THE METABOLISM AND GROWTH OF RAINBOW TROUT, SALMO GAIRDNERI, IN FRESH AND SALINE WATERS.

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SUMMARY

The growth and metabolic responses of rainbow trout to salinities of 10, 20 and 28 ppt and freshwater were investigated under varied dietary conditions.

During the spring and summer, voluntary food consumption was greatest in 10 and 20 ppt and least in 28 ppt. During the winter, salinity had no effect on this parameter.

Energy balance studies showed that total metabolic rates increased directly with feeding rate. Metabolic rates were least in 10 ppt, equal in freshwater and 20 ppt and greatest in 28 ppt.

Growth, as calories retained, was optimal at 10 ppt, equal at 20 ppt and freshwater and least at 28 ppt.

Salinity did not influence the proximate body composition of trout.

No change in apparent digestibility was elicited by salinity except at 28 ppt where lower values were found.

Apparent SDA was unaffected by salinity but increased as feeding rate increased.

Feeding trials showed that specific growth rates and gross growth efficiencies were higher in 10 ppt than freshwater and 20 ppt at six different feeding rates. Maintenance ration levels were least in 10 ppt.

While an increase in feeding rate caused a proportional decrease in net growth efficiencies, no effect of salinity on this parameter was observed.

Nitrogen balance studies showed that salinity caused small changes in apparent and true protein digestibilities at different levels of protein intake.

Total nitrogen excretion was lower in 10 ppt than freshwater and 20 ppt at different protein intakes as was endogenous nitrogen excretion.

Nitrogen retention was optimal in 10 ppt while maintenance protein requirements were least in this salinity.

Salinity produced no marked effect in NPU values.

It was concluded that the changes in both metabolic rate and maintenance energy/nitrogen requirements elicited by seawater resulted from an alteration of the energy requirements for osmotic and ionic regulation.

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Chapter 1. INTRODUCTION

Section 1.1. The Observed Effects of Salinity on the Growth of Fish

The majority of the commercial salmonid farms in this country and the rest of Europe operate on supplies of fresh water. Despite the fact that the first salt water rainbow trout farm was started about fifty years ago in Denmark (Sedgwick, 1973) the development of salt water salmonid farming has been comparatively slow. Recent interest has been aroused in the possibilities of intensive culture of salmon, and possibly sea trout, in sea water because of their high market values and the threatened scarcity of these species. Consequently, over the last fifteen years, there has been an increase in the number of salt water salmonid farms (Jensen, 1955; Petersen, 1957; Vik, 1963; Sedgwick, 1966; Purves, 1968; Murai & Andrews, 1972; Tatum, 1973; and others).

Several advantages may be secured through rearing salmonids in salt water. These are:-

a. Rapid growth occurs all the year round together with a better conversion of food into fish flesh than is obtained in fresh water (Sedgwick, 1970).

b. A reduced disease risk (Sedgwick, 1973).

c. A greater constancy in the conditions of water quality and water supply.

Much observation has been made in the farm situation on the improved growth rates of salmon and trout in sea water. However, relatively few scientific investigations into this phenomenon have been made and it was considered that a thorough study into the effects of water salinity on growth would be of great value. Therefore a

programme of experiments was devised which would examine such parameters as growth, metabolism and appetite under conditions of different water salinities.

Publications of relevance to the main topic of investigation include those observations made in the farm situation, where environmental conditions may be uncontrolled, and laboratory experiments. In the farm situation sea water may affect the growth performance of fish in one or several ways. Firstly, the fish will be subjected to higher water temperatures, on average, than would occur in fresh water. This applies especially to those farms situated on the Western coasts of Norway and Scotland where the influence of the warm North Atlantic Drift maintains winter temperatures above $4^{o}C$ (Møller, 1973). Secondly, fish reared in a floating cage may supplement their normal food intake by eating plankton and small fish so increasing their rate of growth. Finally, the sea water itself may influence the growth of the fish by altering the appetite or the metabolism of the fish in some manner.

Some of the observed changes in growth rate caused by rearing fish in sea water will now be reviewed. Repeated observations have shown that among euryhaline animals those species living in reduced salinities have a smaller maximum size than those living in higher salinities. Whilst this probably results from greater feeding opportunity in the marine environment some researchers maintain that the larger size of the marine animals results directly or indirectly from the effects of the altered osmotic environment on the animal. Much evidence of this nature has been reviewed by Canagaratnam (1959) mostly involving species other than salmonids. Most of the observat-

ions made in the farm situation on the effects of salinity on growth rate involve species of the genus Oncorhynchus, the Pacific salmon. Sato and Kashiwagi (1968) reared chum salmon, Oncorhynchus keta, from fry in fresh water and salt water ponds whose salinity ranged from 20 - 29 p.p.t. Growth was markedly higher in the sea water ponds. Garrison (1965, 1971) demonstrated that the growth rate of juvenile coho salmon, Oncorhynchus kisutch, was faster in a brackish water pond than in a fresh water pond. Milne (1972) reports that in the U.S.A., chinook salmon, Oncorhynchus tschawytscha, grow at their fastest rate in sea water of strength 18 p.p.t., full strength being taken as 34.33 p.p.t salinity (Normal Copenhagen Sea Water). Finally, pre-migratory coho salmon, O.kisutch, held in a saline estuary grew to a length of 15 cm in 90 days whereas similar fish held in fresh water only grew to half this size in the same period, both groups feeding solely on natural plankton (Anon., 1964).

Observations on the growth of Salmo species in sea water include the following. Alm (1934) stated that land locked Atlantic salmon, Salmo salar, never attain as large a size as those that migrate to sea although both have equally good feeding conditions. Falk (1968) reported some results obtained from the brackish water cage culture of rainbow trout, Salmo gairdneri, in Germany. Here, fish were subjected to salinities from 6 - 8 p.p.t and were fed to repletion on a wet fish diet. After 63 days, the fish had grown from an average of 54 g each to an average of 118 g in sea water while those in fresh water only grew to about 100 g from a similar initial weight. Murai and Andrews (1972) attempted to compare the growth rates of rainbow trout, Salmo gairdneri, in fresh water and in sea water of 28 p.p.t. salinity.

Unfortunately, a definite conclusion could not be made due to a large difference in temperature between the groups in fresh water and sea water. However, survival, growth rates and food conversion were good for trout reared in sea water at a temperature of 13.5°C. Two exceptions to these general observations include reports by Koops (1972) and Tiews (pers. comm.) in experiments culturing rainbow trout in cages in the Western Baltic. Both have found no differences in food conversion efficiency, feeding demand, nor growth between trout in sea cages and trout grown in fresh water despite the fact that water temperatures were similar for both.

Other species which have been observed to have a changed growth rate in saline water include catfish. Lewis (1972) demonstrated that by adding salt artificially to inland ponds an increase in the growth rate of channel catfish, Ictalurus punctatus, was observed together with a better food conversion than was obtained with similar fresh water catfish.

In most instances cited above, neither water temperature nor absolute food consumption were strictly controlled and the best conclusion that can be drawn is that most salmonids can be cultured successfully in sea water and they will grow as fast as, or faster than, fish cultured in fresh water. The cause of faster growth in sea water might be salinity per se, or more favourable temperatures or an increase in the food consumed.

Several attempts have been made by research workers to show that salinity does affect the growth of fish under controlled experimental conditions. Canagaratnam (1959), in an experiment with salmon fry, O. kisutch, and goldfish, Carassius auratus, rigidly controlled water

temperature and food intake and measured the growth rate of the fish in different salinities. His results show that for the coho salmon growth was fastest at the highest salinity tested which was 18 p.p.t. Goldfish did not show similar results. This might point to a species difference in the extent of the effect of salinity on growth. Kepshire and McNeil (1972) demonstrated that salinity affected the growth rate of young chinook salmon, O. tschawytscha. Fish held in water of 0, 17 and 18 p.p.t. salinity grew at a faster rate than fish grown in 24, 25 and 28 p.p.t. Bullivant (1960) showed that young quinnat salmon, O. tschawytscha, grew faster in 50% sea water than in fresh water but not significantly so, while those fish held in full strength sea water were significantly slower to grow. He concluded from this that there was little difference in the physiological states of fish held in fresh or in 50% sea water. Saunders and Henderson (1969a, b, c) conducted a series of experiments on the survival and growth of Atlantic salmon (S. salar) fry, parr and smolt in waters of different salinities. They were able to show a definite influence of salinity on growth rates of fry and smolt. Salmon alevins grew faster in sea water of 6 p.p.t.salinity than in either fresh water or sea water of 12 p.p.t. salinity. Salmon smolt and post-smolt grew faster in fresh and brackish waters (7, 15 and 22 p.p.t.) than in sea water (30 p.p.t) over one to two years. Otto (1971) found that pre-smolt coho salmon, O. kisutch grew marginally better in 5 and 10 p.p.t. sea water than in either fresh water or 15 p.p.t. sea water.

Thus, there is a substantial amount of evidence which indicates that salinity has a direct effect on growth rate of salmonids. The optimum salinity appears to differ depending on the experimental

conditions and the species. Generally speaking it is the middle salinities which favour growth the most.

Examples of species other than salmonids which experience changed growth rates in sea water include the following. Kinne (1960) found that the growth rate of the desert pupfish, Cyprinodon macularius increased with increasing salinity up to 35 p.p.t. mainly due to increasing food consumption. Gibson and Hirst (1955) experimenting on guppies, Poecilia reticulata, found that the growth rates were faster in both 25% and 50% sea water than in fresh water over the entire thermal range of the fish. Vallet and co-workers (Vallet, Berhaut, Leray, Bonnet & Pic, 1970) showed a marked influence of salinity on the growth and food conversion of Mugil auratus and M. capito. They concluded that salinity affected the conversion efficiency to produce an optimum growth rate in 20 p.p.t. salinity. Rawson (1946) found that whitefish, Coregonus clupeaformis, grew faster in sea water of 15 p.p.t. salinity than in fresh water. Canagaratnam (1966) showed that the growth rate of Sarotherodon (=Tilapia) mossambica was faster in all water salinities than in fresh water with the fastest growth occurring in 50% sea water. Apparent contradiction of these results was reported by Chervinsky (1961) who found no significant differences in the growth rate of Sarotherodon nilotica in fresh water and various concentrations of sea water. Although a species difference might be evident in this case it is more likely that poor experimental conditions in the latter experiment prejudiced the results.

The effect of salinity on growth rate is a topic still open to research especially concerning members of the genus Salmo. Contradictions in results still arise, the most notable being the recent work of Shaw,

Saunders and Hall (1975) who found no difference in growth rates between Atlantic salmon parr grown in salinities of 0, 10 and 20 p.p.t.

The effects of salinity on a parameter such as growth cannot be examined in isolation from other modifying factors which are of a biotic or an abiotic nature. Biotic factors which may modify the growth response to salinity include size of fish, whether parr or smolt, and physiological conditions. Abiotic factors might include the level of salinity, the temperature, photoperiod and season. Thus, account must be taken of all these factors and their possible influence when investigating salinity effects on growth. The following section reviews some of the effects of salinity on the general physiology of the salmonids together with the modifying influences of some of the factors listed above.

Section 1.2

Salmonids may be subjected to changes in environmental salinity either artificially, in an experimental situation, or naturally, during migration. Here it will be apposite to review some of the physiological adjustments which take place in fish subjected to a changing or changed salinity together with some of the factors which modify these changes.

Osmotic and Ionic Regulation

Osmotic and ionic regulation in fishes has been extensively reviewed by Parry (1966), Black (1951b) and Krogh (1965). Fish rarely exist in a medium where the osmotic pressure of the body fluids of the fish is equal to the osmotic pressure of the surrounding medium. A fresh water teleost exists in a medium which has a lower osmotic pressure than that of the blood and tissue fluids of the fish. As a result of osmosis, there is a tendency for water to enter continuously the body of the fish through its permeable surfaces. Also, body salts will tend to leach into the surrounding water. Fresh water fish adapt to this situation by excreting a very dilute urine in large volumes. For example, resorption of sodium and chloride ions in the distal tubules of the kidney results in the production of hypo-osmotic urine which in the rainbow trout has a chloride concentration of only 5 - 12 mM/l (Holmes, 1961) compared with the plasma chloride concentration of 120 - 140 mM/l (Houston, 1959a; Parry, 1960). Numerous glomeruli in the kidney and a high glomerular filtration rate result in copious urine. Holmes (1961) has given a figure of 75 - 110 ml/kg/day as the rate of urine production in rainbow trout; this figure has been confirmed by Holmes and Stainer (1966).

To compensate for the loss of salts which inevitably must occur in fresh water, salts can be absorbed from the food in the gut and can be actively absorbed by the gills. In the goldfish, Carassius auratus, sodium can be taken up in exchange for ammonia ions and similarly chloride ions for bicarbonate ions (Potts, 1968).

The problems are reversed for fish living in sea water, a hyper-osmotic medium. The relatively low osmotic pressure of the blood and body fluids in marine teleosts mean an obligatory water loss from the fish through both the permeable body surfaces and the urine. This water loss is restored by swallowing the medium and eliminating the divalent ions renally and the monovalent ions extrarenally (Smith, 1930). The physiological activity necessary to maintain the balance of water and ions in these conditions is minimized by limiting the permeability of the integument of the fish and by the resorption of water in the kidney tubules. Hence, the rate of urine flow in marine teleosts is low. Rainbow trout can resorb 99% of the water in the glomerular filtrate (Holmes, 1961) and a reduction in the glomerular filtration rate produces a urine iso- or hypo- tonic to the blood. Interestingly, Stanley and Fleming (1964) showed that Fundulus kansae produced urine remarkably hypertonic to the blood, although hypotonic to sea water during adaptation to sea water.

Drinking rates of rainbow trout in one-third-,half-, and full-strength sea water have been shown to be 42, 95 and 129 ml/kg body wt. per day respectively and 66 - 80% of these loads were absorbed from the intestine (Shehadeh & Gordon, 1969). They also found that during passage through the intestine, ingested sea water was gradually depleted of monovalent ions and calcium and enriched in magnesium and sulphate.

They concluded that the intestine plays a passive role in the osmoregulatory respect of salinity tolerance in rainbow trout although it functions in ionic regulation by restricting the entry of magnesium and sulphate into the blood. Excess divalent ions will be eliminated via the kidney while monovalent ions will be excreted by an extrarenal mechanism situated in the gills.

Several workers, for example Gordon (1963) with rainbow trout and Potts, Foster and Stather (1970) with salmon, have shown that salt fluxes are much greater in sea water than in fresh water or brackish water. However, many workers assumed that salt influx in sea water took place through the gut until Motais and Maetz (1965) showed that in the flounder Platichthys stellatus, only 20% of the salt influx could be accounted for by salt swallowed. The remaining 80% must enter through the body surface.

An augmented excretion of monovalent ions is necessary in marine teleosts as only a small proportion of ingested salt can be excreted by the kidney (Parry, 1966). The locus for salt exchange was first restricted to the gill region (Keys, 1931) and then more specifically to the acidophilic cells, the 'chloride cells', in the gill epithelium at the bases of the lamellae (Keys & Willmer, 1932). The presence of these cells has been confirmed in most salmonid species (Huntsman & Hoar, 1939; Parry, 1958; Houston, 1959a) and their numbers have been reported to increase around the time of seaward migration, (Parry, 1958; Kashiwagi & Sato, 1969) and also after long term existence in sea water (Vickers, 1961; Shirai & Utida, 1970). Also, there is evidence that active handling of sodium or chloride ions occurs in the 'chloride cells' as shown by the abundance of energy-providing mitochondria

within the cells and the presence of rate-limiting enzymes related to sodium or chloride transporting systems (Maetz, 1971).

Thus, there is a large body of information on the physiological adaptations of teleosts to fresh water and sea water environments. These involve several effector organs; the gill, the gut and the kidney. Salmo gairdneri possesses a glomerular, euryhaline kidney with long tubules from glomerulus to collecting duct and large numbers of glomeruli. It can switch from a fresh water 'programme' to a sea water 'programme' by way of changes involving glomerular filtration rates, tubule absorption rates (Holmes & McBean, 1963) and 'chloride cell' adjustments. This switch is far from instantaneous as physiological adjustments and synthesis or destruction of functional cell sites takes time. However, the fact that the steelhead trout, S. gairdneri, is migratory and the rainbow trout is well able to exist in sea water is clear evidence for this adaptation.

Aquatic organisms exist in a dynamic state having a constant interchange of ions and water with the external medium. As previously described, fish must employ a variety of means to maintain their osmotic and ionic integrity. Many of these processes are energy-requiring (Potts, 1954). The amount of energy required for osmotic and ionic regulation at any given temperature depends upon a) the permeability of the interface, and b) the difference between the internal and external concentration of osmotically active particles (Nordlie & Leffler, 1975). Theorizing from here it may be said that if the gradient between internal and external osmotic concentrations changes, as when a fish moves from one salinity to another, the energy expended on maintaining the osmotic and ionic balance will alter, the degree of change

being dependent on any concomitant changes in integumentary permeability. As the costs of osmoregulation are a significant proportion of the total metabolic expenditure (20 - 27%) in the rainbow trout (Rao, 1968, 1971) it may happen that an alteration in this component will result in a decrease or increase in the total metabolic expenditure of the fish. Should a change in metabolic expenditure occur then possibly there will be a change in the energy available for deposition as growth because the proportion of the total energy used for growth is the complement of that dissipated in metabolism (Beamish & Dickie, 1967). This is the hypothesis which was tested in the first experiments of the present series.

The Tolerance of Salmonids to Salt Water and Salinity Change

Almost all salmonids are migratory at some time in their life history. Some members of the group undergo migration between fresh and salt waters while others are restricted to short migrations within the river or lake in which they live. Those salmonids which migrate from fresh water to the sea in their early life and back again to spawn, the anadromous salmonids, appear to fall into two categories, according to Houston (1961), depending on their ability to osmoregulate in different salinities at a particular age. Houston's estimates of adaptive ability were based on the time required for his fish to reduce plasma and tissue chloride levels which had risen, following sea water transfer. This index of adaptive capability stems from the fact that the inability to survive in the marine habitat may be associated with the inability to regulate either the composition or the volume of the internal body fluids or both.

