membranes will be formed as a sphere around discrete droplets, encapsulating them. Capsules formed in this way have already found some application as a vehicle for dietary materials (Jones et al, 1974), as well as a host of potential uses in medical fields (Chang et al, 1966).

Physical methods of micro-encapsulation include deposition of wall material about a nucleus by evaporation of a solution containing the wall-forming material, most often by the techniques of spray-drying or air suspension encapsulation; by centrifugal or pressure atomisation and projection of core material through a pre-formed coating "sheet"; or by pressure extrusion. Many other ingenious methods of encapsulation have been devised, but the majority fall into one or other of the three categories outlined here (Vandegaer, 1974).

Clearly the number of methods of micro-encapsulation that may be of use in aquaculture is enormous, but the physical methods generally require relatively sophisticated apparatus which is not readily available. The methods of encapsulation that could be attempted were therefore strictly limited to those requiring a minimum of equipment, and only one true micro-encapsulation technique was employed in the preparation of diets for carp larvae, although several methods were investigated.

8.2. Materials and Methods.

8.2.1. Diet Preparation.

Ten different dietary formulations/preparations were tested in the experiment, of which seven were manufactured in the laboratory and three were commercial preparations. Two basal diet formulations, employing different major protein sources, were bound and presented in three different forms, of which two involved the conventional process of manufacture and reduction to the desired particle sizes and the third involved the manufacture of "micro-pellets" of the right particle size. In addition, a third diet formulation was employed in the manufacture of nylon micro-encapsulated diets by a process of interfacial polymerisation.

The two basal diet formulations which were incorporated in diets made by three preparation techniques are shown in Table 19. The first (BD1) was based on white fishmeal as the major protein source (Caledonian Fishmeal Co. Ltd), while the second (BD2) contained a potato protein concentrate (Registered Trademark; "Protamyl MF"; AVEBE Starches Ltd., Holland) as the major protein source. Brewers yeast was added in small quantities to each because it was observed in a previous experiment (not included) that this appeared to possess some attractant properties. In addition, a small amount of fishmeal was added to the potato protein diet to enhance palatability because the potato protein had a very bland taste. The potato protein was used because it possessed a good amino acid profile and because it was supplied as a fine powder (particle size $< 45 \mu\text{m}$).

The diets were formulated to contain 55% crude protein and 12% crude lipid on a dry weight basis, but as the experiment was only intended as a qualitative rather than quantitative trial, and the different preparatory techniques used affected the final composition of the diets, all the diets were subjected to a proxim-

Table 19. Basal Diet Formulation.

Ingredient	% of Dry Diet	
	BD1	BD2
White fishmeal	73.6	5.0
"Protamyl MF"	-	63.3
Brewers yeast	5.0	5.0
Cod liver oil	5.5	11.2
Glucose	5.0	2.1
Dextrin	3.0	-
Alpha cellulose	2.0	2.0
Vitamin pre-mix*	2.0	2.0
Mineral pre-mix**	3.9	9.4

*To supply per 100 g dry diet: Thiamin hydrochloride 5 mg; riboflavin 5 mg; Ca pantothenate 10 mg; niacin 20 mg; biotin 0.6 mg; pyridoxine hydrochloride 4 mg; folic acid 1.5 mg; cyanocobalamin 0.01 mg; inositol 200 mg; ascorbic acid 100 mg; choline chloride 400 mg; menadione 4 mg; PABA 5 mg; α -tocopherol acetate 40 mg; vitamin A acetate 200 IU. Bulking agent α -cellulose.

**Ca(H₂PO₄)₂·H₂O 13.58%; Ca lactate 32.70%; Fe citrate 2.97%; MgSO₄ 13.20%; K₂PO₃ 23.98%; Na₂(PO₃)₂ 8.70%; NaCl 4.35%; AlCl₃·6H₂O 0.015%; KI 0.015%; CuCl 0.01%; MnSO₄ 0.08%; CoCl₂·6H₂O 0.10%; ZnSO₄·7H₂O 0.30%.

ate analysis to determine their composition on an "as-fed" basis. The dietary ingredients were ground and sieved to obtain material with a particle size of <50 μ m, and were blended together. The required quantities of mineral pre-mix, calculated to balance the ash content of the basal diets to 12%, were added in solution/suspension to the diets just prior to preparation by the different techniques described below. The proximate analyses of the diets are presented in Table 20.

8.2.1.1. Carboxy Methyl Cellulose Bound Diets.

To each of the basal diet formulations shown in Table 19 was added 2% by weight of carboxy methyl cellulose (Na salt, high

viscosity) and sufficient water containing 0.1% of an orange dye (Sunset yellow FCF) to form a smooth paste. This was air dried at room temperature for 48 hours after extrusion through a syringe to form thin strings of diet. The resultant hard pellets were ground and sieved to obtain feeds having particle sizes of 120 - 250 μm and 250 - 355 μm . All experimental diets were coloured to resemble Artemia.

8.2.1.2. Alginate Bound Diets.

Following the work of Meyers et al (1972) and Metailler et al (1979), the basal diets were made into a high-moisture diet bound with calcium alginate. Prior to the addition of the mineral mix to the basal diets, 1% by weight of sodium alginate (Alginate Industries Ltd.) was added to the dry ingredients and thoroughly mixed in. The mineral mix in a solution containing 0.1% of the dye, Sunset yellow FCF, was then added to the dry mix to give a thin paste containing 45% by weight of water. This paste was immediately extruded through a syringe containing eight 0.8 mm holes into a 0.1% solution of calcium chloride in distilled water. After approximately 2 minutes soaking in the calcium chloride bath, the thin strings of diet, which had now become firm due to the reaction between the alginate and free calcium ions (Andrew and MacLeod, 1970), with the calcium chloride solution were placed in a high speed blender and homogenised for 20 seconds.

The fragments of diet were then washed several times with fresh water and were sieved under water to obtain particles of 120 - 250 μm and 250 - 355 μm . The particles were washed through the sieves by running tap water. The collected fractions of the desired particle size were then stored in a minimum quantity of 0.1% calcium chloride solution at 4°C until needed. They were delivered to the experimental fish automatically via a peri-

Table 20. Proximate Analysis of Experimental Diets on an As-fed Basis.

	CMC* Fishmeal	CMC* Protamyl	Alginate Fishmeal	Alginate Protamyl	Diet Designation					
					Pelleted Fishmeal	Pelleted Protamyl	Nylon Microcap.	Flake 5-A-NA(AR)	Flake 6-B-GF(AR)	BP Larvit B
Moisture %	14.7	9.4	62.4	56.9	11.7	7.7	80.3	7.9	8.3	7.4
Crude Protein %	55.3	57.4	21.2	27.0	57.9	62.2	15.4	45.8	44.9	62.7
Crude Lipid %	9.8	9.1	3.9	3.5	17.9	22.0	1.7	11.8	11.0	5.5
Ash %	9.6	9.9	5.4	5.8	8.9	7.1	2.0	17.5	17.4	14.0

* Carboxy methylcellulose-bound diets

staltic pump from a reservoir in which they were kept in suspension by vigorous aeration.