Those salmonids belonging to Category 1 include O. kisutch, S. salar, S. gairdneri, the steelhead trout, and S. trutta and are generally anadromous, smolting fish. Those belonging to Category 2 include O. keta, O. nerka and O. gorbuscha which are generally anadromous, non-smolting salmonids. Fish belonging to Category 1 are unable to tolerate full sea water as underyearlings but are able to adapt to the new environment at later stages (Huntsman & Hoar, 1939; Conte & Wagner, 1965). For example, Parry (1958) found that the salinity tolerance of S. salar develops gradually during the presmolt life of the fish. Black (1951a) found that both chum, O. keta, and coho salmon, O. kisutch, fry could survive direct transfer into half-strength sea water but only chum

salmon fry could survive transfer into full strength sea water.

The development of the ability to regulate in sea water is correlated, for those species in Category 1, with the phenomenon of parr-smolt transformation. The process of smolting includes profound changes in morphology, physiology and behaviour and usually precedes seaward migration (Vanstone & Markert, 1968; Fessler & Wagner, 1969; Baggerman, 1960b; Hoar, 1965). These changes transform the cryptic coloured bottom-dwelling parr into a silvery pelagic smolt adapted to the marine environment. In steelhead trout the parr-smolt transformation is markedly size-dependent and seasonal in occurrence (Wagner, 1968). Such observations have led to Houston (1959a, 1960) suggesting that parr-smolt transformation is a premigratory adaptation to the osmoregulatory requirements of marine life.

Fish belonging to Category 2 display the ability of surviving direct transfer into sea water at the fry stage. Hoar (1958) has suggested that these species represent an evolutionary trend towards the abbreviation of the fresh water period with the loss of parr-smolt transformation.

The movement of juvenile salmonids from fresh to salt water is followed by transient, but marked disturbances of the water-electrolyte balance (Black, 1951a; Kubo, 1953; Gordon, 1959; Houston, 1959a; 1960; Parry, 1960, 1961; Conte & Wagner, 1965; Miles & Smith, 1968; Leduc , 1972). The ability of salmonids to survive sea water transfer is dependent on their ability to restrict changes in the water-electrolyte balance and to restore this balance to 'normal'. In general, salmonids respond to changes in their osmotic and ionic environment by regulating to compensate for movements of water and solutes caused by external

changes.

Houston (1959a) has divided the adaptation period into two phases. Following sea water transfer there is an initial 'adjustive' phase which is characterised by the marked departure of blood electrolytes and water from levels found in fresh water fish. In the steelhead trout this phase lasts for 3 - 7 days after fish have been transferred to full sea water (Houston, 1959a). Secondly, there is a 'regulative' phase when the composition of the internal body fluids is maintained at approximately the same level as is found in fresh water fish by osmoregulatory mechanisms. Both the adjustive and regulative phases of sea water adaptation are strongly linked with the active and passive methods of osmoregulation mentioned below. It must be realized that all fish used in the following experiments were in the regulative phase of sea water adaptation having been given ample time to adjust to the sea water medium.

One of the most important features in the adaptation of fish to sea water is their ability to alter the permeability of the gills and other permeable parts of the integument to water. This was suggested by Gordon (1963) and supported by work of Shehadeh and Gordon (1969). Alteration in surface permeability is one of the means cited by Houston (1964) where fish adapt passively to salinity change during the initial adjustive phase of sea water adaptation. Other passive responses include the redistribution of tissue water and the uptake of inorganic ions by soft tissue and bone. All these processes act as a buffer during the adjustive phase to restrict the rates and magnitudes of changes in electrolytes and body fluid composition. These processes require little or no energy expenditure and so provide an interval during which the

development of the specialised energy-consuming regulatory mechanisms may occur. These energy-consuming regulatory activities include changes in extrarenal salt excretion and kidney function. For instance, the number of 'chloride cells' believed to be involved in active handling of sodium and chloride ions (Maetz, 1971) increases during and after sea water adaptation (Kashiwagi & Sato, 1969; Shirai & Utida, 1970). Alterations in kidney function after sea water transfer include rapid decreases in the glomerular filtration rates and increases in water resorption and divalent ion secretion by the renal tubules (Parry, 1966).

Successful adaptation to sea water is dependent upon several factors; size of the fish, the species of fish, often the time of year, the value of the salinity, the time over which the salinity change is spread, and possibly the water temperature.

With regard to species difference in sea water adaptation, Parry (1960) investigated the rise in blood concentration of three species of salmonid after sea water transfer and found that the salinity tolerance decreased in the following order:

S. salar > S. gairdneri > S. trutta

When acclimated to sea water, the salt content of their blood rose sufficiently to raise the freezing point of the blood by only 10% over that found with fresh water fish. This indicates excellent osmotic regulation in all three species.

If it is intended that fish should be reared in high salinities it is often advisable to acclimate the fish to a low salinity and then to gradually raise the salinity to the desired level. Wagner, Conte and Fessler (1969) found chinook salmon, O. tschawytscha, survived sea

water transfer better if tested by gradual transition through intermediate salinities to full sea water. Saunders and Henderson (1969b) found that acclimation of young S. salar to low salinities (7 ppt) increased their ultimate survival time in higher salinities (15 ppt). Jones (1947) found smolts of S. salar could not withstand sudden sea water transfer but could tolerate a gradual transition over a period of 10 hours. Other workers have found that gradual transition increases survival in full strength sea water (Huntsman & Hoar, 1939). Wagner et al. (1969) suggested that gradual sea water transition stimulates the existing extrarenal system towards its full capacity. As the salt load is increased slowly through gradual sea water transfer the regulatory mechanisms have time to become fully compensatory.

Repeatedly it has been demonstrated that survival in sea water is size dependent i.e. the larger the fish, the better its ability to hypo-osmoregulate. This is probably caused by the reduction in the surface to volume ratio with increasing body size particularly in the gill area. Also it has been shown that it is size and not age or sexual maturity which governs salinity tolerance (Huntsman & Hoar, 1939; Parry, 1958, 1961; Koroleva, 1960; Conte et al, 1965; Saunders & Henderson, 1969a). Parr-smolt transformation and sea water tolerance are distinct and unrelated processes as was shown by Conte et al. (1965, 1966) and Wagner et al. (1969). These authors showed conclusively that the capability of surviving in brackish and marine waters develops before migration in several salmonid species and is a function of fish size. This takes place before the overt expression of smoltification. The two processes are unrelated as has been shown by the acclimation to sea water of fish before the onset of parr-smolt transformation. This

has been demonstrated for O. tschawytscha (Wagner et al, 1969; Kepshire & McNeil, 1972), for cutthroat trout, Salmo clarki and steelhead trout, S. gairdneri (Giger, 1972).

In apparent contrast to these findings, Conte and Wagner (1965) found the steelhead trout experienced a regression of the marine osmoregulatory system starting in the summer which they associated with desmoltification during the terminal stages of the migratory period. However, in a later paper Wagner (1974b) demonstrated only a slight regression in euryhalinity during the summer with a resumption in the autumn. His conclusion was that the extent of the summer regression of euryhalinity is governed in part by the coefficient of condition of the trout. The higher this coefficient, the less marked is the summer regression. A seasonal fluctuation in sea water tolerance has also been shown by Houston (1957, 1961), Hoar (1958) and Baggerman (1960b). Houston (1961) obtained similar results to Conte and Wagner (1965) in comparing the sea water tolerance of steelhead trout and chum salmon, O. keta. He found that sea water tolerance was reduced in the steelhead after smoltification whereas the chum salmon, a non-smolting member of category 2, showed increasing sea-water tolerance with increase in size. It is possible that the influence of season on sea water tolerance will be as small for rainbow trout as chum salmon. Although the rainbow trout will adapt to sea water it does not normally smoltify while in fresh water in contrast to the migratory form of the same species, the steelhead trout. Thus the sea water tolerance of rainbow trout may be unaffected by season (Landless, 1976b).

Finally, temperature may have some importance in the tolerance of salmonids to salt waters. Gordon (1959) found an

indefinite survival of brown trout, S. trutta in sea water if they were gradually transferred at rates inversely related to the temperature. This view is supported by Adams, Zaugg and McLain (1973) who found that parr-smolt transformation and sea water tolerance of steelhead trout were inhibited at water temperatures of 15 and 20°C.

To summarize some of the factors involved in the tolerance of young salmonids to sea water it can be said that the mechanism for hypoosmoregulation is present to the degree that young fish can survive an immediate transition from fresh to sea water before parr-smolt transformation. This early and increasing tolerance of sea water, which is facilitated by growth, can be explained by the development of osmoregulatory mechanisms which are passively self-restrictive (Houston, 1964). These passive elements of adaptation appear to be unaffected by the season or the photoperiod (Wagner, 1974b). By contrast Zaugg and McLain (1972), Adams et al. (1973) and Zaugg and Wagner (1973) present evidence that Na^+- and K^+- activated ATPase in gill microsomes during smolting appears to be sensitive to temperature and photoperiod. Possibly this enzyme system is involved in the active transport of ions across the gill membrane and if so, might be the basis of the active regulating mechanism in sea water. Hence, the gradual acclimation of small trout to increments in salinity may serve to stimulate the physiological processes necessary for full sea water tolerance so that there is less immediate dependency on the size-related passive mechanisms.

1.3. The Aims of the Research

In the previous sections the effects of salinity on the general physiology and growth rate of salmonid fish have been reviewed. The evidence relating to the effects of salinity on the growth rates of members of the genus *Salmo* is a little conflicting (cf. Saunders *et al.*, 1969a, b, c; Shaw *et al.*, 1975) and it was decided that an investigation into the effects of salinity on the growth of the rainbow trout, *Salmo gairdneri* would be most valuable. The rainbow trout was chosen as the experimental animal because of both its general importance as a cultured fish and the increasing practice of culturing this fish in sea water. In addition to growth, it was decided to investigate as many associated parameters as possible, e.g. growth rates, appetite, metabolic rates, specific dynamic action of food fed, and protein turnover, in an attempt to construct an overall picture of the effects of different salinities on the metabolism of the trout. Consequently, the following experiments were devised and undertaken.

In Experiment 1 (Chapter 3) an endeavour was made to quantify the effects of different salinities on the voluntary food intake, or appetite of trout held in fresh water, 10, 20 and 28 ppt sea water. This was accomplished by using an automatic demand feeding system.

An altered environmental salinity has been shown to affect the metabolic rate of rainbow trout (Rao, 1968, 1971). Hence, it was considered that much value could be derived from a study which combined growth and metabolic rate investigations. Experiment 2 (Chapter 4) was undertaken to perform such an investigation on trout held in fresh water, 10, 20 and 28 ppt sea water.

Following the results of Experiment 2 a series of feeding trials was undertaken in Experiment 3 (Chapter 5) to investigate the growth of trout at six different ration levels in fresh water, 10 and 20 ppt sea water. From the results it was anticipated that the effect of salinity on the maintenance and optimum ration levels would be determined. A correlation between maintenance ration levels and total metabolic rates from the previous experiment, would be valuable.

In response to the results obtained in the previous two experiments Experiment 4 (Chapter 6) attempted to determine the metabolic cost of specific dynamic action of food fed to trout in fresh water, 10 and 20 ppt sea water. It was hoped from this that possible effects of salinity on the specific dynamic action of food fed would support the theory that the proportion of basic substrates metabolized was changed in different salinities.

Finally the effect of salinity on the protein requirements of trout held in fresh water, 10 and 20 ppt sea water was investigated in Experiment 5 (Chapter 7). Nitrogen rather than energy turnover was examined in this experiment as protein synthesis is the most characteristic feature of growth and it was anticipated that a comparison of the maintenance and optimum protein levels between different salinities would be valuable.

CHAPTER 2. MATERIALS AND GENERAL METHODS

The following chapter describes the various experimental systems which were used together with the general conditions of the experiments.

2.1. The Recycling System

Two water recycling systems were designed and built in order to achieve two basic requirements for experiments. The first was the maintenance of constant environmental conditions, in particular temperature, dissolved oxygen and salinity. The second was the holding of healthy stocks of fish. The correct use of recycling systems enables these two conditions to be fulfilled in that deleterious changes in water quality, caused by the fish, were rectified. If fish are held in a restricted volume of water, changes in water quality occur which must be corrected. These changes include increases in the concentrations of dissolved ammonia, dissolved organic material, solid faecal matter, dissolved carbon dioxide and a decrease in the dissolved oxygen level of the water.

To these ends, two independent water recirculation systems were constructed, one to hold fresh water and the other to hold sea water, the value of the salinity being dependent on the experimental requirements. As far as possible the two systems were constructed to be identical so that fish held in the two systems would be subjected to similar environmental conditions except for salinity. In this way it was expected that direct comparisons of growth and metabolism could be made between fish held in fresh and sea waters.

Each of the two systems consisted of a number of fish holding tanks, a single tank in Experiment 1 and six tanks in Experiments 2,

3, 4 and 5. The system used in Experiment 1 will be described and reference should be made to Figure 1. In each of the two systems water was pumped from the filter to a header tank by means of a Beresford PV 21 water pump capable of delivering a flow of 530 g.p.h at a head of 10 feet. From the header tank water was returned to the fish-holding tank. Water entered the circular fish-holding tank at the perimeter and was directed at the surface of the water in a tangential manner. This created a circular flow of water within the tank. This, together with a centrally placed drain, ensured both an efficient changeover of water in the tank and clearance of faeces from the bottom of the tank. Water and waste left the fish-holding tank via a constant-level pipe, through a faecal trap and then into the filter tank. Each filter tank was an open-topped box of about 60 gallons capacity, constructed of marine plywood coated with varnish and epoxy resin. Inside each filter was a horizontal, corrugated plastic sheet supported four inches from the bottom of the tank and bearing five cubic feet of $\frac{1}{4}$ inch gravel. The plastic sheet had slots cut into it at one inch intervals along the troughs of the corrugations. Water entered the filter at the water surface level and was drawn down through the gravel by the suction of the water pump which was drawing water from under the corrugated plastic sheet. As the water passed through the gravel, suspended solids were removed by mechanical action while ammonia, the principal nitrogenous excretory product of the fish, was oxidized by the action of the nitrifying bacterial flora growing on the surface of the gravel particles.

The water was pumped up a distance of eight feet into the header tank where it was sprayed down on to the surface of the water con-

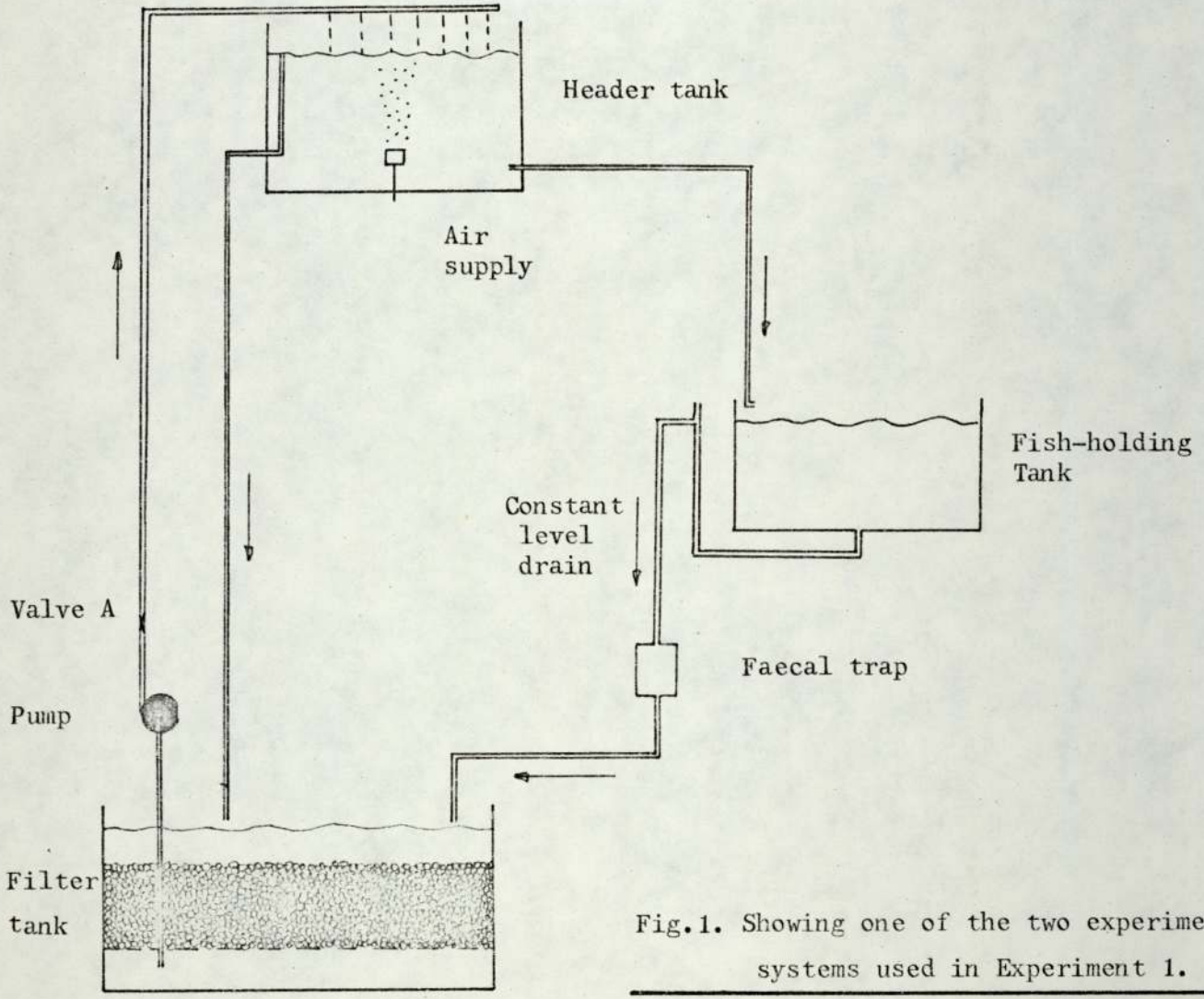


Fig.1. Showing one of the two experimental systems used in Experiment 1.

tained in the tank. This action together with an auxiliary air supply situated in the header tank effected the reoxygenation of the water depleted by the fish. The head of water was maintained at a constant level by a stand-pipe inside the header tank. Excess water in the header tank was returned to the filter tank by way of this stand-pipe.