8.2.1.3. Micro-pelleted Gelatin Bound Diets.

The preparation of micro-pellets bound with gelatin basically involved the emulsification of a core/gelatin solution in an oil, gelling and hardening of the gelatin droplets, and separation to yield a stable, dry pellet. The size of the resultant pellets was governed by the speed of stirring during the emulsification stage.

The method used was developed from a technique described by Tanaka et al (1963) originally designed to sustain the release of pharmaceutical substances in vivo, although it was subsequently discovered that a very similar technique had been patented specifically for the preparation of larval fish diets (Bayless, 1975).

A combination of both methods was used to prepare the experimental diets. A 6% w/w solution of gelatin in distilled water at a temperature of 50°C was mixed with an equal weight of the basal diets. The resultant paste was emulsified in thrice its volume of corn oil contained in a 2 litre round-bottomed flask held in a water bath at a temperature of 50°C, by means of an overhead stirring device rotating at 150 rpm. This stirring speed was chosen because it was found to yield particles in the desired size range, namely 150 - 350 µm.

The emulsion was then cooled rapidly to <10°C by addition of ice to the water bath, and 50 ml of cyclohexane was added to each litre of corn oil as a diluent when the temperature of the emulsion fell below 15°C. This was necessary to prevent aggregation of the particles as the viscosity of the oil increased with falling temperature. At the same time, 5 ml of a 25% solution of gluteraldehyde was added for each litre of corn oil, in order to harden the surface of the particles and facilitate later separat-

ion (Green,1960). Stirring was continued for a further 10 minutes after addition of the gluteraldehyde, then the pelleted material was separated from the oil by vacuum filtration. The resulting mass was re-suspended in twice its volume of acetone in order to remove adhering surface oil, and again vacuum filtered. Two acetone rinses were effected before the filter cake was spread out on aluminium foil sheets and allowed to air dry at room temperature.

A dry powder resulted from this process, which consisted of particles of diet lodged within a matrix of cross-linked gelatin, the pellets possessing an even spherical appearance. The desired size ranges were achieved by sieving.

8.2.1.4. Nylon Micro-encapsulated Diets.

Nylon micro-capsules were prepared by the interfacial polymerisation procedure described by Chang et al (1966), with the modifications suggested by Jones and Gabbot (1975). The technique was unsuitable for encapsulating the basal diets (D.Jones, pers. comm., 1978), and it was therefore decided to use the avian egg-based diets described by Jones and Gabbot (1975) and Jones et al (1976) with a slight modification of the internal phase.

The internal phase of the diet comprised equal proportions of whole homogenised chicken egg and a solution containing 10% w/v soluble casein, 2% w/v soluble starch, 2% w/v of mineral mix (without calcium lactate), 2% w/v white fishmeal and 1% w/v vitamin mix. The fishmeal and vitamin components were suspended in fine particulate form in the diet.

Capsules were manufactured by mixing a 50 ml portion of diet with 50 ml of 0.4M diamino hexane in 0.45M carbonate/bicarbonate buffer of pH 9.8, also containing 6mM 4,4'-diamino-2,2'-biphenyl-disulphonic acid. The water soluble diamine/diet mixture was then emulsified in 500 ml of mixed solvent (4:1 cyclohexane/chloroform)

containing 1% v/v Span 85. A further 500 ml of mixed solvent containing 0.036M sebacyl chloride was then added to the emulsion. The reaction was carried out in a 2 litre capacity round bottomed flask held in a water bath packed with crushed ice, and emulsification was effected by an overhead stirring device rotating at 70 rpm. This stirring speed yielded capsules of the desired size. The reaction was quenched after three minutes by the addition of 600 ml of mixed solvent.

The capsules were allowed to settle for three minutes, after which time the supernatant was carefully syphoned off and discarded. The capsules were re-suspended in 400 ml of 50% v/v Tween 20 solution, which was then diluted with 800 ml of distilled water. Separation of the capsules was effected by washing through sieves with running tap water for 15 minutes, after which capsules of the desired particle size were immersed in a boiling water bath (in a minimum of water in a separate beaker) for 8 minutes. This coagulated the egg protein and produced a semi-solid core. The capsules were then stored in a minimum quantity of 0.9% sodium chloride solution at 4°C until required for use.

The water soluble diamine was made up in buffer as a means of removing hydrogen ions liberated during the condensation reaction with the acid chloride, and 4,4'-diamino 2,2'-biphenyl disulphonic acid was included to impart a strong negative charge to the nylon micro-capsule membrane, which facilitated separation of the capsules. The 1:4 cyclohexane/chloroform solvent was found by Chang et al (1966) to be the best combination for thin, strong capsule walls. Increasing the proportion of chloroform renders the membranes brittle. The oil-soluble detergent Span 85 promoted the emulsification of the aqueous phase in the organic phase, while the water-soluble detergent Tween 20 was used to remove the mixed

solvent contaminating the capsules. Thorough washing was necessary to eliminate residual Tween 20, which might have affected the palatability of the capsules (Jones and Gabbot, 1975).

8.2.1.5. Commercial diets.

Two flake diets manufactured by the drum drying process (Meyers and Brand, 1975) were kindly supplied by S. Meyers and were designated by his coding as 5-A-NA(AR) and 6-B-GF(AR). These were ground and sieved to the desired particle size before use. In addition, a commercial experimental larval diet designated as "Larvit B" was supplied by BP Nutrition (UK) Ltd. This was understood to be designed for the feeding of larval turbot, and was produced by a process termed micronisation. It was coloured to resemble Artemia and was of a suitable particle size for direct feeding to carp larvae.

8.2.1.6. Experimental Design.

Twenty-four batches of 200, forty-eight hour old carp larvae with absorbed yolk sacs were placed in separate compartments of the experimental tank lattice described in section 2.5. All of the experimental diets were given to duplicated experimental populations of larvae and were distributed continually over 24 hours via the automatic feeding equipment described in section 2.6 in excess of requirement. In addition, two duplicated controls of Artemia-fed and starved larvae were carried out. Artemia, nylon micro-capsules and alginate bound diets were delivered to the experimental tanks via a peristaltic pump from aerated feeder reservoirs.