Water at a constant head passed down a delivery pipe by gravity and was directed to the circular fish holding tank. The water flow rate from the pump was regulated by valve A (Figure 1) situated downstream of the pump and was adjusted so that the delivery was just in excess of that removed from the header tank to the fish tank. This excess was returned to the filter tank via the stand-pipe and this method compensated for any irregularities in delivery rate from the water pump. The total capacity of one system was 120 gallons.

A variety of construction materials was used in the recirculation systems. The header tanks and fish-holding tanks were supported with 'Bartangle' steel frames. All fish tanks were constructed from polypropylene, and the header tanks were of fibre-glass. All pipework was polyvynylchloride (PVC), $\frac{3}{4}$" nominal diameter, brand names 'Durapipe' and 'Bartol'. In all cases non-toxic materials were used for constructional purposes.

The two systems used in Experiment 1 (Figure 1) were modified and improved for later experiments to include more fish-holding tanks and temperature control facilities. On examining Figure 2 it can be seen that the layout of header tank, pump and filter has remained constant but the number of circular fish-holding tanks has increased to six tanks in each system. The size of the fish tanks was reduced to 2 feet in diameter in the later experiments compared

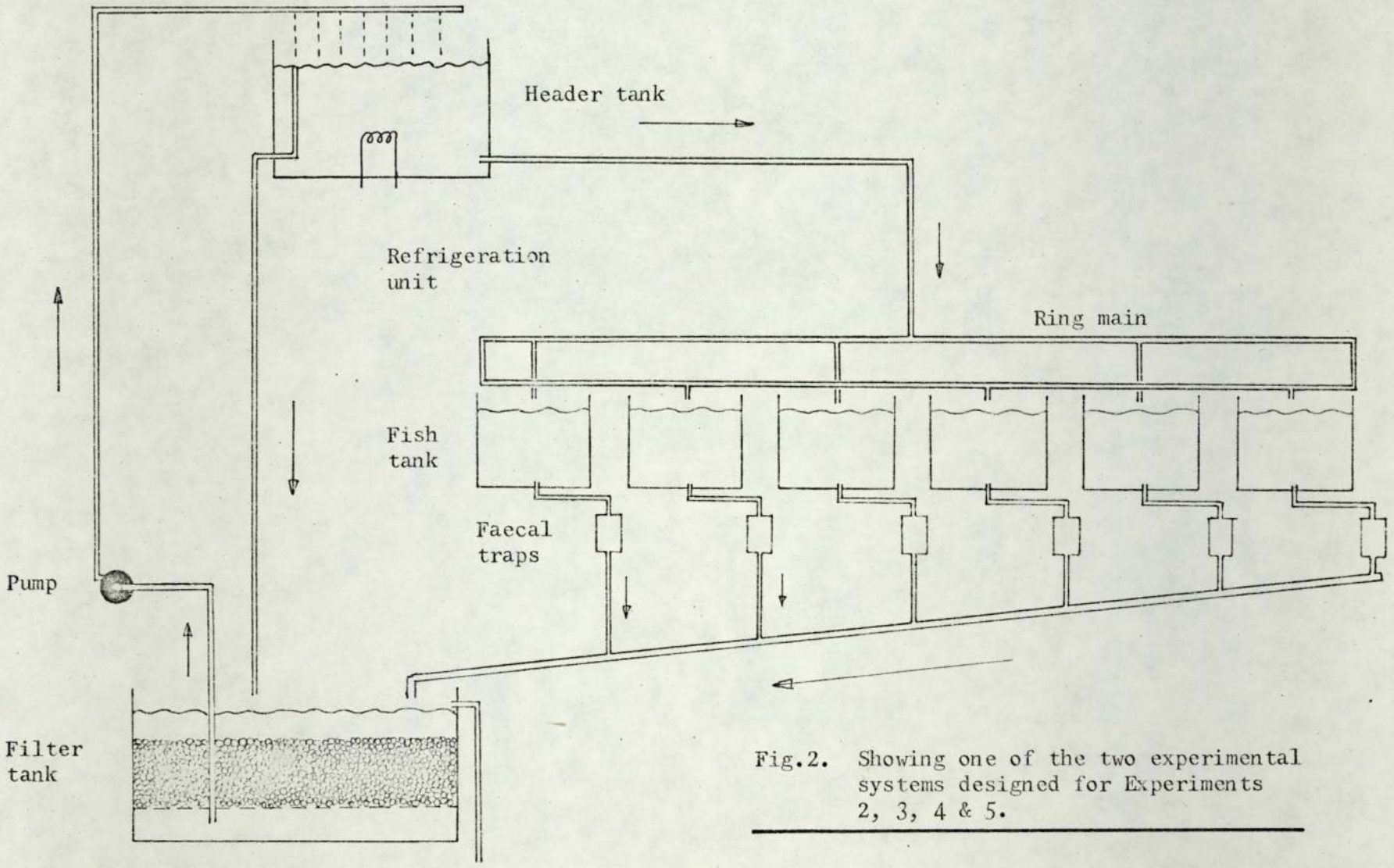


Fig.2. Showing one of the two experimental systems designed for Experiments 2, 3, 4 & 5.

to 3 feet in diameter in Experiment 1. Examination of Plates 1 and 2 will clarify the improvements. Other alterations in design include a ring-main which supplied a constant pressure of water to each fish-holding tank and also temperature control facilities. This equipment comprised a refrigeration unit which consisted of thermostatically controlled cooling coils, one in each header tank, linked to an air-cooled compressor unit sited at an adjacent window. Minor corrosion of the cooling coils necessitated further modifications of the system. This involved isolating the cooling coils from the circulating water in each system. In Figure 3 it can be seen that the cooling coil was immersed in a large tank of water through which a proportion of the circulating water passed by means of glass tubing. This tubing was $\frac{1}{2}$ inch in diameter and 60 feet of it was arranged in the cooling tank in a single helix.

All dimensions of experimental tanks are listed in Table 1.

Water Supplementation

In theory it is possible to operate a recycling system without any supplementary water except that required to replace the loss by evaporation. However, users of recirculating systems recommend that supplemental water should be added to the system at a minimum rate of 2% of the total recirculation rate, and Burrows & Combs (1968) maintain that a 5% water supplementation rate is desirable for, at a rate lower than 2% the water becomes viscous and discoloured and tends to foam when agitated. Also, it is necessary to minimize the levels of nitrate (NO_3 - N) and nitrite (NO_2 - N) compounds produced by the nitrifying bacteria in the filter bed. Nitrite is the more toxic of the two compounds as exposure of trout

Plate 1 The arrangement of the two-foot diameter tanks used in Experiments 2, 3, 4 and 5. There are two tiers of six tanks per tier. Fresh water tanks are above sea water tanks. Note the difference between the growth tanks and the growth/metabolism tanks, the latter being equipped with sealable lids with perspex viewing windows.


growth tank
growth / metab tank

Plate 2 The disposition of water inlet and double standpipe drain within one two-foot fish tank. Note the fish swimming against the circular flow of water.


F. 3.
S. 3.

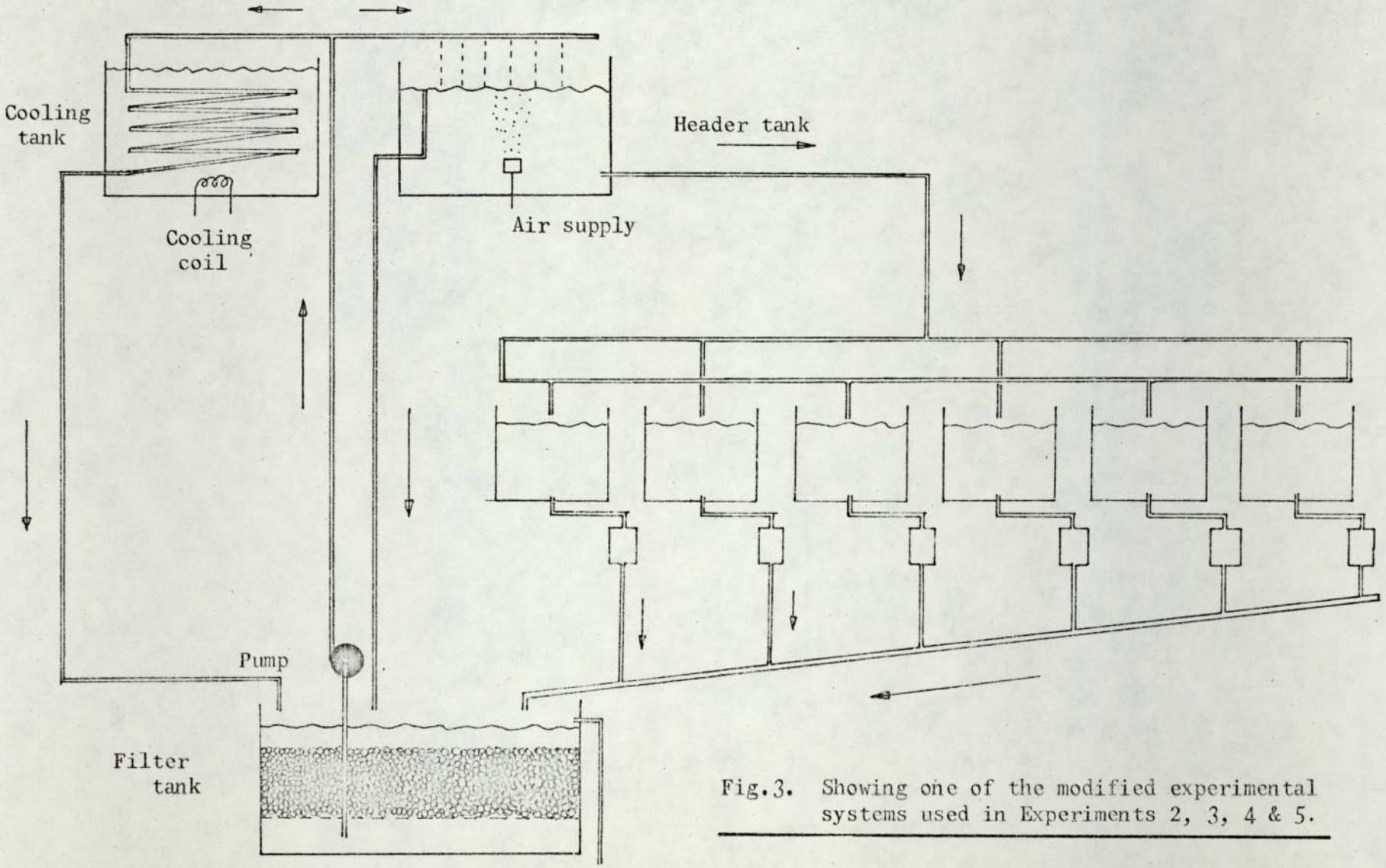


Fig.3. Showing one of the modified experimental systems used in Experiments 2, 3, 4 & 5.

TABLE 1. SPECIFICATIONS OF EXPERIMENTAL TANKS

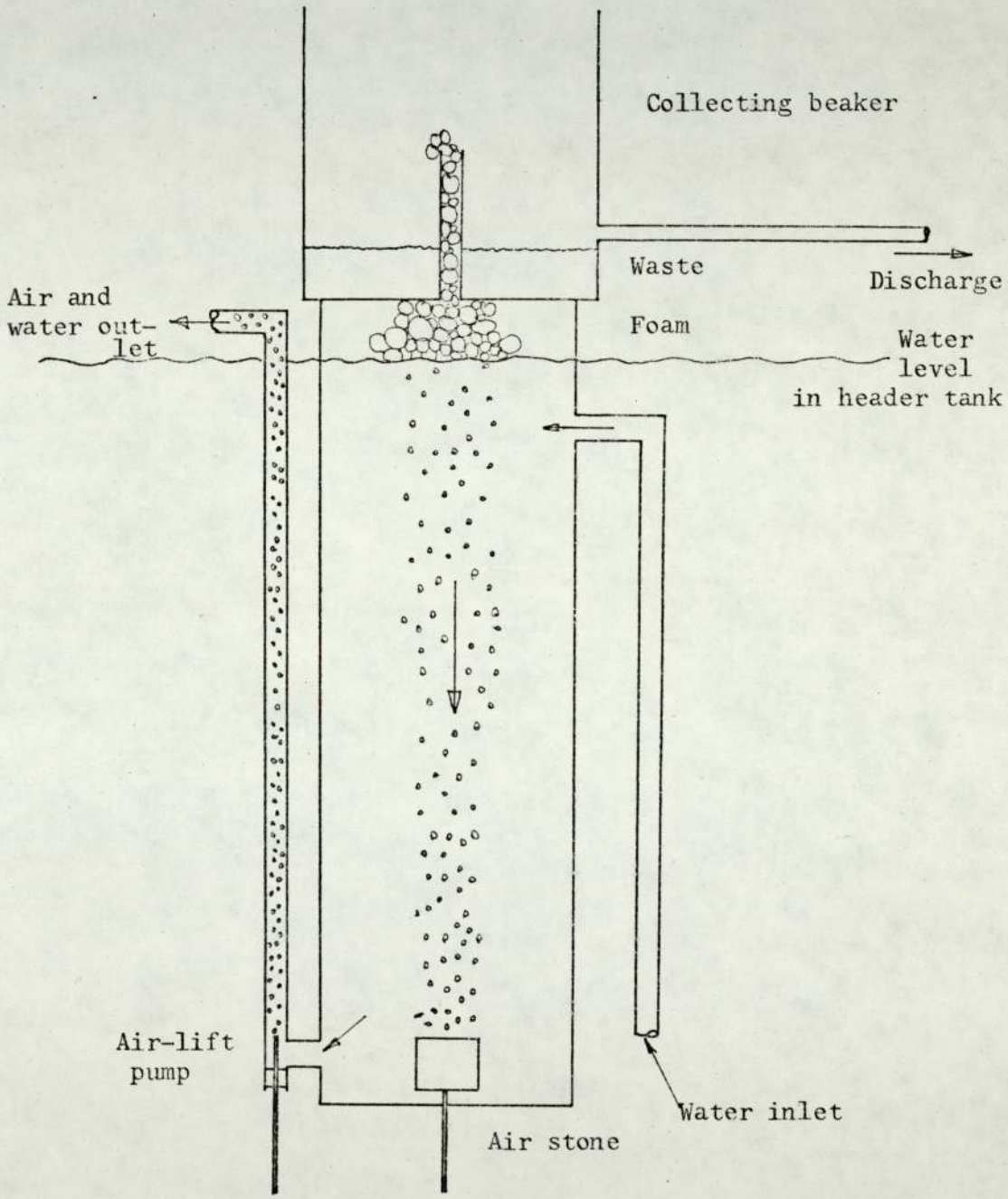
Tank	Dimensions cm.	Capacity - Full	Capacity - working
Experiment 1			
Filter	81 x 81 x 50	72.29 gal. (330 l.)	25 gal. (114 l.)
Three-foot dia. fish tank	91 dia x 38 h	55 gal. (250 l.)	45 gal. (200 l.)
Header tank (Exp.1)	81 x 71 x 61	77.3 gal. (350 l)	50 gal. (227 l.)
Faecal trap	25 dia x 20 h	2.16 gal. (9.8 l.)	2 gal. (9.1 l.)
	Total Capacity =		122 gal. (555 l).
Experiments 2, 3, 4 and 5			
Filter	as before	as before	25 gal. (114 l.)
Two-foot dia. fish tank	57 dia x 40 h	18 gal. (81.8 l.)	15 gal. (68 l.) x 6
Header tank	55 dia x 60 h	30 26 gal. (137.5 l)	28.8 gal. (131 l.)
Faecal traps	12 dia x 21 h	0.55 gal (2.5 l.)	0.44 gal (2 l.) x 6
	Total Capacity =		146.44 gal. (665.7 l.)

to nitrite results in an oxidation of haemoglobin to methaemoglobin (Smith & Williams, 1974). This compound cannot transport oxygen and fish may die from anoxia. Russo, Smith & Thurston (1974) showed that for trout the 96-hour LC50 (median lethal concentration) values ranged from 0.19 - 0.39 mg/litre NO_2 - N. However, trout are much more tolerant of nitrate. Jones (1964) reported that fish will tolerate as much as 800 p.p.m. of calcium nitrate.

In all the experiments water was replaced with supplementary water as frequently as possible and it was found that replacing 25 gallons of water each day in each system prevented undue discolouration and foaming, provided an air-stripping device was used. The use of airstrippers is discussed below. Replacing water in the volumes mentioned above resulted in a rate of approximately 1% in Experiment 1 and approximately 0.5% in the other experiments. In order to standardize routine procedure supplementary fresh water was added to the fresh water system in the same volumes as sea water was added to the sea water system.