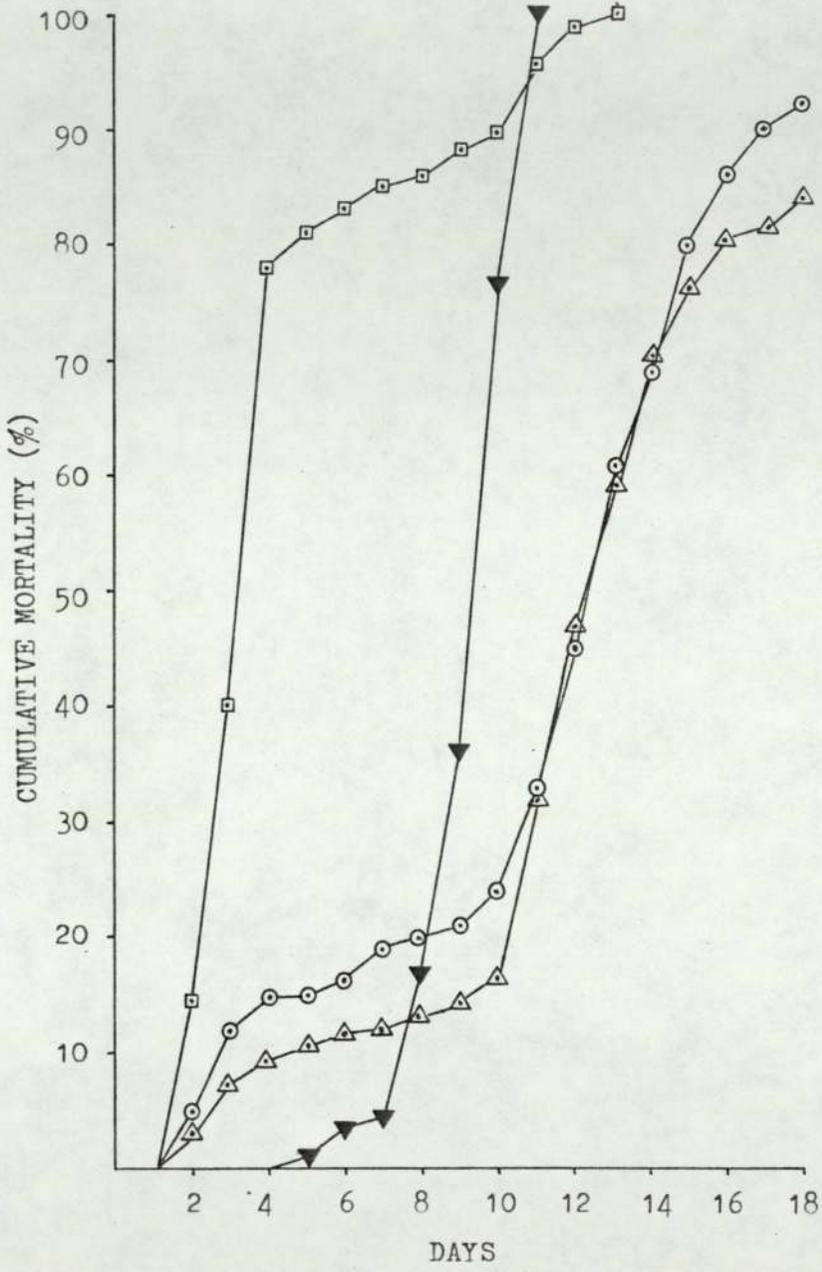
Samples of larvae were taken at three-day intervals and their length, wet and dry weights determined in the manner described in section 2.10. Daily mortalities were noted, and faecal samples were taken and mounted in glycerol jelly for microscopical examination.

8.3. Results.

Without exception, all of the experimental diets failed to adequately support the growth of carp larvae, although some diets did slightly improve survival. The cumulative mortality of larvae fed the experimental diets prepared in the laboratory is shown in Fig. 19 and that of larvae fed the commercial diets in Fig. 20. It can be seen from Fig. 19 that the basal diets bound with carboxy methylcellulose and micro-pelleted with gelatin, together with the fishmeal basal diet bound with sodium alginate, proved more fatal to the carp larvae than starvation, massive mortalities occurring among the experimental fish before the onset of mortalities in the starved controls. However, a lower level of mortality was obtained with larvae fed the potato protein basal diet bound in sodium alginate and nylon microcapsules containing the egg/casein diet, although these diets did not promote substantial growth (Table 21).

The commercial diets proved equally unsuitable for the larvae, as can be seen from Fig. 20. Although Larvit B was apparently well accepted by the larvae, it produced heavy mortality almost immediately after the experiment was begun, 94% of the experimental larvae dieing before the demise of the starved control larvae. The flake diets initially showed a low level of mortality, but this began to accelerate after the tenth day of the experiment such that between 80 and 90% of the larvae had died by the end of the experiment. The two flake diets had almost identical proximate composition (Table 20) and performed very similarly with the larvae; it was not known what differences existed between these diets. Neither Larvit B or the flake diets supported satisfactory growth, but larvae fed Larvit B gained the greatest weight of any of the experimental fish (Table 21).

Figure 20. Cumulative Mortalities of Carp Larvae II. Commercial Diets.



○ Flake 5-A-NA(AR)

△ Flake 6-B-GF(AR)

□ Larvit B

▼ Starved Larvae

All of the experimental diets were accepted by the carp larvae, with varying degrees of avidity. As suggested by Appelbaum (1976), the satiation and gut emptying time for each diet was investigated as a measure of the palatability of the diets, but very inconclusive results were obtained. It generally took larvae approximately 12 minutes before feeding activity on Artemia diminished, giving a reasonable indication of appetitive behaviour, but larvae offered dry diets took several hours to achieve an apparently full gut, and never showed any observable diminution in feeding activity. However, larvae offered the nylon micro-capsules showed appetitive behaviour similar to that observed for Artemia, and the capsules were actively sought. Dry diets, on the other hand, were frequently rejected by the larvae. Gut emptying time was not found to be a useful parameter for assessing diet suitability, as proposed by Appelbaum (1976), because larvae tended to retain food in the gut until they were offered more, whereupon they would defaecate.

Microscopical examination of faecal matter obtained from larvae fed on the different diets revealed that the gelatin micro-pellets passed through the gut unchanged, suggesting that the larvae were not able to digest the cross-linked gelatin matrix. Why the micro-pelleted fishmeal basal diet should have produced the most rapid mortality of any of the experimental diets, while the micro-pelleted potato protein basal diet yielded the best survival (Fig. 19) cannot be explained. The residual corn oil from the pelleting process contaminating the surface of the pellets was greatest on the pelleted potato protein diets, so this could not be invoked as an explanation. All of the other experimental and commercial diets were apparently fragmented in the gut, implying that some level of digestion was achieved. The

Table 21. Growth and Survival of Experimental Carp Larvae.