Slight discolouration and foaming occurred in the sea water system. This was caused by dissolved surface-active organic material aggregating at the surface of the water and forming a foamy scum. This phenomenon was utilized in a method for removing most of this dissolved organic material which involved an 'airstripper'. These devices have been described by Sander (1967) and are in common use among amateur aquarists. Consequently an adapted version of a counter-current airstripper was constructed in glass and was installed in the seawater header tank. The method of operation was straightforward (Figure 4). Three-quarters of the

Fig.4. Diagram of the 'Airstripper' used in conjunction with the sea water system



Dimensions 68 cm high x 7 cm dia.

length of the airstripper was immersed in the sea water and a current of water through the tube was induced by the action of the air-lift pump. The direction of the water current is shown by arrows on the diagram. In the main body of the airstripper the water current passed down against an upward flow of bubbles from the air-stone. The movement of these bubbles resulted in strong agitation in the column of water causing the dissolved organic material to aggregate and foam on the surface of the water inside the column. When the foam reached the level of the bottom of the collecting beaker it was forced up the stand-pipe where it accumulated inside the collecting beaker. Here it condensed into a viscous liquid which flowed to waste along the discharge pipe. The rate of waste discharge from the airstripper was approximately 1.5 litres/hr. It was found that the use of such a device considerably reduced the discolouration and foaming in the sea water system.

The Biological Filter

When aquatic animals are held for any length of time in a constant volume of water, the water will undergo a progressive deterioration in its ability to support life unless corrective measures are taken. These measures may vary depending on the type and magnitude of the changes in water quality which occur. In this case, the first limiting change which takes place is the depletion of oxygen by the metabolism of the fish. This was corrected by aeration and agitation of the water.

The second limiting change is the production of ammonia by fish which is their primary means of nitrogen elimination. Unless steps are taken, potentially toxic levels are reached quickly. The upper

'safe' level of total ammonia for trout is given as 1 mg/l by Smith (1972) with oxygen levels in excess of 7 mg/l. As the dissolved oxygen level of the water falls, so the threshold of ammonia toxicity falls (Larmoyeax & Piper, 1973). On the basis of the combined results of Liao & Mayo (1974) and results from the Bozeman Fish Cultural Development Centre, U.S.A, an ammonia level of 0.5 mg/l has been considered the permissible upper level for extended exposure given a pH of 7.7 - 7.8 at a water temperature of $10^{o}C$. It must be borne in mind when studying figures of threshold levels that the toxicity of an aqueous ammonium solution depends mostly on the pH value of the water since only the unionized component(NH_3)is toxic to fish (Downing & Merkins, 1955; Lloyd, 1961). Toxicity is also affected to a lesser extent by dissolved oxygen and temperature.

Several methods are employed, both on the laboratory and commercial scales, to keep total ammonia levels within tolerable limits. These include air-stripping, biological filtration and the use of ion-exchange resins. The most practical method for the current research was the use of a biological filter. This method involves the passage of ammonia laden water through a vessel which usually contains a solid substrate such as gravel, sand or plastic media. The function of the substrate is to provide a large surface area on which the nitrifying bacteria may grow. Such filters may be of the trickling variety or the submerged variety, the latter sub-dividing into the up-flow and the down-flow type. The filters used here were of the sub-merged down-flow type and they were built to be as large as possible in the space available. The dimensions of the filters have been given in Table 1 and their construction has been outlined earlier.

Water which had been used in the fish tanks was made to pass through the filter under the action of the water pump. The speed with which water passes through a gravel bed is important. If too fast, all the ammonia will not be oxidized. If too slow, there is a danger that the water will lose too much oxygen part-way through the bed and the bottom portion of the bed may become anaerobic. In the present case, the water was drawn through as slowly as possible being equal in flow to the summed flow rates to each fish tank plus the overflow from the header tank. This rate was approximately 18 l/min. at the pump.

Dissolved oxygen levels were determined, using Winkler's method, for water before and after passing through each filter to check that anaerobic conditions were not approached. It was found that approximately 10 - 15% of the oxygen was lost in passing through each filter. Provided that the water flowing into the filter had a minimum dissolved oxygen level of 6 mg/l no hazard was foreseen. Liao & Mayo (1974) state that the minimum dissolved oxygen level under which biological oxidation of ammonia will still occur is 5 mg/l. To ensure this minimum level, well oxygenated water was discharged into each filter from the header tank overflows and this was mixed well with the ammonia laden water from the fish tanks.

Using this system enabled total ammonia levels to be maintained within acceptable limits. The maximum value recorded was 0.72 mg/l and usually values ranged between 0.4 and 0.5 mg/l (NH_3/NH_4^+).

Bacteria which grow in a filter bed are chemosynthetic autotrophs which oxidise simple inorganic compounds producing more complex carbohydrates, proteins and fats. They use the oxidation of ammonia as their energy source. Such a system also requires a source of a base to combine with the nitrate ions produced. Hence, a thin layer of crushed cockle shell was spread on the surface of the filter-bed. If absent, the production of nitrous and nitric acids would have occurred which would have resulted in a fall in the pH. The calcium carbonate reacts with the nitrate produced to stabilize the pH. Hence:

$$4NH_3 + 7\,O_2 \xrightarrow{\text{Nitrosomonas}} 4\,NO_2^- + 6\,H_2O$$

$$2\,NO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2\,NO_3^-$$

$$CaCO_3 + 2\,NO_3^- \longrightarrow Ca(NO_3)_2$$

Backflushing of the filter, as described by Burrows & Combs (1968) was not attempted. Due to the persistent growth on the surface of the filter bed of filamentous bacteria, probably Sphaerotilus sp., each filter was cleaned by removing three quarters of the gravel and washing it under tap water. Monitoring of ammonia levels in both systems was practised for several days after washing the gravel using an ammonia probe and meter. This revealed an initial rise in the concentration of total ammonia lasting 2 - 3 days which then fell to 'normal' levels. Thus, leaving some gravel undisturbed during cleaning ensured the rapid re-colonisation of the washed gravel which would undoubtedly have lost most of the bacterial flora. Kawai, Yoshida & Kimata (1965) have shown that strong washing removes 70% of the bacteria attached to their sand substrate.

2.2. General Experimental Conditions

The Sea Water

Artificial sea water was used in all experiments because of the need to maintain the constancy of composition of recirculated water on a long-term basis. In Experiment 1 sea water was made from a brand of hobbyists' sea-salt named 'Tropic Marin'. This powder concentrate has been used in other scientific investigations (Farmer & Beamish, 1969). It was not possible to obtain quantitative details of its composition from the manufacturers but it was appreciated that in addition to the major chemical constituents included in its composition, many trace elements were included in order to simulate as closely as possible the composition of natural sea water.

In Experiments 2, 3, 4 and 5 sea water was made from individual chemical components dissolved in water. The formulation of this salt mix was obtained from Spotte (1970) and is shown in Table 2. The formulation used was taken originally from another brand of hobbyists' sea-salt known as 'Instant Ocean' obtainable in the U.S.A. Reconstituting both the 'Tropic Marin' and this latter sea water formulation was carried out by dissolving a weighed amount of salts in 25 gall of tap water contained in a tank separate to the experimental systems. The mix was then aerated vigorously for 24 hours before it was added to the main body of recirculated experimental water.

The Chemistry of the Water

Important water parameters were monitored regularly during and after experiments. These environmental variables included temperature, dissolved oxygen, total ammonia, pH, salinity and total nitrite

TABLE 2. COMPOSITION OF SEA WATER SALT MIX

Dissolved in 25 gall of tap water:-

Major Elements		
Sodium chloride	$NaCl$	3129.8 g
Magnesium sulphate	$MgSO_4$	782.4 g
Magnesium chloride	$MgCl_2$	612.3 g
Potassium chloride	KCl	68.0 g
Calcium chloride	$CaCl_2$	156.4 g
Sodium bicarbonate	$NaHCO_3$	23.8 g
Minor Elements		
Strontium chloride	$SrCl_2$	2.2500 g
Manganese sulphate	$MnSO_4 . H_2O$	0.4500 g
Sodium phosphate	$NaH_2PO_4 . 7H_2O$	0.4500 g
Lithium chloride	$LiCl$	0.1125 g
Sodium molybdate	$Na_2MoO_4 . 2H_2O$	0.1125 g
Sodium thiosulphate	$Na_2S_2O_3 . 5H_2O$	0.1125 g
Trace Elements		
Potassium Iodide	KI	0.0101 g
Aluminium sulphate	$Al_2(SO_4)_3$	0.0975 g
Potassium bromide	KBr	3.0476 g
Cobalt sulphate	$CoSO_4$	0.0101 g
Rubidium chloride	$RbCl$	0.0169 g
Cupric sulphate	$CuSO_4 . 5H_2O$	0.0011 g
Zinc sulphate	$ZnSO_4 . 7H_2O$	0.0109 g

(NO_2-N) concentrations. Generally, these variables were similar in both systems with the exception of salinity and absolute dissolved oxygen levels.

Salinity in the seawater system was determined twice-weekly by using a chloride meter (Evans Electroselenium Ltd., Halstead, Essex) which had an accuracy of ±5 mEq Cl^-/l. Readings obtained were converted from mEq Cl^-/l units into salinity values in terms of g/l by using a nomogram. Normal Copenhagen sea water was used as the standard for 100% sea water, this being 34.33 g/l salinity.

Dissolved oxygen was determined daily using either the Azide modification of Winkler's method taken from the publication 'Analysis of Raw, Potable and Waste Waters'(1972) or using an oxygen meter (Simac Instrumentation Ltd., Walton-on-Thames, Surrey, Model 65) calibrated against samples checked by Winkler's method. When the meter was calibrated in this manner the accuracy was ±0.1 p.p.m. dissolved oxygen. Water samples were taken from the fish tank inlets and the dissolved oxygen levels usually varied between 92 - 94% saturation for both fresh and salt waters at all experimental temperatures.

The pH of the recycled water was measured twice-weekly using a pH meter (Pye Unicam, Cambridge, Model 291, Mark 2). Values tended to fluctuate irregularly between 7.1 - 7.5; these levels are quite acceptable for holding trout and were low enough to prevent un-ionized ammonia (NH_3) levels from attaining dangerous concentrations. For example, at a temperature of 12°C and a pH of 7.3, the percentage of total ammonia occurring in the un-ionized form would be only 0.43% of the total ammonia concentration (Trussell, 1972). Thus, a total

ammonia concentration of 0.5 mg/l in the above conditions would result in only 2.15 µg/l un-ionized ammonia. Total ammonia concentrations (NH_3/NH_4^+) were determined twice-weekly with an ammonia probe and meter (Electronic Instruments Ltd., Chertsey, Surrey, Probe Model 8002, Meter Model 7030). Average values varied from 0.4 - 0.5 mg/l and the accuracy of these determinations was approximately ±0.01 mg/l.

Nitrite (NO_2 - N) levels were measured in both systems during the course of experiments using a colorimetric test based on the Griess-Ilosvay method and it was found that levels were always negligible. Accordingly, it is probable that nitrite was being efficiently oxidised to nitrate (NO_3-N) in both systems.

Finally, temperature was measured twice-daily in both systems by means of thermometers which were positioned in the line of the outflows from the header tanks of both systems. In Experiment 1, temperature was 15 ±1°C for both systems and in all other experiments the temperature was maintained at 12±0.5°C.

It was evident during all experiments that the recycling systems were maintaining healthy stocks of fish as was evidenced by good appetites and food conversion rates. Also, the environmental conditions were well within acceptable limits for trout.

General Conditions and Methods

Rainbow trout, Salmo gairdneri Richardson, were used in all experiments and were obtained from the 'Vortex' fish farm at Donnington, Gloucestershire. Before and during all experiments, with the exception of Experiment 5, trout were fed on 'Beta' floating trout pellets, grade 4, manufactured by Cooper Nutrition Products Ltd.,

Witham, Essex. The size of the trout was always in excess of 17 cm in length to ensure rapid adaptation to sea water. This minimum length was used after Conte & Wagner (1965) who found that steelhead trout were fully euryhaline at 14 - 15 cm long and by Jensen (1966) who found that rainbow trout had to be 15 - 20 cm long before they would adapt successfully to full sea water.

Weighing of fish was undertaken in all experiments. This was carried out by one of two methods, either individual weighing or batch weighing. When fish were weighed individually they were removed from their tank and anaesthetised in a solution of MS222 (Sandoz Ltd., Basle) at a concentration of 50 mg/litre. They were removed from the anaesthetic, lightly dried on a paper towel and placed on a small rectangular pad of absorbent material situated on the pan of a Sartorius 1100 top-pan balance (model 1106). The fish and absorbent pad were then weighed with an accuracy of 0.1 g and the fish was returned to the fish tank where complete recovery ensued within three minutes. The absorbent pad was then weighed and the difference of this weight from the weight of pad and fish was taken as the live weight of that fish.

Weighing fish on a batch basis involved no anaesthetic. The difference in weight was obtained between a plastic bin containing about 8 litres of water and the bin and water containing ten fish. The fish were netted from the fish tank individually and subjected to a standard draining time of 5 sec in the net before placing in the bin of water. Bin and fish weights were determined on a triple-beam balance in Experiment 1 and on a Sauter 10 kg side-pan balance in all other experiments, both balances being accurate to 1 g. In all cases fish were starved for 12 hr prior to weighing.

The temperature of the water in both systems during Experiment 1 was $15 \pm 1^{\circ}C$. This temperature was chosen as it was the most convenient to maintain constant in the absence of constant-temperature equipment. Temperature stayed at this level because ambient air temperatures were similar. Slight cooling of the water in the systems could be achieved by the addition of relatively cold supplementary water.

The temperature of the water in both systems during Experiments 2, 3, 4 and 5 was maintained at $12 \pm 0.5^{\circ}C$ by the use of refrigeration equipment. This temperature was chosen because several authors have found temperatures of 12 - $13^{\circ}C$ to be optimum for trout growth (Swift, 1955, 1961; Baldwin, 1957; Atherton & Aitken, 1970). The changes in growth and metabolism caused by environmental salinity were anticipated as being relatively small. Therefore it was felt that such changes would be more apparent at a temperature which promoted a faster rate of growth than at one which promoted a slower rate of growth as the duration of most experiments was relatively short.

The lighting conditions were similar for both experimental systems both being illuminated by the same single six-foot fluorescent tube together with two forty-watt incandescent bulbs situated on the ceiling of the experimental room. During Experiment 1, the photoperiod was regulated by a "Venner" time switch giving 13.5 hr on and 10.5 hr off. In addition, there was also 0.75 hr 'dawn' and 'dusk' at the beginning and end of each day which was effected by linking a control box with the lighting circuit. The control box operated an automatic timer which at dawn gradually decreased the electrical resistance of part of the lighting circuit which increased the current passing so brightening the two in candescent bulbs. When these bulbs had reached the point of their

maximum brightness, the fluorescent light was switched on. At the end of the day, the reverse occurred resulting in a 0.75 hr period of 'dusk'. During Experiments 2, 3 and 5 this control box was dispensed with and the photoperiod maintained at 14 hr on, 10 hr off by a 'Venner' time switch. In this case the onset of 'day' and 'night' was abrupt. During Experiment 4, photoperiod was maintained at 12 hr on and 12 hr off.

2.3. The Demand Feeding System used in Experiment 1

Experiment 1 (Chapter 3) involves the determination of the voluntary feed intake of trout held in different salinities using a demand feeding system. Accordingly, a demand feeding system comprising two triggers and feed dispensers for each of the two fish tanks was constructed.

The specific design of the triggers, air valves, and food dispensers was conceived and developed by Smith (1976) and their operation is described below. Reference should be made to Figures 5 and 6.

After a preliminary period of learning, a fish would nudge the red tip of one of the triggers, the tip of which was just immersed in the water of the fish tank. This initiated a brief pulse of 12 volt current in the electrical circuit which was then prolonged into a pulse of 0.6 sec duration after passing through a part of the circuit designed to have this prolonging effect (see Figure 7). This 'delay' circuit is termed a monostable multivibrator and the prolongation of the short contact made by the fish into a 0.6 sec duration pulse was essential for the correct functioning of the rest of the apparatus. The 12-volt pulse was then transformed into a 240 volts pulse which was used to actuate a small solenoid. The retraction of the slide into the solenoid operated an air valve made of plastic disposable syringes by pulling the plunger of the centre syringe to the left (see Fig. 6) past an air inlet aperture. This allowed compressed air from a bottle at a pressure of 25 p.s.i. to enter the inner syringe via the air inlet and to pass along several feet of plastic tubing to the feed dispenser situated over the fish tank. This pulse of compressed air actuated the feed dispenser by forcing the plunger to the left (see Fig. 6) against the tension of the

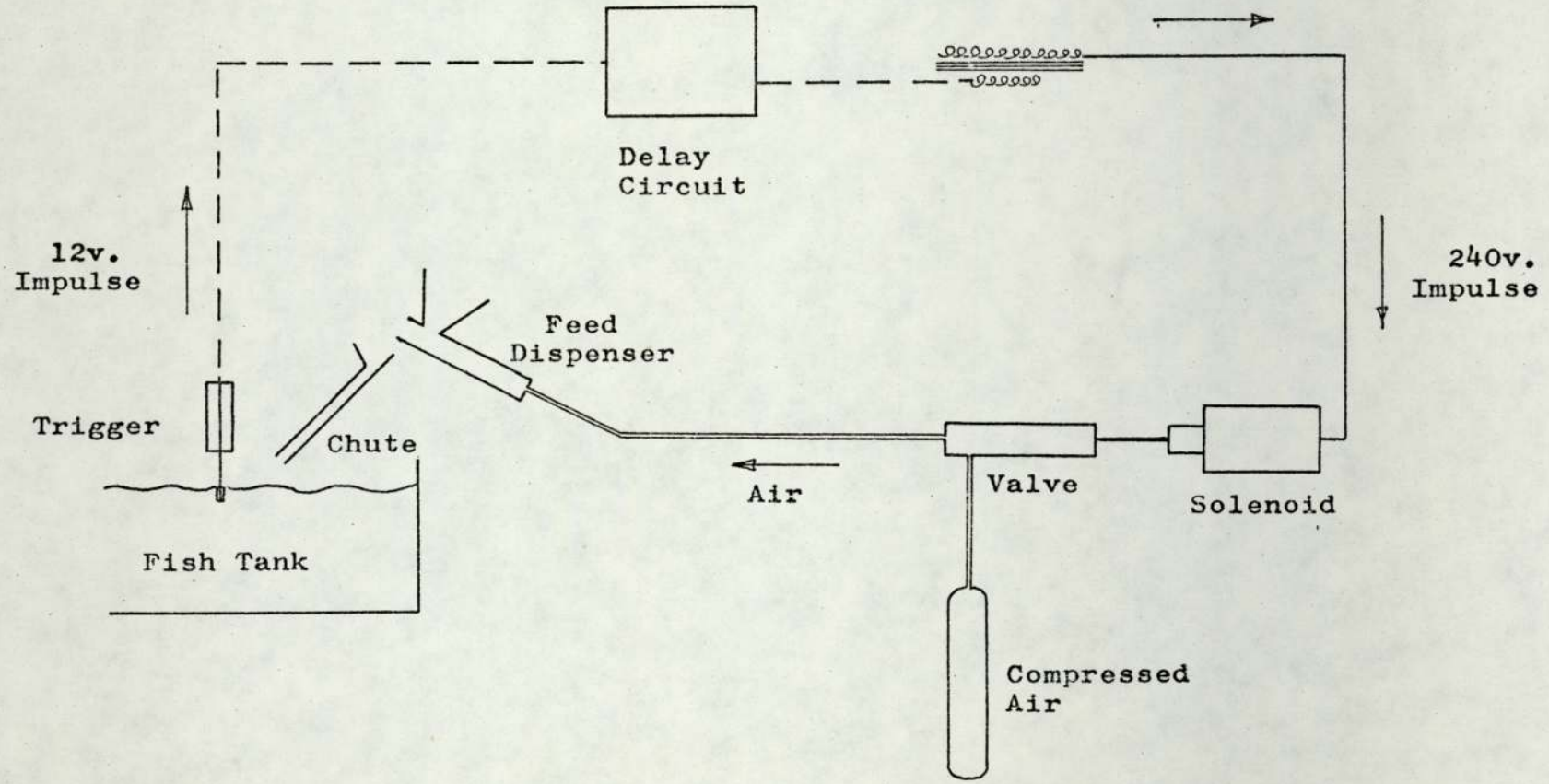


Figure 5. Diagram of the path taken by an impulse created by a trigger actuation which causes feed to be dispensed.

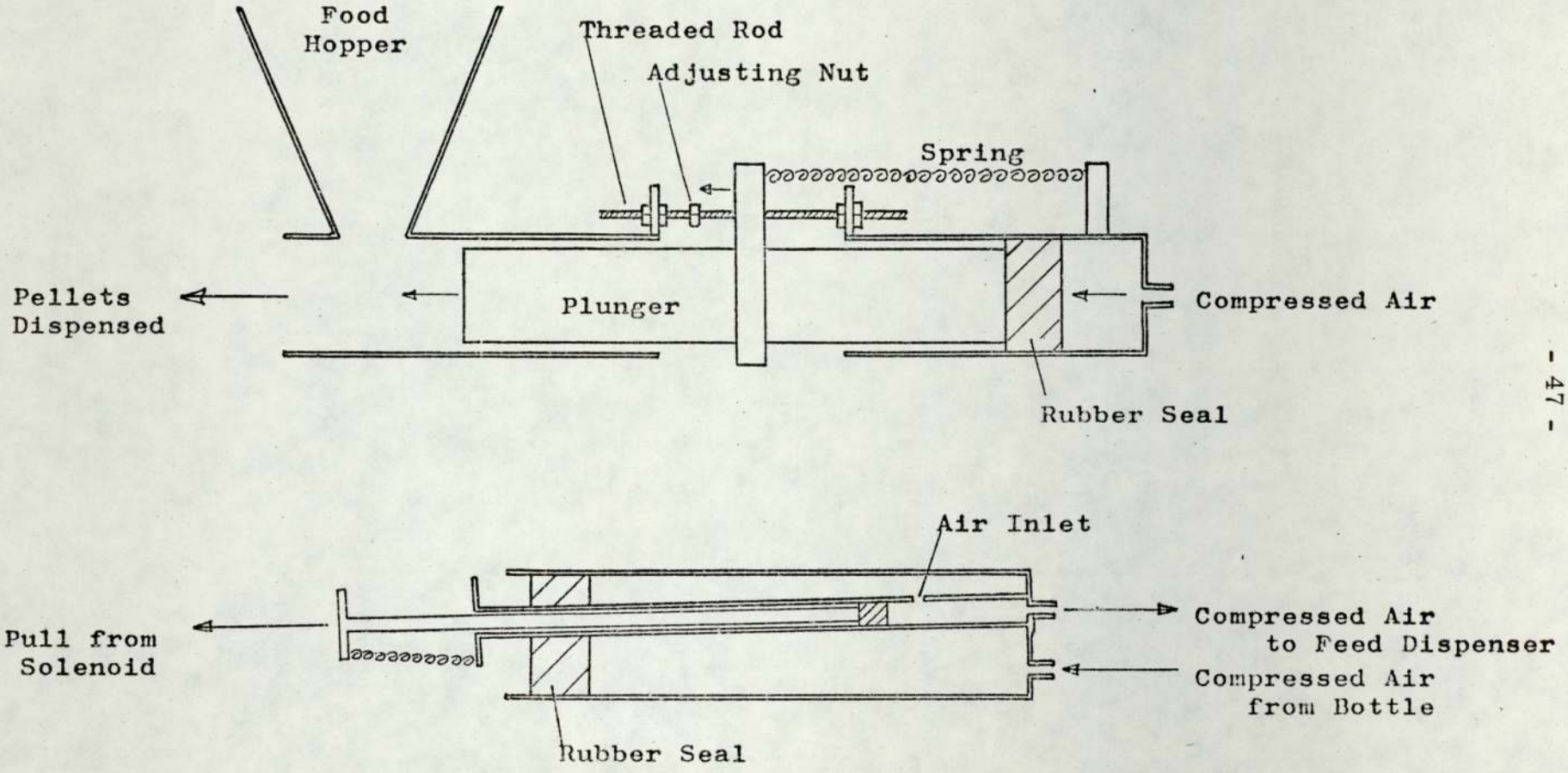
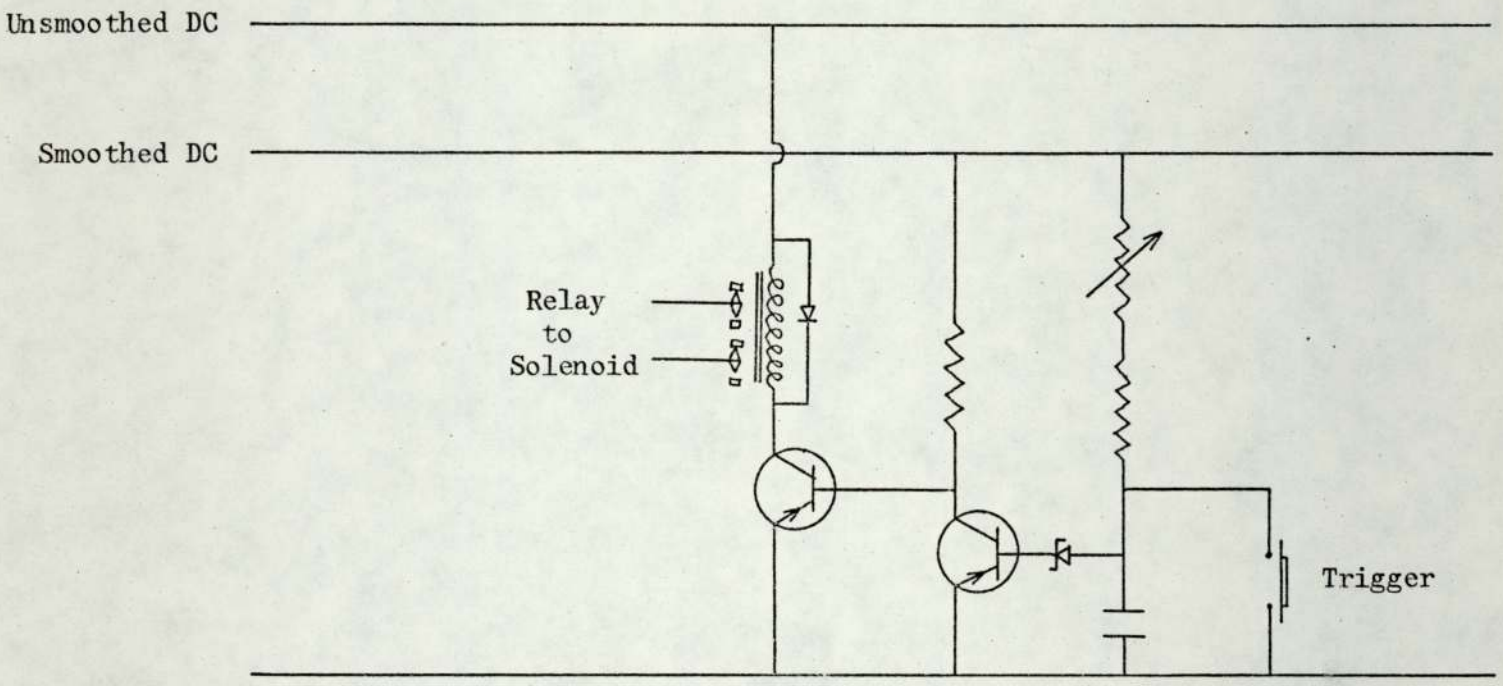


Figure 6. Diagrams of a compressed-air operated feeder together with the valve operated by a solenoid.

Figure 7. Diagram of the delay circuit used in conjunction with a demand feeder



spring so pushing feed pellets out of the large aperture at the end of the feeder. These pellets then fell down a chute to land on the water surface near the tip of the trigger. At the termination of the pulse of current, the slide of the solenoid returned to its resting position and the plunger of the air valve moved right, passing the air inlet, under the action of springs. Thus the valve was now closed to compressed air from the bottle. Immediately the compressed air in the feed dispenser was released to the atmosphere via the valve and the plunger of the feed dispenser returned to its resting position by means of springs.

Each contact made by the fish could be recorded on a digital counter linked into the circuit. The number of pellets dispensed at every contact could be regulated quite accurately by adjusting the nuts on the threaded guide rods of the feed dispenser which controlled the distance of travel of the plunger. When adjusted correctly, the dispenser could be set to release 2-3 pellets at every actuation. The actual food dispensed over a period of 24 hours was determined by measuring the difference in weight between a quantity of pellets placed in the food hopper at the start of the period and the weight of the food left at the end of the period. The results obtained for the two feeders in one tank were summed to give a total weight of food dispensed every day. As a precaution against unintentional activation of the triggers at night the feeding circuits were deactivated automatically at the commencement of each dark period.

A possible source of error in equating food dispensed with food consumed was that possibly not all pellets dispensed were eaten. The only way to circumvent this problem was to observe for long periods

the feeding of the trout at the demand feeder and by careful adjustment of the feed dispensers to restrict the dispensed food to 2 - 3 pellets per activation. This ensured that the particular fish which activated a trigger consumed all of the food dispensed so that only on very few occasions were pellets left by that fish. Usually when this occurred the pellets were eaten rapidly by other fish in the vicinity of the trigger. Thus, there remains the possibility that a few fish in each tank never learned to activate the trigger but fed solely from the pellets neglected by those fish who had fed themselves. It was anticipated that this source of error in measuring ad libitum feeding levels would be similar in both tanks and as the results would be relative, valid comparisons could be made. Therefore, it was proposed that food dispensed should be equated with food consumed. As a check on this, daily inspection of the contents of both faecal traps (Figure 1) showed that either none or very few pellets remained uneaten to be carried out of the tanks. This number was negligible.

Plates 3 and 4 give a good representation of the disposition of triggers and feed chutes within one of the fish tanks.

Plate 3 The arrangement of two three-foot diameter fish tanks used in Experiment 1. Note the disposition of triggers, feeders and dispensing chutes. The chute in the centre of the picture is just upstream of the associated trigger.

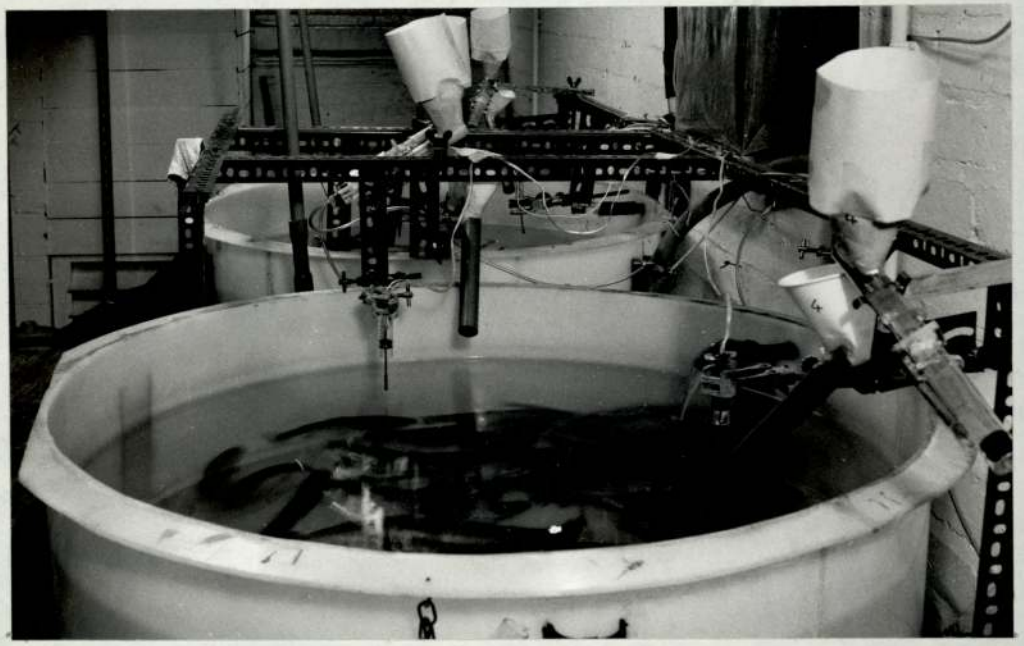


Plate 4. Plan view of one fish tank showing the disposition of triggers, water inlet and one dispensing chute (A).


A

2.4. Tank Modifications

This section describes the modifications undertaken on converting some of the two-foot diameter fish tanks into metabolic rate determination chambers (Experiments 2 and 4) and later on into nitrogen-balance determination tanks.

The Metabolism Chambers used in Experiments 2 and 4

The determination of the metabolic rate of aquatic animals by indirect calorimetry requires that the rate of oxygen consumption is measured. This is usually accomplished by one of three methods:-

a. By a manometric method suitable for small fish.

b. By determining the decrease in the dissolved oxygen concentration of water in a sealed container over a period of time. This method is perhaps the most frequently used (Ege & Krogh, 1914; Fry & Hart, 1948; Wohlschlag & Juliano, 1959; Brett, 1964; Smit, 1965).

c. By utilizing continuous-flow respirometers (cf. Keys, 1930; Wells, 1935; Power, 1959; Roberts, 1964).

In Experiment 2, the sealed container (type 'b') was used and in Experiment 4, continuous respirometry (type 'c') was used.

Experiment 2

The 'closed chamber' method was used in this experiment in the modified manner first described by Brett & Sutherland (1970) and Brett, Sutherland & Heritage (1971). This method combines the functions of a metabolism chamber and a growth tank into one chamber, the metabolism/growth tank, where studies on the metabolism and growth of one or more fish may be made synchronously. This system has the immediate advantage that fish need not be moved from growth tank to metabolism tank and back which means that unnecessary disturbance,

often resulting in abnormal metabolic rates, is avoided. The avoidance of such 'stress' means that fish will feed normally so giving a truer picture of their total metabolism.

The construction of this tank is shown in Figure 8. In normal use, water entered the tank via the inlet. The flow rate was controlled by valve A. Water was directed in a manner which created a helical flow of water in the tank. This flow resulted in 100% clearance of faeces from the tank bottom together with a continuously low level of swimming activity by the fish against the flow. Water drained by way of a double stand-pipe via valve D into the faecal trap where settling of solids occurred. The water flowed out and into the main filter. Normally valves C and B were closed.