Initial live body weight 1.04 ± 0.06 mg

	Diet designation				
	CMC Fishmeal	CMC Protamyl	Alginate Fishmeal	Alginate Protamyl	Pelleted Fishmeal
Final weight after 18 days	3.96	3.09	1.89	2.52	1.04
<u>+S.D.</u> (mg)	0.70	0.59	0.62	0.16	0.05
Survival (%)	5.4	18.0	1.5	24.5	0

	Pelleted	Nylon	Flake	Flake	BP
	Protamyl	Microcap	5-A-NA(AR)	6-B-GF(AR)	Larvit B
Final weight after 18 days	0.81	1.86	2.12	2.61	5.90
<u>+S.D.</u> (mg)	0.16	0.11	0.66	0.26	0.37
Survival (%)	65.1	45.8	7.5	16.0	0

Final live body weight Artemia-fed control: 67.96 ± 6.54 mg

nylon micro-capsules were ruptured, the nylon membranes being indigestible.

While not supporting growth, the micro-pelleted potato protein diet and the nylon micro-capsules did prolong survival, the nylon micro-capsules enabling the larvae to maintain their weight. This can be ascribed to the excellent water stability of these diets in comparison with all the other experimental diets, which tended to fragment and pollute the water. The carboxy methylcellulose-bound diets were the least water stable of the experimental diets, and the heavy water pollution coupled with the high stocking density at the beginning of the experiment was the most likely cause of the initial heavy mortalities. As was discussed in section 2.5, the experimental facility proved difficult to keep clean without excessive disturbance of the larvae, which did not aid the performance of the less water-stable diets. Of all the experimental diets,

the nylon micro-capsules seemed to hold the most promise for further investigation.

8.4. Discussion and Conclusions.

If anything, the results served to emphasise the difficulties of rearing carp larvae directly on artificial diets, as experienced repeatedly by other researchers (Kainz, 1974, 1976; Appelbaum, 1976; Jirasek, 1976; Mires, 1976; Anwand et al, 1976; Albrecht et al, 1977; Dabrowski et al, 1978; Grudniewski et al, 1979; Dabrowska et al, 1979). The difficulties encountered have been ascribed to many and various reasons.

In this experiment, the slightly improved survival obtained with the gelatin micro-pellets and the nylon micro-capsules did underline the importance of the physical properties of the diet in relation to water stability, although the importance of this factor was to some extent exaggerated by the shortcomings of the experimental system. The relatively high stocking densities employed in the experimental tanks, coupled with unhygienic tank conditions must to a large extent be blamed for the experimental mortalities. These conditions would have made the performance of a poor diet worse. It is of interest to note that the stocking densities employed in the experiment were comparable with those routinely used in commercial rearing of carp with live food (Huisman, 1973), but were very much higher than those used by researchers obtaining some degree of success in rearing carp larvae entirely on artificial diets (Anwand et al, 1976; Appelbaum, 1977; Dabrowska et al, 1979). Many feeding experiments with artificial larval diets have been carried out in static or low-flow aquaria, and serious consideration must be given to the possibility that development of natural food in these aquaria on uneaten food residues may partially account for the success of the experimental diets.

The failure of all of the experimental diets to support growth must be attributed to the inadequate nutritional composition of the diets, since all of the diets with the exception of the micro-pellets were ingested and apparently digested to some extent by the larvae. The micro-pellets were apparently indigestible, and further evidence to support this observation was obtained from a subsidiary experiment with rainbow trout hatchlings.

Eight groups of 25 rainbow trout (Salmo gairdneri) fry with freshly absorbed yolk sacs were fed in duplicate to satiation by hand four times a day with three different diets, the fourth duplicated group being starved as a control. Two of these diets comprised a fishmeal-based experimental diet formulated to contain 55% crude protein and 10% crude lipid, bound with carboxy methylcellulose and micro-pelleted in the manner described in section 8.2.1.3, the third being a commercial trout fry diet. The trout had an initial weight of 67.7 ± 1.4 mg, and samples of ten fish per tank were weighed in the manner described in section 2.10 for carp fry at weekly intervals, the experiment being continued for four weeks.

All of the starved control fish had died by the 21st day of the experiment. Trout fed the commercial fry diet reached a mean weight of 220.5 ± 4.5 mg by the end of the experiment, and showed $8 \pm 4\%$ mortality. Trout fed the experimental carboxy methylcellulose bound diets reached a significantly ($P < 0.05$) higher mean weight of 261.1 ± 3.1 mg in the same time, and showed 16% mortality, while trout fed the micro-pelleted fishmeal diet had reached only 85.1 ± 0.3 mg with a mortality rate of 20%. The pelletation process therefore rendered the nutrients in the experimental diet much less available than in conventionally bound diets, thus explaining the failure of the diets so treated to support the growth of carp larvae, assuming that the unbound diet were able to do so.

Despite the fact that the nylon micro-capsules were the most successful diet in terms of palatability, this diet also failed to support growth of the carp larvae. Although the nutritional suitability of the micro-capsule core material for the larvae is open to question, some blame for the poor performance of the capsules may rest with their permeability to small molecules. Nutrient leaching from fish diets is a serious problem (Goldblatt et al, 1979), and is exacerbated by decreasing particle size of feeds. Murai (1966, cited by Murai and Andrews, 1975) showed that the pantothenic acid content of a trout diet of 1 mm pellet size was reduced from 500 to 250 mg/kg in 10 seconds and completely disappeared after 1 minute in water. Similar leach rates would probably apply to most of the water soluble ingredients in a larval diet, if not more rapid. The rapidity with which ingredients could leach out of the capsules was illustrated during their preparation. The water soluble dye Sunset yellow FCF added to the dietary ingredients was incorporated in the capsules quite visibly when they were first separated from the organic solvents, but this colouration was lost within 1 minute of washing to remove residual detergent from the capsules, implying that other water soluble ingredients were lost in the same time before the diet was even presented to the larvae.

Nylon micro-capsules have an effective pore diameter of < 1.7 nm (Jones et al, 1976), so molecules smaller than this would leach out of the capsules. However, the nylon capsules have given some encouraging results in trials with Mytilus edulis and Paenus japonicus (Jones and Gabbot, 1975; Gabbot et al, 1976; Jones et al, 1979) and it is possible that the problem of their permeability may be overcome. As well as release of their contents by rupture, it has been shown (Jones and Gabbot, 1975; Gabbot et al, 1976) that the capsule wall may be digestible, since some incorporation of the core

protein into the capsule wall occurs by cross-linking of nylon and protein molecules. It is possible to increase the amount of wall bound protein by decreasing the concentration of water soluble diamine during the preparation of the capsules (Gabbot et al,1976). There are also many other possible condensation reactions which can be used to produce micro-capsules with walls made of a variety of synthetic polymers (Wittbecker and Morgan,1959; Morgan and Kwolek,1959) and investigation of some of these processes could give micro-capsules with properties more suited to the delivery of larval diets.