When starting a metabolism determination the tank lid, normally in position, was secured to the top of the tank by six bolts. Valve D was then closed. Both stand-pipes were then removed, via the central port in the lid and the small 'Totton' centrifugal water pump was connected to the universal joints. Next, valve C was opened and the pump suction and delivery pipes allowed to flood with water. As the water level in the tank rose, air was forced out of the central port and vents in the lid until after about five minutes the whole chamber was flooded. Then valve A was closed and the bungs were positioned in the central port and lid vents. Valve B was opened and the small water pump was started. When the pump had been running for five minutes a water sample was taken by opening valve E and filling a 125 ml bottle by displacement. The dissolved oxygen concentration of this sample was determined by the azide modification of Winkler's method. After a suitable time interval of 30 - 60 minutes a further water sample

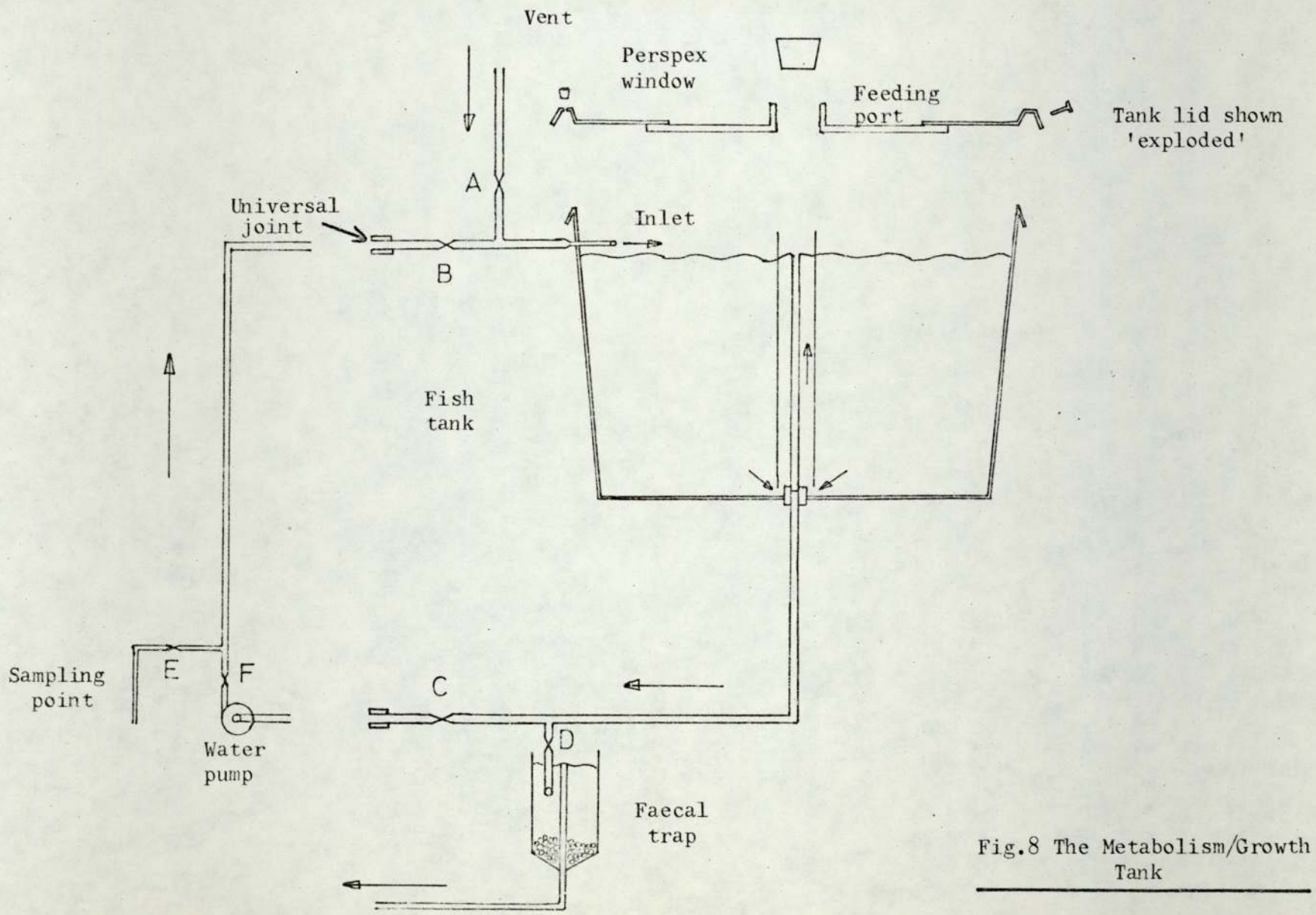


Fig.8 The Metabolism/Growth Tank

was taken and the determination finished by stopping the pump, replacing the stand-pipes, closing valves B and C and opening valves A and D.

Using the following equation the metabolic rate could be determined over the period of the test.

$$\text{Metabolic Rate} = \frac{V \times D}{\text{Total live fish weight, g.}} \times \frac{60}{\text{test time, mins.}}$$

where V = the total volume of the tank (83 l)

D = Fall in dissolved oxygen concentration, mg/l

Observation of fish in the tank was carried out through a 10 inch diameter 'Perspex' disc which replaced a cut-out portion of the tank lid in the centre of the lid. Feeding of the fish was undertaken between metabolism tests using a hand-held feeding tube inserted into the central port.

Particular care was exercised during each experimental test as maintaining fish in a restricted volume of water causes environmental conditions to change; oxygen becomes rapidly depleted, carbon dioxide levels increase, and temperature may rise. Therefore, each experimental test duration was from 30 - 60 min. This ensured a reliable measure of oxygen consumption without water conditions becoming restricting. Dissolved oxygen concentrations were never allowed to fall below 5 mg/l during one test. This ensured that the total metabolic rates of the fish were unaffected by low dissolved oxygen levels as has been mentioned by Beamish & Dickie (1967) and Fry (1957). The figure of 5 mg/l is above the value of 50% saturation which is the value below which the metabolic rates of rainbow trout fall (Kutty, 1968). Also, the relatively short duration of each test ensured that water temperature fluctuations were negligible.

A maximum of four tests could be made on one tank in any one day. This was because an adequate time for flushing the tank with 'new' water had to be maintained. This flushing time was generally one hour and this ensured that dissolved oxygen levels reached a maximum value before a further test was carried out.

It was hoped that this particular design of chamber was an improvement over the design of the Brett & Sutherland chamber. Their tanks have separate inflows for the closed recirculated water and the normal inflow. In the present design of tanks, using the same inlet for both recirculated water and for 'normal' water the same flow of water in both direction and magnitude was maintained. This ensured that the fish swam in the same direction and expended approximately the same amount of energy for swimming during the test and during normal periods. It was essential to adjust carefully the small water pump outflow at valve F to ensure that flow rate was identical to the normal flow rate of water from the tank inlet.

Experiment 4

The design and operation of the metabolism/growth tanks was changed from that in Experiment 2 for the purpose of determining the specific dynamic action of food fed to trout held in different salinities. The metabolism tanks were modified from being used intermittently to being used continuously so providing an even greater reliability in the measure of metabolic rate. Other advantages secured in using a continuous-flow respirometer include the constancy of water conditions and the decrease in stress inflicted on the fish by periodic changes in the water level in the tank. Alterations in the design of the metabolism chambers include changes in the method of draining water from the tank

together with the loss of the small water pump (see Figure 9).

Normally, when not being used for respiration tests, water entered the fish tank via valve A and the tank inlet. It drained to waste through the double standpipe and via valve D into the faecal trap and then to the filter tank (solid arrows). When being used as a respirometer over a period of several days the tank was constantly flooded and water drained to waste through a constant level drain (broken arrows). To change the method of draining, valve D was closed and the tank allowed to flood until some water was expelled from the drain vents and feeding port in the lid. These apertures were then sealed with rubber bungs leaving the standpipes in place. This ensured that the outer standpipe, being unfixed, floated up to rest against the underside of the rubber bung so forming an effective seal. This allowed water to drain to waste via the bottom of the outer standpipe and allowed a thorough flushing of the chamber. Also, maintaining the standpipes had the advantage of not disturbing the fish. Waste water drained past the closed valve D and through open valve C. The water passed out through a constant level drain, the height of which had been adjusted beforehand so that water drained away from the tank at the same rate as it entered. To determine this flow rate, valve G was closed, valve F opened and the flow of water measured using a volumetric flask and stop-watch.

In order to take a water sample for dissolved oxygen determinations, valve E was opened and a sample taken by filling a 125 ml bottle by displacement. The dissolved oxygen concentration was then found by the azide modification of the Winkler method. When a metabolism test was being undertaken, water samples were taken hourly from 8.00 a.m.

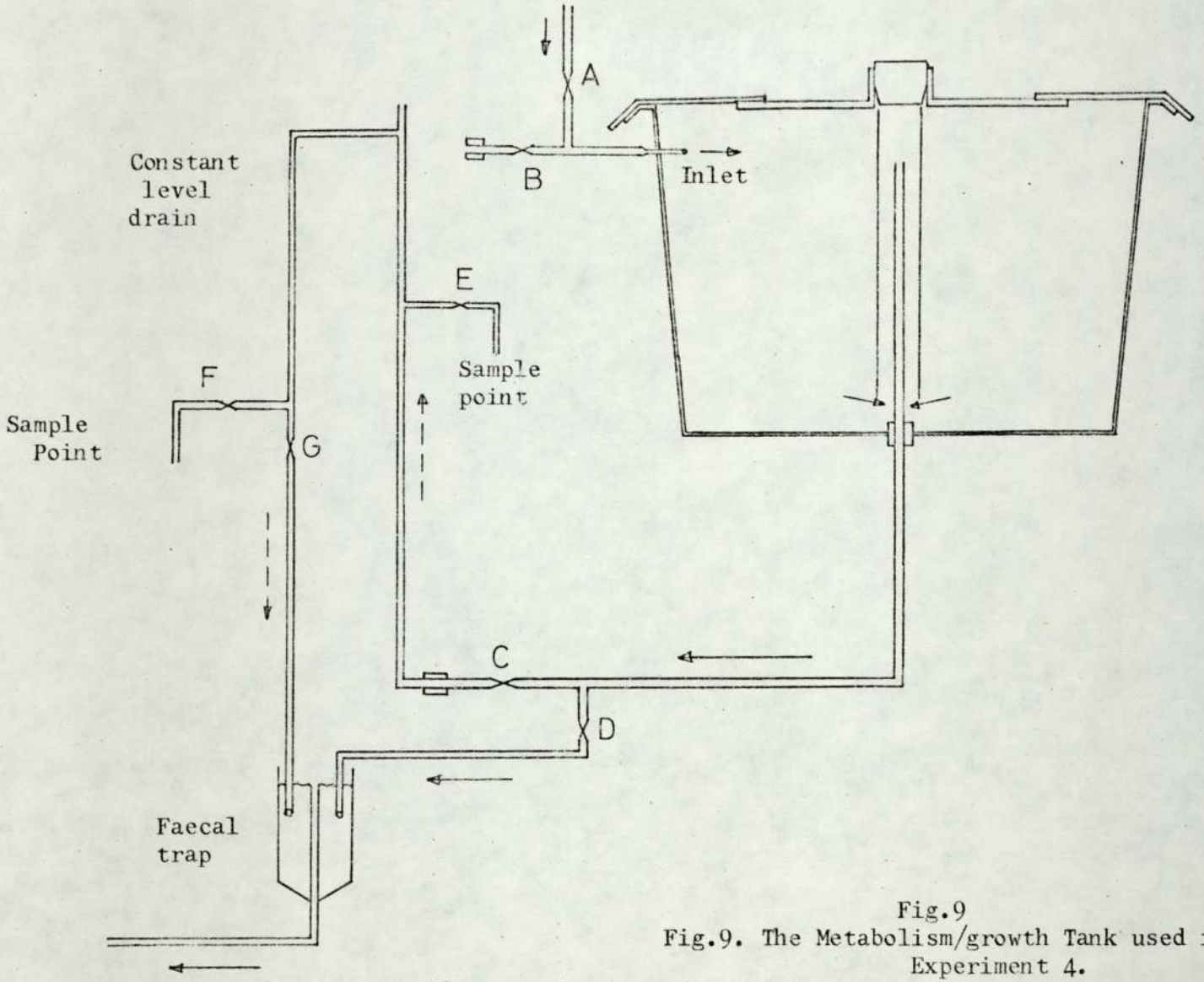


Fig.9
Fig.9. The Metabolism/growth Tank used in Experiment 4.
The tank is shown flooded for use in metabolic rate determinations

to 9.00 p.m. from both the outflowing sample point (valve E) and an inflowing sample point. For the purposes of the experiment, an inflowing water sample was taken from an adjacent tank. It had been shown beforehand that the dissolved oxygen concentration of inflowing water of adjacent tanks was identical. Also, the water temperature and flow rate was determined hourly. Reference should be made to Plate 5 which shows the disposition of the constant level drain and outlet sampling point.

The following equation, given by Evans (1972) was used to determine the respiration of the fish:-

Respiratory Rate, mg. O_2 / hr. =

$$F\,(Y_1 - Y_2) + V\,(Y_{2TO} - Y_{2TI})$$

where F = Flow Rate, l/hr

$Y_1 - Y_2$ = Mean difference in oxygen concentration between inflowing and outflowing water, mg/l

V = Volume of water in the respirometer, l

$Y_{2TO} - Y_{2TI}$ = The oxygen concentration of the outflowing water at the beginning and end of the period respectively, mg/l

Changes in activity may cause an alteration in the rate of oxygen consumption which will not appear immediately in measurements owing to the delaying or damping effect of the volume of water in the chamber. This 'lag' in measurements is corrected for by the latter half of the equation = $V(Y_{2TO} - Y_{2TI})$.

Feeding of the fish was undertaken three times per day. To accomplish this when a metabolism test was being made, the movable constant-level drain was lowered slightly which caused the outflowing water to increase in rate. When the water level in the chamber had dropped so that it was about $\frac{3}{4}$" from the lid, the constant-level drain was re-

Plate 5 The arrangement of draining tubes for one metabolism/growth tank as used in Experiment 4.

A - Drain from tank

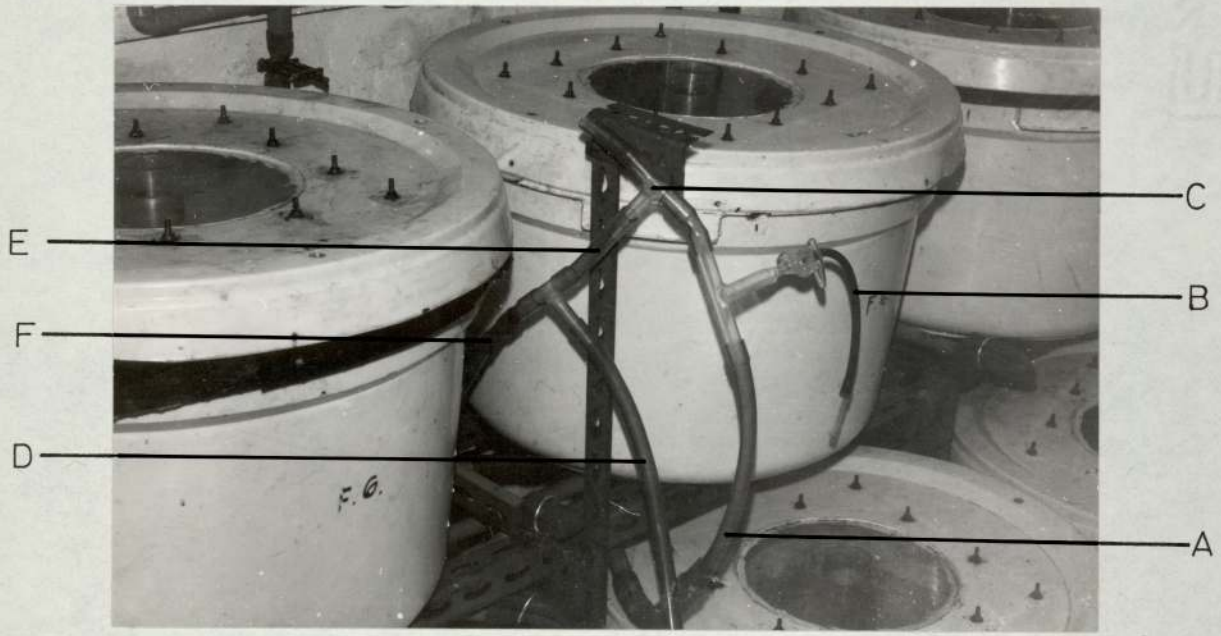
B - Water sampling point

C - Constant level device

D - Water bypass for measuring water flow rate from drain

E - Drain to faecal trap

F - Valve G of Figure 9


E
F
D
C
B
A

positioned so that the water level was maintained at the lower position. The central bung was removed and the ration administered through a hand-held feeding tube. When all the food was consumed, the constant-level drain was raised to its original position and the chamber reflooded. Care was taken to ensure that all air bubbles were expelled from the central port and the four minor air vents around the perimeter of the tank lid.

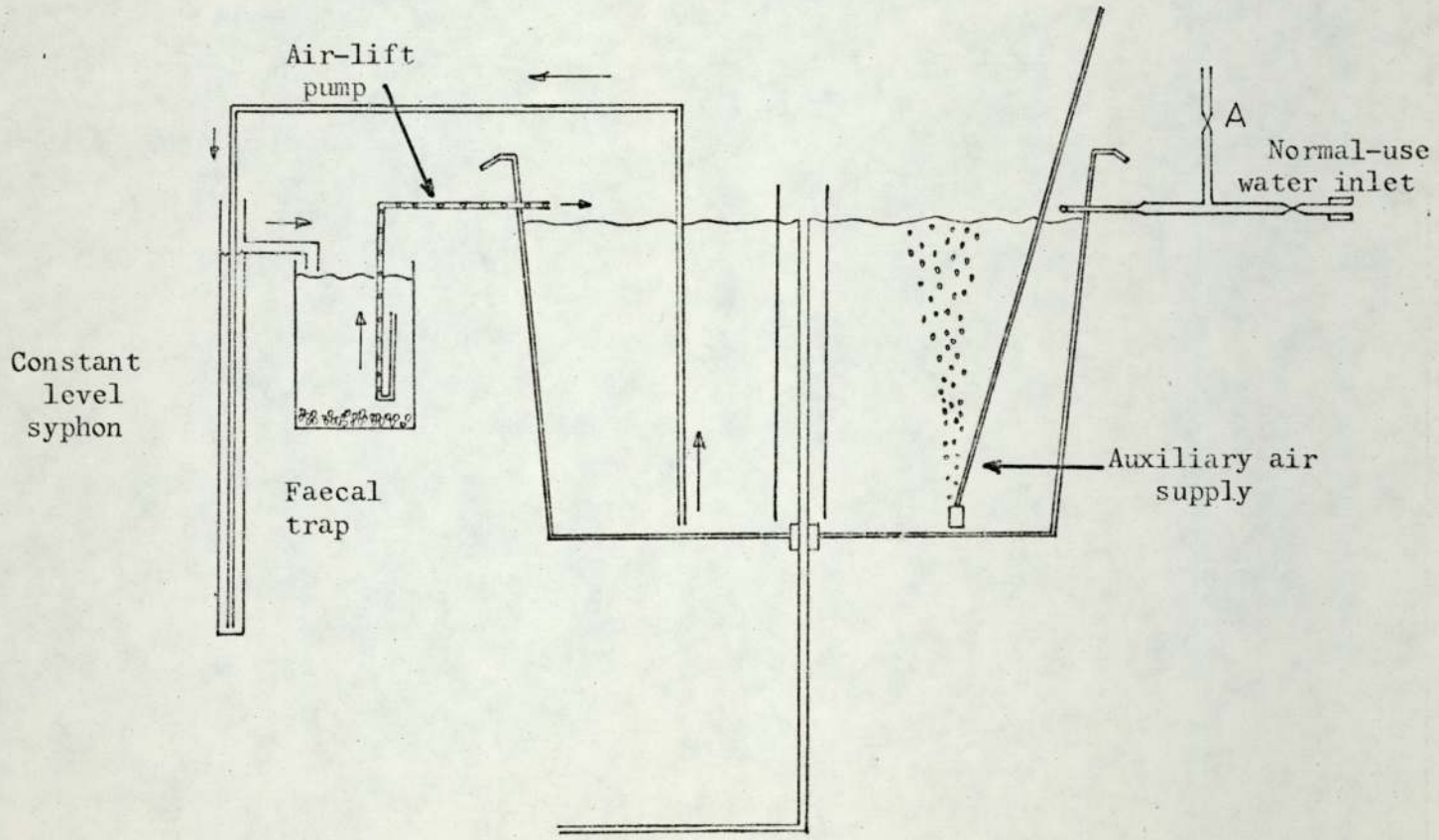
Experiment 5 - The Nitrogen-Balance Tank

This experiment involved the determination of the nitrogen balance of trout held in different salinities and fed on pelleted diets of varying protein contents. Accordingly, most of the fish tanks were modified in the manner detailed below and five diets were formulated and pelleted each having a different protein content.

Ten of the fish holding tanks were adapted to permit each tank to hold a static volume of water for a period of time and to allow faeces to be separated from the main volume of water. The main considerations in the design of the modifications were to maintain soluble faecal nitrogen at a low level, to have the fish consume all the food presented, and to maintain temperature at a constant level. In pursuance of point two, all fish were fed at a rate of 1% of the live body weight/day and supplementary aeration of the water in the tank was maintained.

Each experimental tank was altered by the addition of a faecal trap of 5 litres capacity, a constant-level siphon and a small air-lift pump (Figure 10). In normal use, water entered the tank from the inlet and the flow was regulated by valve A. Water drained to waste through the double standpipe as described in the previous sections. When a nitrogen balance test commenced the inflow of 'fresh' water was stopped by closing valve A. Immediately the auxiliary air supply from a 'Rena' diaphragm air-pump was started. The siphon was flooded and the air-lift pump switched on. The action of the siphon was to draw water and faeces out of the fish tank into the faecal trap via a constant-level device. This allowed fluctuations in the flow of the siphoned water to compensate for irregularities of water flow

Fig.10. Diagram of the nitrogen-balance tank used in Experiment 5



maintained by the air-lift pump. As water entered the faecal trap, the faeces settled out by gravity and water was replaced into the fish tank by the air-lift pump. There was a 'U' bend in the suction end of the air-lift pump which prevented faeces from re-entering the fish tank.

Each nitrogen balance test was of 25 hours duration. It had been found previously that the temperature of the water in the fish tank fluctuated by $1^{o}C$ over this period and it was considered that this variation was acceptable for experimental purposes. At the end of each test, valve A was re-opened and the tank flushed with 'fresh' water. The siphon was drained and the air supply to the air-stone and air-lift pump stopped.

CHAPTER 3. Experiment 1 - The Quantitative Determination of the Effect of Salinity on Food Consumption

3.1. Introduction

Food consumption, metabolic rate and environmental conditions are intimately related factors in fish. Optimum growth conditions can be achieved for many fish species by manipulation of the environment usually with respect to temperature and food supply (Kinne, 1960; Pandian, 1967; Brett, Shelbourn & Shoop, 1969; Brett & Sutherland, 1970; Brett, 1971a.) The objective of this study was to investigate the possible relationship between environmental salinity and the appetite of rainbow trout together with the effects of any changes in appetite on growth rate and food conversion efficiency.

The evidence relating to the effect of salinity on voluntary food consumption of fish is scarce and somewhat contradictory. For example, Peters and Boyd (1972) with the hogchoker, Trinectes maculatus, and Vallet et al. (1970) with Mugil auratus and Mugil capito found that different environmental salinities exerted no effect on the voluntary food consumption of these fish. However, Kinne (1960) with the desert pupfish, Cyprinodon macularius found the food consumption was highest in 35 ppt sea water and lowest in fresh water. Similarly, MacLeod (personal communication) found with rainbow trout that appetite increased gradually with increase in salinity up to 28 ppt after which the appetite declined. In contrast to these latter results, Otto (1971) found that voluntary food consumption of coho salmon, O. kisutch, was highest in 10 ppt sea water and lowest in 20 ppt sea water. It was therefore considered of great interest to determine whether salinity could elicit an alteration in appetite of rainbow trout under the condit-

ions of the present experiment.

As an integral part of this experiment it was proposed that 'demand feeders' be used instead of hand feeding. This was to eliminate those experimental errors inherent in hand feeding fish to repletion which may subject fish in different experimental tanks to different feeding opportunities. Using demand feeders allows fish to take food at any time of the day in quantities which entirely satisfy their appetites. In addition, the use of demand feeders frees the experimenter from lengthy periods of hand feeding fish to satiation. Ishiwata (1968) found that 60 min was required to satiate rainbow trout weighing 135 g at 10^{o}C using hand feeding.

The use of demand feeders in the fields of nutrition and metabolism has been practised for several years. Rozin and Mayer (1961, 1964) found that individual goldfish could be trained to obtain food by pressing a lever. This capacity of fish (or any other animal) for performing tasks in order to obtain a reward or avoid an unpleasant shock is termed operant conditioning. More recently it has been observed that populations of trout could be similarly conditioned (Adron, 1972; Adron, Grant & Cowey, 1973; Landless, 1976a). Adron and coworkers found that groups of thirty trout could be self-trained within a period of about ten days to feed themselves on a demand routine. They proposed that demand feeding of fish had a potential for studying the factors which control the feeding response in fish. These factors may be dietary or environmental. The reason for using a population of fish rather than individual fish is that greater reliability in results is obtained where replicated treatments are absent.

Accordingly, an experiment was designed and undertaken to

investigate the feeding response of two populations of rainbow trout using a demand feeding system.

3.2. Materials, Methods and Experimental Procedure

The experimental systems and fish tanks have already been described in Chapter 2 together with details of the operation of the demand feeding system. One system contained fresh water throughout the experiment and was taken to be the control situation while the other system held waters of different salinities depending upon the phase of the experiment. The temperature of the recirculating water was maintained at 15 $\pm$ 1°C in both systems. Photoperiod was 13.5 hr. on, 10.5 hr. off with a $\frac{3}{4}$ hr. 'dawn' and 'dusk' at the beginning and end of each day.

Fifty rainbow trout of about 18 cm in length each were placed in each of the two 45 gal. fish tanks. Each population was hand-fed for one week on 'Beta' floating trout pellets, grade 4, in order to allow the fish to acclimate to the environmental conditions. At this point in time, each system was holding fresh water.

After this acclimation period hand-feeding was discontinued and the demand feeding apparatus set up. Two independent demand feeders and triggers were allocated to each tank. This resulted in an average of 25 fish to a trigger, a figure close to that used by Adron et al. (1973) who used 30 fish per trigger in their experiments. Each trigger was positioned carefully so that the red tip of the actuation lever projected about 5 mm below the surface of the water. The angle and position of the feeding chutes were also carefully adjusted (Plates 3 and 4) so that food could be dropped 'upstream' of each trigger. This enabled a trained fish to nudge the trigger and continue swimming on to obtain

food pellets. A constant check was maintained on this apparatus to ensure correct functioning.

The experiment was then carried out in five phases, each phase being continuous with the next. The duration of the whole experiment was six weeks and the phases proceeded in the following order:-

a. Two weeks - Both tanks containing fresh water - Training period

b. One week - Both tanks containing fresh water -

c. One week - Control tank containing fresh water, test tank containing 10 ppt sea water -

d. One week - Control tank containing fresh water, test tank containing 20 ppt sea water -

e. One week - Control tank containing fresh water, test tank containing 28 ppt sea water.

When the demand feeders were in use pre-weighed amounts of food were put into the hoppers of the food dispensers every day. Food consumed by each tank of fish was measured every day by weighing the food left in the hoppers at the end of the day and subtracting this from the initial weight of food. The food dispensed by both feeders for one tank was summed and the total weight of food consumed per tank per day was found. Faecal traps were inspected daily for evidence of uneaten food. At all times this quantity was negligible.

Following the initial acclimation period the trout in both tanks were weighed individually under anaesthetic as described in Chapter 2. At this time, two or three fish were changed from one tank to another so keeping the total numbers in each tank equal but adjusting the total weight of fish in each tank so that they were as similar as possible. Later comparison of the mean fish weights between tanks by Student's

't' test revealed no significant difference. Subsequent weekly weighings were carried out on a 'batch basis' as described in Chapter 2.

During phase 'a' the trout in each tank learned to feed themselves by nudging the tips of the triggers and obtaining the reward of food. When it was observed that the trout in both tanks were using the demand feeders successfully, the experiment commenced.

The second phase of the experiment was undertaken with both systems containing fresh water. Its purpose was to investigate the possibility of the existence of inherent differences in food consumption between the two populations of trout under identical environmental conditions.

Following this second phase, the fish were reweighed and the salinity of the water in tank number 1 was increased over a period of eight hours to 10 ppt. This period of time was considered to be sufficient to allow changes in the food requirement to be registered in changes in food consumption the next day. Rozin and Mayer (1961, 1964) demonstrated that the mechanism responsible for the homeostatic control of nutrient intake was fairly rapid in goldfish when compared to homoiotherms (Janowitz & Hollander, 1955). Goldfish compensated to changes in the caloric density of their diet within one day. A week was then allocated to the comparison of feeding rates between fish in fresh water and 10 ppt sea water. Following this third phase, the fish were reweighed and the salinity of the water in tank 1 was increased over a period of eight hours to 20 ppt. A week was then allocated to the comparison of feeding rates between fish in fresh water and 20 ppt sea water. Finally, after this fourth phase, the fish were reweighed and the salinity of the water in tank number 1 was increased

over a period of eight hours to 30 ppt sea water. After three days of the final week had elapsed, the salinity of the water in tank number 1 was decreased to 28 ppt by the addition of fresh water in order to prevent the occurrence of mortalities, three of which had happened during the early stages of the final phase. This week was allocated to the comparison of feeding rates between fish in fresh water and fish in 28/30 ppt sea water. At the end of the final week, the fish in both tanks were reweighed.

Several parameters were determined at the end of each week from the following equations.

1. Conversion Ratio (Conversion Factor) $= \frac{\text{Food Fed, g 'dry' food}}{\text{Weight Gain, g live fish}}$

2. Specific Growth Rate % per day $= \frac{{}^{1}_{n}W_2 - {}^{1}_{n}W_1}{T_2 - T_1} \times 100$

Where W_2 is the total weight of fish per tank at time T_2 and W_1 is the total weight of fish per tank at time T_1, T_2 being later than T_1.

T_1 and T_2 are measured in days (from Brown, 1957).

3. Weight - adjusted Feeding Rate, g. food consumed/day/100 g live fish

$$= \frac{\text{Total Food Consumed per week, g} \times 100}{7 \times \text{Mean Total Fish Weight, g.}}$$

Where the Mean Total Fish Weight is given by the equation:

4. $$\frac{\text{Total Live Fish Weight, g at time } T_1 + \text{Total Live Fish Weight, g, at Time } T_2}{2}$$

Where T_1 and T_2 are the start and the end respectively of each week.

3.3. Results

The period of time was determined for both populations of fifty trout to self-train itself to actuate a trigger and obtain a reward of food. Absolute daily food consumption during this training fortnight is shown in Figure 11. Both tanks of fish show approximately the same overall pattern of food intake. The increase in food consumption rose from under 10 g per day per tank to 60 - 70 g per day per tank in about 8 - 10 days. After this period, the food intake increased very gradually over the course of the entire experiment.

In order to facilitate comparison, these results were plotted as three-day running means of daily food consumption per tank plotted against time (see Figure 12). Smooth curves were fitted to both sets of data by eye. Both curves reached a plateau of food consumption after 9 - 10 days. The small difference in daily food consumption between tanks was taken as a reflection in the difference in the total weights of fish between tanks.

It was concluded that after the period of 14 days training, both tanks of fish were well experienced in self-feeding. This was inferred from the fall in the gradients of the curves in Figure 12 after 9 - 10 days. Also the behaviour of the fish had changed after this time. During the first few days of the training period, fish would attack triggers quite vigorously from various angles of approach and often tried to swallow the triggers. This was possibly due to the fish mistaking the trigger tip for a floating food pellet. After about 6 days the speed and angle of approach of the fish towards a trigger was more purposeful. Trout would swim towards the trigger against the current of the water, nudge it gently with mouth or nose, and continue swimming.

Fig.11. Food consumption by two tanks of fish over the training fortnight.
Tank 1, closed circles; tank 2, open circles

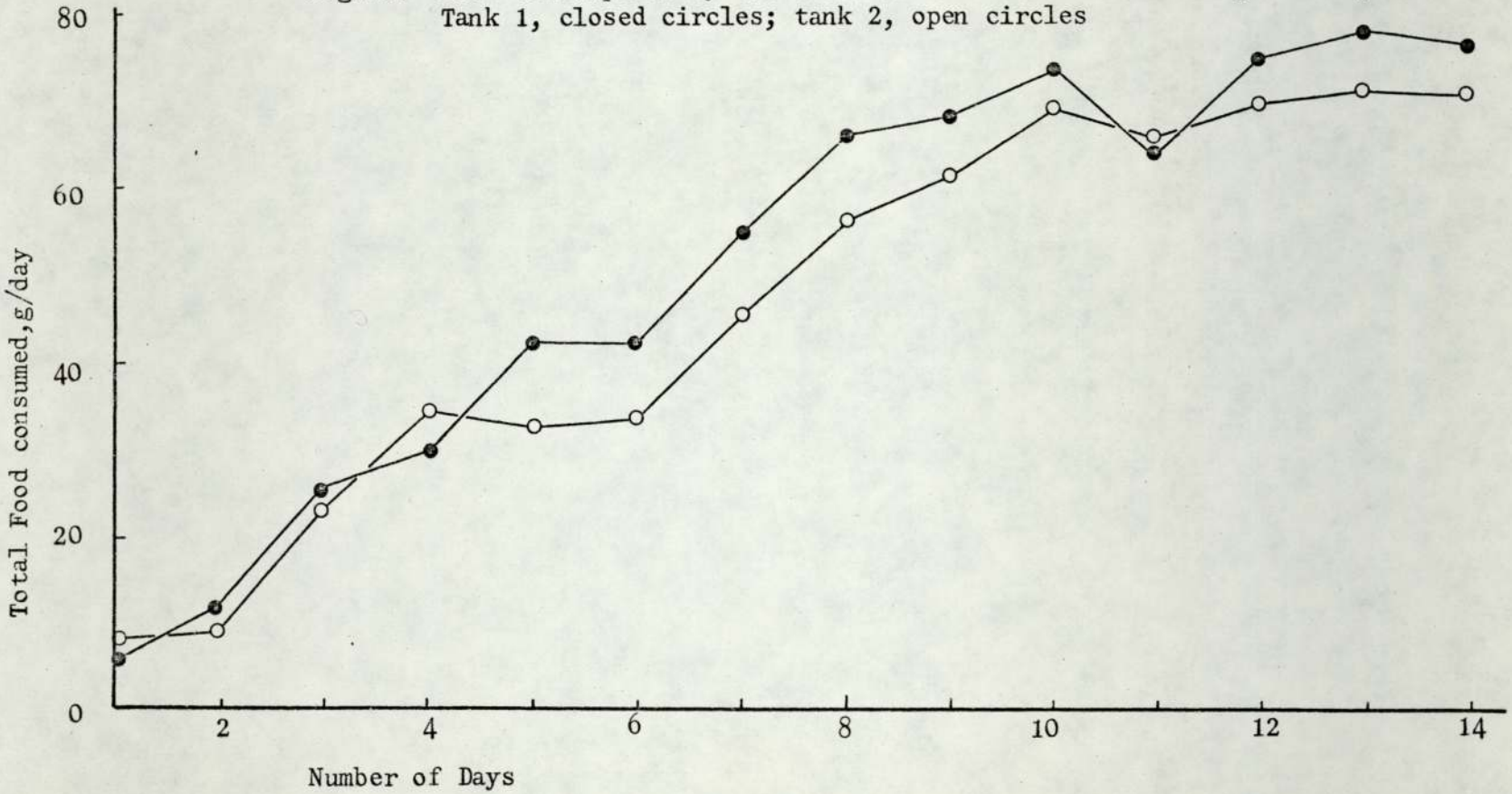
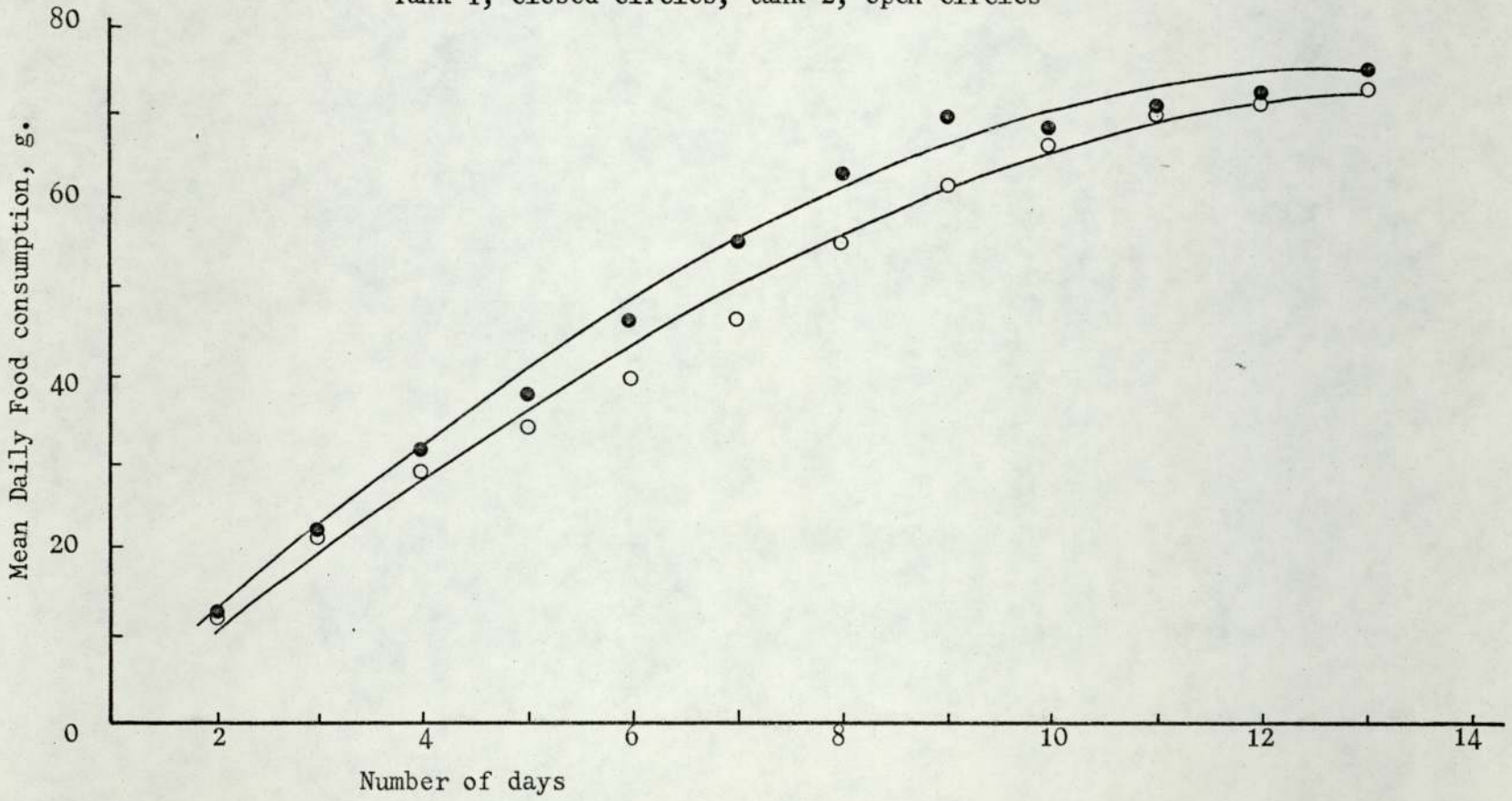