Apart from the loss of nutrients by leaching, there are many other possible nutritional failings of larval diets, some of which have been considered by other researchers. Dabrowski and Glogowski (1977a) demonstrated that ingestion of exogenous proteolytic enzymes contributed significantly to the overall enzymatic activity of fish guts, and went on to show (Dabrowski and Glogowski,1977b) that considerable quantities of potentially ingestible proteolytic enzymes were present in aquatic invertebrates of the type forming the first food of fish larvae in the wild. This caused them to postulate that exogenous proteolytic enzymes may play an important role in the digestion and assimilation of food during the larval stages of fish. However, they were unable to show that addition of exogenous enzymes in the form of bovine trypsin (Dabrowski et al,1978) or carp intestinal/hepatopancreal extracts (Dabrowska et al,1979) had any appreciable affect on growth of carp larvae, although they did obtain a slight improvement in survival (Dabrowska et al,1979). Anwand et al (1976) obtained similarly inconclusive results when adding alkaline proteases, neutral proteases and α -amylase to carp larval diets.

Following the work of Nose et al (1974), who showed that the

pH of an amino acid test diet markedly affected its assimilation by young carp, Dabrowski et al (1978) adjusted the pH of their carp larval diets to obtain a mildly alkaline value, but this had little apparent effect on the growth or survival of the larvae.

Other authors have paid attention to the protein component of artificial diets for carp larvae (Anwand et al, 1976; Lukowicz and Rutkowski, 1976; Lukowicz, 1977; Grudniewski et al, 1979). The use of freeze-dried whole fish, or fish muscle in artificial diets has resulted in some slight improvements in growth and survival (Lukowicz, 1977), but these have always been markedly inferior in comparison with the growth and survival of carp larvae fed natural zooplankton or Artemia. Amino acid analyses of partially successful and unsuccessful diets (Anwand et al, 1976; Grudniewski et al, 1979) have generally not revealed differences sufficient to be invoked as explanations for the success of one diet as opposed to another. The amino acid compositions of the majority of experimental diets used by different authors have been excellent (Dabrowska et al, 1979), in excess of the known requirements of carp for essential amino acids (Nose, 1979) and certainly as favourable as the amino acid compositions of natural food organisms (Albrecht and Wunsche, 1972; Watanabe et al, 1978b). In addition, rearing experiments with other species of fish larvae (Dabrowski et al, 1979; Seidel et al, 1980) have produced poor results with artificial diets apparently well-endowed in amino acid content. The inadequacies of artificial diets for fish larvae cannot therefore be easily explained in terms of their quantitative amino acid contents, at least within the framework of current knowledge relating to the amino acid requirements of fish.

More recently, attention has been focussed on the lipid content of natural and artificial larval foods (Watanabe et al, 1978c,d,e;

Grudniewski et al,1979; Watanabe et al,1980; Seidel et al,1980) with particular regard to the content of poly-unsaturated fatty acids (PUFA) and higher unsaturated fatty acids (HUFA) of the linolenic (ω_3) series, which have been shown to exhibit essential fatty acid functions in fish (Cowey and Sargent,1977). In contrast to some species such as rainbow trout, which have a dietary requirement for linolenic acid or HUFA of the ω_3 series, adult carp have been shown to require both linoleate and linolenate in their diet (Watanabe et al,1975a,b). They are also evidently able to chain elongate and desaturate 18 carbon fatty acids with reasonable efficiency because growth is only marginally improved by the addition of ω_3 HUFA (20:5 ω_3 and 22:6 ω_3) to the diet (Takeuchi and Watanabe,1977), although ω_3 HUFA was found to possess greater essential fatty acid activity than linolenic acid (18:3 ω_3) alone. Takeuchi and Watanabe (1977) recommended that levels of 1% of linoleate (ω_6) and 1% linolenate (ω_3) should be included in diets for carp in order to satisfy their essential fatty acid requirements.

Fatty acid analyses of Artemia (Watanabe et al,1978d) have shown that these invertebrates contain a relatively high amount of 18:3 ω_3 and HUFA of the ω_3 series, although their content of 18:2 ω_6 was fairly low. In terms of their fatty acid content therefore, Artemia appear to meet the essential fatty acid requirements of carp, although their content of 18:2 ω_6 may be sub-optimal. The fatty acid composition of some unsuccessful carp larval diets tested by Grudniewski et al (1979) compared favourably with that of plankton and did not in any way appear deficient. These authors therefore ruled out the fatty acid content of their diets as being a responsible factor for their poor performance, although they did propose that the balance between dietary amino acids and fatty acids may be more critical for larvae than for older fish. Seidel et al

(1980) could not find any obvious fatty acid deficiencies in unsuccessful diets they fed to Atlantic silversides (Menidia menidia) and concluded that this could not have been a contributory factor to the poor performance of the diets. While the fatty acid composition of larval foods merits more attention, it is unlikely that artificial diets containing reasonable and balanced proportions of oils of marine and vegetable origin (rich in ω_3 and ω_6 PUFA respectively) would not satisfy the essential fatty acid requirements of larval fish.

One other possibility regarding the lipid content of larval diets as a possible source of larval mortalities concerns their susceptibility to biochemical damage by oxidised lipids. It is well known that the long-chain poly-unsaturated fatty acids are very susceptible to oxidation, and that the products of lipid oxidation are detrimental to the health of mammals and fish (Castell, 1979). Watanabe and Hashimoto (1968) demonstrated the marked growth depressing effects of highly oxidised fish oil in carp diets and also showed that alpha-tocopherol added to the diet could alleviate these symptoms. Similarly, Fowler and Banks (1969) found that rancid herring oil caused gill clubbing, kidney malfunction, anaemia and lethargy in Chinook salmon, symptoms which could again be prevented by administration of alpha-tocopherol.

Fishmeals prepared by the normal oven-drying process will always contain some level of oxidised fat, the level depending on the level of lipid in the meal, its drying temperature and the storage times before and after manufacture, as well as the level of added anti-oxidants. Use of such meals in larval diets therefore raises the question of the effect of these rancid lipids on the larvae. The enormous surface area/weight ratio of larval feeds also exposes ingredients to rapid oxidation, and to what extent

even freshly added lipids are subject to deterioration before diets reach the fish is unknown. Very much higher levels of anti-oxidants may be needed in larval diets than in adult fish feeds.

Many authors have reported on the beneficial effect of small supplements of live food in addition to artificial diets on the growth and survival of fish larvae (Jirasek,1976; Mires,1976; Albrecht et al,1977; Lukowicz,1977; Beck and Bengston,1979; Girin, 1979; Seidel et al,1980). Seidel et al (1980) demonstrated that Atlantic silversides (Menidia menidia) could be successfully reared on a combination of Artemia and a dry food,a feeding ratio of 7 days dry food: 1 day Artemia giving as good survival as an Artemia-fed control and approximately three-quarters of the growth rate. Fluchter (1980) reports that whitefish larvae (Coregonidae) can be similarly reared,although the required interval between Artemia feeds is shorter (every other day) if mortality is to be kept to a minimum.

It has also been observed on several occasions that frozen zooplankton and Artemia lose their nutritive value to fish larvae (Dabrowski et al,1978; Beck and Bengston,1979; Fluchter,1978),as do freeze-dried plankton. However,Artemia nauplii or plankton that have been shock frozen in liquid nitrogen possess as good a nutritive value as living organisms (Lukowicz,1977; Fluchter,1980) and this property persists for some 2 hours after thawing (Fluchter,1980). These observations have led some authors (Beck and Bengston,1979; Seidel et al,1980; Fluchter,1980) to suggest the presence of an unknown "growth factor" in live organisms,and to recommend the isolation of this factor for biochemical characterisation.

Fluchter (1980) has suggested that the substance in question must be water insoluble because liquid nitrogen frozen Artemia or

plankton are ruptured during this process, but retain their food value to larvae for up to two hours after thawing in water, and that it must be connected with intermediate metabolism because of its slow disappearance (attributed to enzymatic processes). However, there is no real need to invoke the presence of a particular substance in live food at all. The resumption of enzymatic activity after thawing would almost certainly result in proteolysis, which can lead to considerable loss of amino acids (Hale, 1972). Aoe et al (1974) have shown that hydrolysis of wheatgerm by either pronase or trypsin results in a significant reduction in the nutritive value of this protein source to young carp in comparison with unhydrolysed wheatgerm. As suggested by Dabrowski et al (1978), fish larvae may require proteins in a specific biochemical form that is only found in the living state.

These speculations are to some extent negated by the work of Appelbaum (1977, 1979), who showed that it was possible to rear carp and grass carp larvae with reasonable success on a diet composed largely of the petro-protein yeast, Candida lipolytica. Although the growth of larvae fed this diet was by no means as good as larvae fed Artemia or zooplankton, growth was nevertheless better than has been reported for an artificial diet by any other author, and survival also appeared to be extremely good (> 70%). Although some doubt may be expressed as to the long-term survival of larvae fed on this, followed by more conventional artificial diets, because the experimental fish were apparently returned to live food or stocked in ponds at the end of the experiments (Appelbaum and Dor, 1978), the fact that carp larvae can be fed at all on a dry diet without significant mortality and with reasonable growth is a substantial achievement in the light of previous results.

It would not seem plausible in terms of current knowledge to suggest that the protein component of the yeast is markedly different to that of any compounded diet screened by other researchers. A comparison of the amino acid profile of the yeast with other experimental diets and with live food (Dabrowska et al, 1979) does not yield any clues to its success from this point of view. The lipid content of the yeast is also very low (own analysis, not included) and would appear deficient in essential fatty acids. Considerable further research with this diet would therefore appear worthwhile, since it could provide valuable information on the nutritional requirements of the larvae and explain why other apparently excellent diets have failed.

CHAPTER 9.

Chapter 9. General Discussion and Conclusions.

9.1. Experimental Results.

The declared aim of this programme of research (Chapter 1) was the development of an artificial larval diet capable of supporting the growth and survival of carp larvae immediately after hatching; this objective was not achieved. However, both the approach to the problem and the results obtained are considered to have made some contribution towards its solution.

Early unsuccessful attempts to rear carp larvae on various artificial diets (Chapter 8) merely added to the catalogue of failures reported by other researchers (Imam and Habashy, 1972; Jirasek, 1976; Anwand et al, 1976; Dabrowski et al, 1978; Dabrowska et al, 1979), and it was decided that a different approach to the problem might be more fruitful. Firstly, it was considered that the husbandry of the larvae was inextricably linked with the success or failure of artificial diets, and that optimisation of husbandry might improve the performance of artificial diets. Secondly, it was thought that determination of the nutritional requirements of slightly older fish would help to indicate dietary formulations more suited to larvae than the arbitrary formulations employed before. Whether this approach proved successful or not, it was hoped that the results obtained would be of practical value for the intensive rearing of carp larvae.

The first experiment (Chapter 3) sought to define the optimal feeding rates for Artemia, in order to both improve the economics of cyst use and to set a growth standard for assessing the performance of artificial diets. It was shown that a rapid decrease in growth rate was exhibited by carp larvae and post-larvae during the first ten days after commencement of feeding, irrespective of the amount of Artemia available, and that maximum growth was elic-

ited during the first five days after commencement of feeding when Artemia were offered in amounts ranging from 200 - 250% of the body weight per day. During the following five days, optimal growth and food conversion were exhibited at lower feeding rates of between 100 and 120% of body weight per day. These results were in broad agreement with those obtained by other researchers, who used different species of live food. Since many of the growth rates for carp larvae fed on Artemia and other living feeds reported by various workers were below those obtained in the experiment, it was reasoned that many researchers were underfeeding their fish. Thus it would appear that application of the results to commercial rearing of carp larvae could result in improved growth and earlier weaning onto artificial diets before stocking out into ponds, which is the normal practice in intensive carp rearing (Appelbaum and Dor, 1978), as well as significant savings of Artemia cysts.

Although monitoring of Artemia numbers is necessary if feeding rate is to be controlled, which would at first sight appear to be a rather onerous task, several simple techniques can be used. Apart from mechanical counting of nauplii, which requires sophisticated apparatus beyond the means of most hatcheries (van Outryve and Sorgeloos, 1976), Sroczynski et al (1977) have shown that the concentration of a suspension of Artemia nauplii or other zooplankton can be estimated with reasonable accuracy ($\pm 5\%$) by colourimetry, the value of the extinction coefficient being linearly related to Artemia concentration for up to 1600 nauplii/ml. Even more simply, approximately correct numbers of nauplii can be obtained by incubation of a weighed quantity of cysts; provided Artemia hatchery conditions remain reasonably constant, hatching efficiency should also remain constant and determination of this would allow prediction of the numbers of nauplii to be obtained from a given

weight of cysts. Estimation of the numbers of larvae to be fed, which may be obtained by weighing eggs before fertilisation, followed by estimation of fertilisation and hatching success, would permit calculation of the approximate numbers of nauplii required, and thus the necessary quantities of cysts to be incubated.

The second experiment (Chapter 4) sought to determine the earliest point at which carp larvae could be transferred with reasonable success to a commercial fry diet. It was found that post-larvae of approximately 15 mg live weight could be reared with 80% survival entirely on an artificial diet, although it was appreciated that this was very much dependent on the commercial diet chosen. However, it was considered that duplication of this result with other dietary formulations should be possible. Following upon this finding, the experiments described in Chapter 5 were designed to establish the correct feeding rates for the artificial diet, since it was thought that feeding to excess may have been a contributory factor to the mortalities usually observed among carp larvae and post-larvae fed on artificial dry diets.

It was found that carp post-larvae fed on the commercial fry diet from a weight of approximately 15 mg demonstrated optimal growth and food conversion for feeding rates ranging from 15 - 17.5% of their body weight per day, and that carp fry showed a slight reduction in feed intake and growth compared with the post-larvae. Optimal growth and food conversion for fry ranging in weight from approximately 100 - 500 mg was exhibited for feeding rates ranging from 10 - 15% of body weight per day. It was also shown that excessive feeding did indeed increase the mortality rate of post-larvae, thus the results held some practical significance in relation to the intensive commercial production of carp larvae. Provided that a similar commercial diet were to be used, it would be possible to

feed larvae on an artificial diet at a lower weight (and thus an earlier stage) than the 50 - 70 mg normally used (Huisman, 1973); control of feeding rate during this period would enhance survival and possibly produce more even population growth, as well as economising on the use of dry food.

An investigation of the effect of feeding frequency (Chapter 6) on the growth of carp fry fed on a commercial diet revealed that it was necessary to present the optimal daily ration in at least six equal portions throughout the day (24 hours) in order to obtain the best growth and food conversion. A feeding frequency of 12 meals per day produced the most even population growth, but this reflected the design of the experimental system, and it is probable that continuous distribution of food until the fish weigh approximately 500 mg is the best procedure. Hand feeding throughout the hours of daylight, a frequent commercial and experimental practice (Mires, 1976; Appelbaum and Dor, 1978; Dabrowski et al, 1978), would not appear to be satisfactory if the growth of the larvae is to be maximised. A study of the effect of food colour on diet utilisation by carp fry (Chapter 6) demonstrated that the fry had a significant preference for green diets, and that matching of diet colour to tank background also appeared to be beneficial. Both of these findings have practical application.

Incorporating the above findings into subsequent experimental practice (with the exception of the findings relating to diet colour), it was attempted to formulate semi-purified experimental diets and determine the optimum ratio of dietary protein to lipid in diets for carp post-larvae and fry. (Chapter 7). Unfortunately, it proved impossible to manufacture an experimental diet which duplicated the success of the commercial trout fry diet for rearing carp post-larvae and fry, although a degree of success was obtained in

determination of the nutritional requirements of fry. It was tentatively suggested that optimal growth was exhibited by fry weighing between 100 and 500 mg fed diets containing 45% crude protein, and that inclusion of lipid in the diet at levels above 5% of the dry diet did not appear to offer any advantages. These results were somewhat contrary to the findings of other researchers for carp weighing between 2 and 10 g (Ogino and Saito, 1970; Takeuchi et al, 1979a,b; Jauncey, 1979) and require confirmation.

It was considered that the design of the experimental system (Chapter 2, section 2.5) overcame many of the problems associated with the feeding of artificial diets to fish larvae and the use of small fish as experimental animals (Houde, 1973; Girin, 1979). It was possible to ensure that virtually identical environmental conditions prevailed in each experimental treatment and that uneaten food presented little hazard to the fish, a problem which has been encountered by several researchers (Gatesoupe and Luquet, 1977; Girin, 1979). The system also eliminated the possibility of extraneous food affecting the growth and survival of larvae, a criticism levelled at some authors by Dabrowska et al (1979). The major disadvantage of the system, the short residence time of food particles before elimination, could have been remedied by an increased tank volume and possibly the use of aeration to re-suspend food particles (Girin, 1979).

It is believed that the major contributions of the research briefly summarised above have been the demonstration of the feasibility of carrying out quantitative studies with fish larvae, a possibility that has been excluded by some authors (van Limborgh, 1979), and an identification of factors which may exert a negative influence on the success of artificial diets for carp larvae. However, the lack of attention paid to the physiological and biochem-

ical reasons for the failure of artificial diets to support the growth and survival of carp larvae is viewed as an important deficiency in the research.

9.2. Recommendations.

The desire to develop an artificial live food substitute for the rearing of fish larvae is currently an academic rather than practical necessity, since it will be possible to rear fish larvae in the foreseeable future entirely on live food which can be made available in adequate quantity by extension of existing technology. Although the manufacture of successful artificial larval diets would be of benefit to the fish culture industry, their greatest contribution will be in the implied understanding of larval nutrition.

Numerous attempts to rear fish larvae entirely on artificial diets have met with little success; while the literature pertaining to these attempts has been discussed in detail in the relevant chapters, it will be useful to summarise here the explanations offered by various authors for the failure of compounded artificial diets to support the growth and survival of fish larvae.

- (1) Live food possesses an as yet unidentified "growth factor" which is rendered unavailable or destroyed upon the death of the living organism (Beck and Bengston, 1979; Fluchter, 1980).
- (2) Poor physical presentation of diets, combined with loss of water-soluble ingredients lead to ingestion of an inadequate fraction of the dietary formulation (Meyers, 1979; Girin, 1979; Goldblatt et al, 1979).
- (3) Exogenous enzymes play an important role in the digestion and assimilation of the protein component of live food, and which are absent in prepared feeds (Anwand et al, 1976; Dabrowski and Glogowski, 1977a, b, c; Dabrowski et al, 1978; Dabrowska et al, 1979).

- (4) The fatty acid composition of the food is an important factor in the nutrition of larvae, and the lability of the polyunsaturated lipids required may lead to the production of toxic compounds in the processed larval diets (Watanabe et al, 1978d,e; Seidel et al, 1980).
- (5) Processing of dietary protein sources renders them unassimilable by larvae due to changes in protein structure, and proteins are only digestible in their natural biochemical form (Dabrowski et al, 1978; Grudniewski et al, 1979; Dabrowska et al, 1979).

The possibility that live foods contain some special "growth factor" is difficult to refute, but it has already been pointed out (Chapter 8) that deterioration in the nutritive value of dead or processed live food can equally well be explained by the changes in protein, lipid or vitamin components that are already known to occur without the need to invoke the presence of an unknown "factor". At present, it would seem best to dismiss this idea.

Loss of ingredients before ingestion cannot be entertained as a major factor in the poor performance of compounded diets with carp larvae, since it is possible to feed the same diets, presumably still subject to the same degree of leaching, to slightly older fish with good results (Chapter 4). The feeding behaviour of carp larvae would also tend to mitigate this problem. In Chapter 7, it was also mentioned that increasing the level of vitamins in the diet had no apparent effect on growth or survival, which would suggest that loss of water soluble vitamins was not a serious occurrence.

Dabrowska et al (1979), summarising three years work on the importance of exogenous proteolytic enzymes in larval fish feeds, reported that they had been unable to demonstrate any improvement in growth or survival of carp larvae fed diets containing mammalian

or piscine proteolytic enzymes, thus there is currently little evidence to support this suggestion.

There is no evidence which argues against the last two suggestions. In relation to the present knowledge of fish nutrition, it would appear that most experimental larval diets have contained adequate levels of essential nutrients, and have been biochemically as good or better than live food. The considerable variation in the biochemical compositions of living feed organisms (Dutrieu, 1960; Gallagher and Brown, 1975; Benijts et al, 1975; Watanabe et al, 1978a,b,c,d), many of which are equally suitable for the rearing of carp larvae, would suggest that carp larvae at least are tolerant of a wide range of nutrient intake provided that it is in the form of live food.

The evidence accumulated in the course of this research and by other authors would seem to indicate an inability of newly-hatched larvae to assimilate processed diets. An improving ability to utilise artificial diets would appear to develop rapidly as the larvae grow (experiment 2, Chapter 4), and it was remarked in the discussion of experiment 3 (Chapter 5) that food intake on a dry weight basis was very similar for carp post-larvae fed Artemia or a commercial trout fry food, although growth on the commercial fry food was poor in comparison with Artemia-fed fish. The only symptom of inadequate nutrition among fish larvae would appear to be death, which is in many cases more rapid than that of starved larvae. Determination of the physiological cause of death among larvae fed on artificial diets must therefore be seen to be of paramount importance to an understanding of larval nutrition. A histological comparison of moribund and healthy Artemia-fed fish during the course of this research did not reveal any differences in appearance, but this merely reflected the inadequacies of the

histological examination.

The fact that larvae accept and die or do not grow, or both, on diets which have reasonable nutritional value to older fish points to a considerable difference in the digestive processes of larval and adult fish. The mechanisms of absorption of fat and protein in larval teleosts have been studied by Iwai (1968, 1969) and Iwai and Tanaka (1968a, b), and they suggest that absorption of these nutrients is effected by two different processes occurring in discrete regions of the gut.

In carp larvae with absorbed yolk sacs, the gut is a simple straight tube, slightly widening and bending along the ventral surface of the swim bladder and gradually decreasing in diameter towards the anus. The gut mucosa is thrown into low annular ridges and consists of simple columnar epithelium with a luminal border of microvilli, infrequently interspersed with secretive goblet cells. Iwai (1969) divides the larval gut into two regions, an antero-mid portion and a posterior portion, on the basis of a slight constriction some 900 - 950 μm before the anus and the different appearance of the epithelial cells in these regions during digestion. After ingestion of a meal of mixed casein and corn oil by carp larvae, Iwai (1969) showed that the epithelial cells of the mid-gut contained large vacuoles filled with lipid, and that the cells of the posterior portion of the gut showed vigorous pinocytotic activity associated with the absorption of protein. The absorption of lipid was not associated with pinocytotic activity in the columnar epithelial cells of the mid gut. These observations prompted the author to conclude that fat was absorbed by diffusion of free fatty acids and monoglycerides into the epithelial cells from luminal micelles produced by the digestive action of lipases in the intestine, and that proteins were partially absorbed in macro-molecular form and

digested intracellularly. This latter supposition was supported by the observation that increased numbers of lysosomes occurred in cells exhibiting vigorous pinocytotic activity.

Both fat and protein absorption are known to be effected by pinocytosis in post-natal mammals (Rubin, 1966; Selkurt, 1971), but this does not substantially contribute to their absorption in the adult; in fact, the mechanisms of absorption of protein in mammals are poorly understood. It is thought that basic, neutral, carboxylic and imino acids all possess separate active transport mechanisms in man, but that some direct absorption of peptides also takes place because of the presence of peptidases in the intestinal villi (Selkurt, 1971; Passmore and Robson, 1973).

Yamamoto (1966) observed that protein macromolecules were apparently absorbed by pinocytosis in the posterior portion of the gut of adult goldfish, which might provide an explanation for the inability of young carp to efficiently utilise protein hydrolysates and free amino acids (Aoe et al, 1974; Nose et al, 1974). Kawai and Ikeda (1973) have shown that carp larvae possess trypsin-like digestive enzymes, whose activity gradually increases with growth. It is interesting to speculate whether these are active in the intestinal lumen or are purely associated with intracellular digestion. If this were the case, and processing of protein sources interferes with their absorption by pinocytosis in carp larvae, then it is possible that ingested proteins may never come into contact with intracellular digestive enzymes and so cannot be assimilated. It is also of interest to note that presumed pinocytotic absorption of protein has been reported in a wide variety of fish larvae, including the alevins of rainbow trout (Iwai, 1968), but that this ability seems to be lost in the adults of the stomached

species such as trout while being retained in the stomachless species (Yamamoto,1966).

In view of the specialized method of protein absorption in carp larvae, it seems plausible to suggest that during the first few days after hatching some particular configuration of proteins may be necessary to initiate absorption, and that presentation of proteins not conforming to requirement could damage the gut and lead to the death of the larvae. It is presumed that an improving ability to handle diverse configurations develops as the fish grow to account for the observed ability of older fish to cope with diets previously unsuited to larvae.

Several indications of a metabolic change occurring at about the larval to post-larval transition weight of approximately 15 mg were gained during the course of this study of juvenile carp. In experiment 1 (Chapter 3) it was noted that no significant decrease in growth rate was observable until this weight was achieved and that the inferred carcass lipid content of the larvae appeared to remain approximately static up to the same point. Thereafter, post-larvae of greater weights showed both a rapid decrease in growth rate and an increase in carcass lipid. In experiment 2 (Chapter 4), it was discovered that carp post-larvae of greater than 15 mg body weight exhibited an improving ability to utilise the commercial trout fry diet, in terms of both survival and growth. While all of these observations may have been artefacts, they should not be ignored.

In conclusion therefore, it is considered that a fundamental study of the structure and function of the larval gut would be of great value in the development of artificial diets for fish larvae, with particular emphasis on changes, if any, at the larval transition weight of approximately 15 mg in carp, and differences between the

absorption and assimilation of live food and processed diets.

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