Fig. 12. Food consumption by two tanks of fish over the training fortnight.
3 -point moving averages plotted.
Tank 1, closed circles; tank 2, open circles



This would place them in the correct position for receiving dispensed pellets. Thus, it appeared that trout conditioned themselves to feed by at first attacking a trigger mistaking it for food and then gradually associating their action with the resultant food reward.

The results obtained during the experimental weeks are shown in Tables 3 and 4. Table 3 gives the total live weights of fish per tank at the beginning and end of each week, together with the mean daily food consumption rates. Comparison of these daily consumption rates for each week was accomplished by using either Student's 't' test or the Fisher-Behrens test depending upon the significance of the variance ratios. Table 4 gives the weekly conversion ratios, specific growth rates and weight-adjusted feeding rates for both tanks of fish.

The pattern of food consumption during the training period, weeks 1 and 2, has already been described. During week 3, when both systems contained fresh water, food consumption was similar for both populations of fish. No significant difference was found between the two mean food consumption rates for tanks 1 and 2 these being 70.38 and 71.40 g/day respectively. Both tanks of fish had identical conversion ratios and identical specific growth rates.

During week 4 comparing fresh water and 10 ppt sea water, mean food consumption was significantly higher ($p < 0.01$) in 10 ppt sea water than in fresh water. These rates were 82.40 and 70.34 g per day respectively. Also, the weight-adjusted feeding rates (Table 4) were higher in 10 ppt than in fresh water. The conversion ratio was marginally lower for fish in 10 ppt sea water (1.80) than for fish in fresh water (1.85). Consequently the specific growth rates were higher for fish in 10 ppt sea water than in fresh water being 1.01 and 0.83%/day

Table 3. Mean food consumption and weekly weight changes during the training and experimental weeks.

Tank Number	Week Number	Salinity	Total Live wt. g/tank Start of week	Total live wt. g/tank End of week	Mean Food Consumed a. g/day/tank ± Standard Error of Mean Weekly Food Consumption rate	Probability
1	1	Fresh	3683	3734	29.71 ± 1.93	Not significant
2		Fresh	3863	3890	25.93 ± 0.59	
1	2	Fresh	3734	4100	70.04 ± 0.96	$p < 0.05$
2		Fresh	3890	4160	66.00 ± 1.23	
1	3	Fresh	4100	4374	70.38 ± 0.63	Not significant
2		Fresh	4160	4438	71.40 ± 0.81	
1	4	10 ppt	4374	4694	82.40 ± 1.99	$p < 0.01$
2		Fresh	4438	4704	70.34 ± 1.11	
1	5	20 ppt	4694	4989	80.10 ± 1.18	$p < 0.02$
2		Fresh	4704	4992	74.20 ± 1.74	
1	6	28 ppt	4675[b]	4879[b]	55.57 ± 5.45	$p < 0.01$
2		Fresh	4992	5299	76.73 ± 0.66	

a n = 7

b n = 47 due to 3 mortalities

Figure 13. Mean weekly food consumption rates of two tanks of fish
Means shown ± one standard error

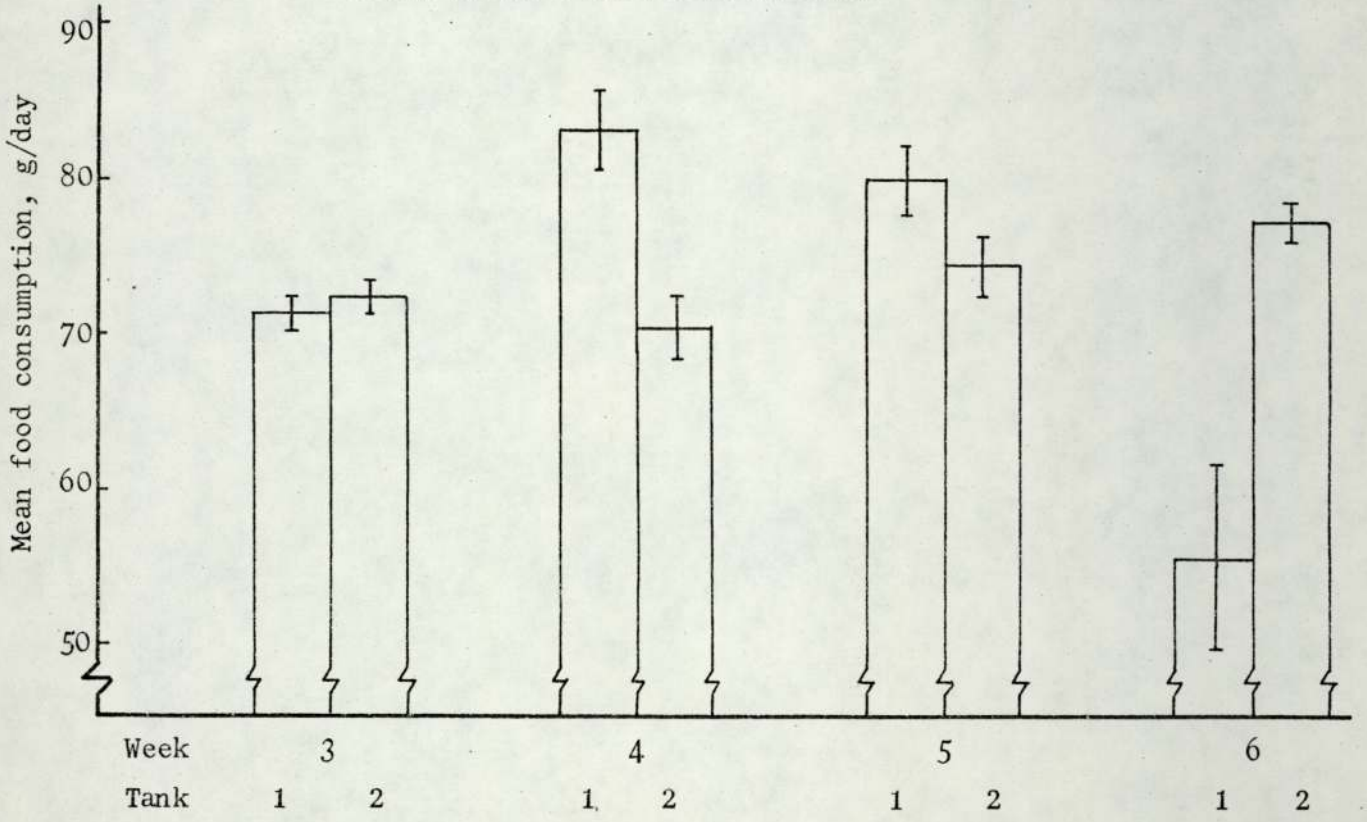
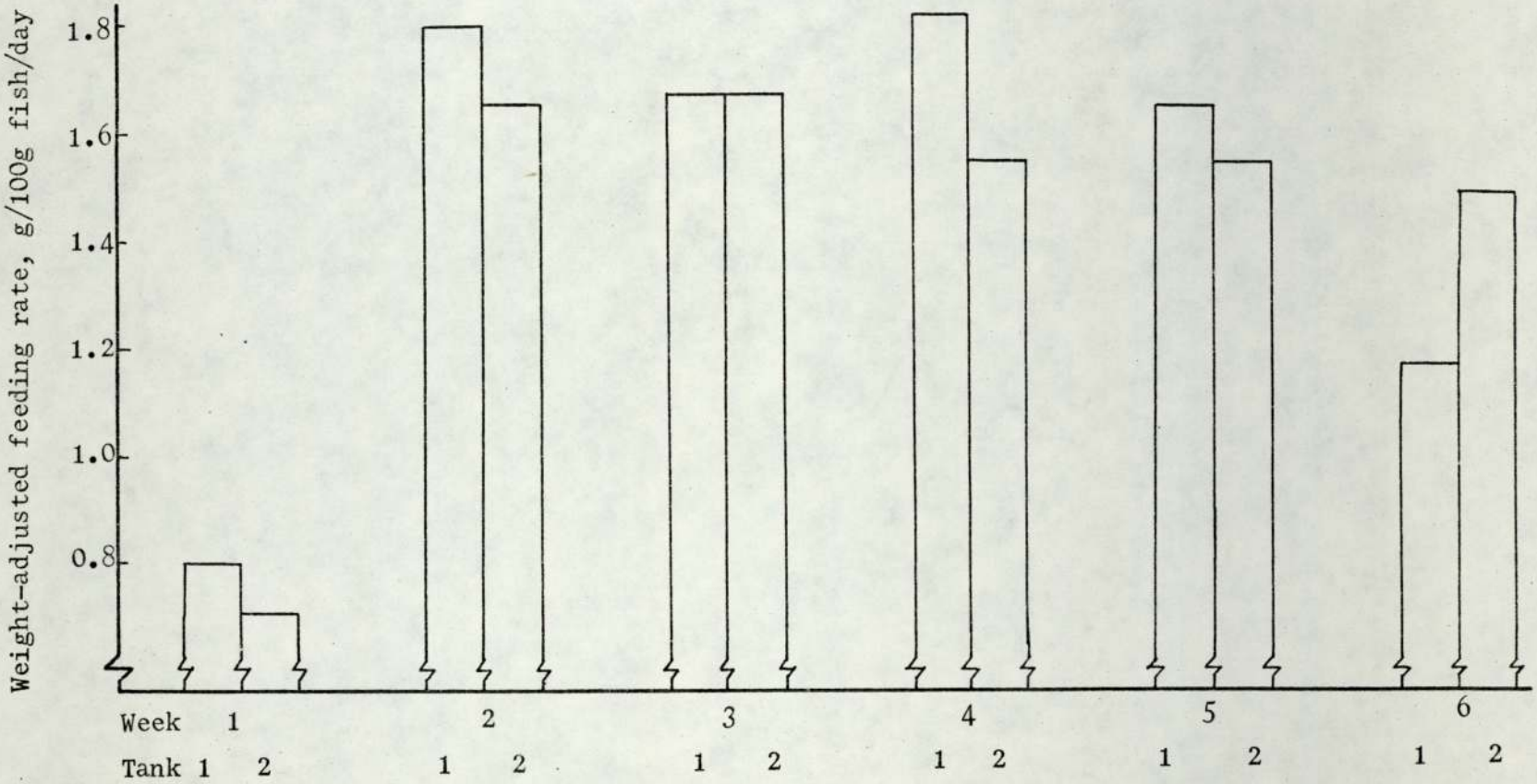


Table 4. Conversion ratios, specific growth rates, and weight-adjusted feeding rates during the training and experimental weeks.

Tank No.	Week No.	Salinity	Conversion ratio	Specific Growth Rate %/ day/tank	Weight Adjusted[a] Feeding Rate g/100g.fish/ day/tank
1	1	Fresh	4.05	0.20	0.80
2		Fresh	6.71	0.10	0.67
1	2	Fresh	1.34	1.33	1.79
2		Fresh	1.71	0.96	1.64
1	3	Fresh	1.80	0.92	1.66
2		Fresh	1.80	0.92	1.66
1	4	10 ppt	1.80	1.01	1.82
2		Fresh	1.85	0.83	1.54
1	5	20 ppt	1.90	0.87	1.65
2		Fresh	1.80	0.85	1.53
1	6	28 ppt	1.91	0.61	1.16
2		Fresh	1.75	0.85	1.49

a Weight-adjusted feeding rates calculated from the mean weekly total fish weight per tank

Fig.14. Mean weight-adjusted weekly food consumption rates of two tanks of fish.



respectively.

During week 5 comparing fresh water and 20 ppt sea water, mean food consumption was significantly higher ($p < 0.02$) in 20 ppt sea water than in fresh water. These rates were 80.10 and 74.2 g per day respectively. Again, the weight-adjusted feeding rates were higher for fish in 20 ppt sea water than in fresh water. The conversion ratio was higher for fish in 20 ppt sea water (1.90) than for fish in fresh water (1.80). This together with the different feeding rates brought about similar specific growth rates these being 0.87 and 0.85 % per day for 20 ppt and fresh water fish respectively.

During the final week, it was found that mean food consumption was significantly lower ($p < 0.01$) in 28/30 ppt sea water than in fresh water. These rates were 55.57 and 76.73 g per day respectively. The weight-adjusted food consumption rate was also lower in 28/30 ppt sea water than in fresh water. The conversion ratio was higher in 28/30 ppt sea water (1.91) than in fresh water (1.75). This together with the different feeding rates resulted in a specific growth rate in 28/30 ppt sea water (0.61) compared with fresh water (0.85).

Mean weekly food consumption rates and weight-adjusted food consumption rates are shown in Figures 13 and 14 respectively.

3.4. Discussion and Conclusions

There seems little doubt that salinity exerted a marked effect on the voluntary food consumption of rainbow trout in this experiment. The extent of this effect was also dependent upon the value of the salinity. The results of phase 'b', the third week's experiment, showed that food consumption in both tanks was very similar. In this case, both tanks contained fresh water and the results show that no environmental factor was affecting the feeding response of fish in one tank and not the other. It follows that a change in the voluntary food intake during subsequent weeks was due to the change in salinity in the sea water tank.

The most marked effect was observed for the trout in 10 ppt sea water where food consumption was considerably increased over that of fresh water fish. Similar but less pronounced results were obtained for 20 ppt sea water. In contrast, trout held in 28/30 ppt sea water consumed less food than fish in fresh water. The explanation of these results is a little difficult in view of the fact that only food consumption and growth were measured and it is considered now apposite to review some of the publications relevant to the control of voluntary food intake.

Intake of food is closely related to the metabolic needs of the organism (Miner, 1955) and variation in the environment or dietary composition causes disturbances in appetite which will eventually settle to a new equilibrium position either similar to or distinct from the previous position. Paloheimo & Dickie (1965, 1966 a, b) have suggested that metabolic rate is proportional to food intake, a proposition which received support from the work of Edwards, Finlayson & Steele (1972) on the cod, Gadus morhua. Hence, any factor which changes the total metabolism of a fish will result in an altered food intake and probably

vice versa. However, it cannot be said whether appetite changes as a result of an alteration in metabolic rate or an alteration in another factor such as gastric evacuation or absorption rates. Wallace (1973) has suggested that feeding rate is more directly related to gastric evacuation rate than to overall metabolic rate. Gastric evacuation has been shown to increase in rate with a rise in temperature in Oncorhynchus nerka (Brett & Higgs, 1970) and in Salmo trutta (Elliott, 1972). Although rates of gastric evacuation and metabolism are undoubtedly associated there may be a degree of independence in the relationship. The two are possibly linked by the speed of release of gastric enzymes. The rate of secretion of digestive enzymes has been shown to be temperature dependent in Ictalurus nebulosus (Smit, 1967) and this may have resulted from alterations in metabolic rate caused by temperature. The rates of secretion of gastric enzymes will contribute to changes in rates of digestion (Brett et al., 1970) and absorption which may lead to alterations in the 'appetite drive'. It has been postulated that the degree of stomach distention provides one method of regulating the appetite (Rozin & Mayer, 1961; Brett, 1971a).

The caloric density of ingested food has been shown to affect the appetite of goldfish (Rozin et al., 1961). The alteration in appetite was probably a response to changes in the circulating levels of essential nutrients in the blood, for example, glucose. The homeostatic responses of intake of particular foods are linked with the levels of essential nutrients in the body in humans (Mayer, Gross & Walker, 1946) and the same is true of fish according to Rozin et al. (1961, 1964).

To summarise the various mechanisms which control food intake it appears that metabolic rate is the chief factor which influences

appetite possibly through the rate of evacuation of stomach contents together with the fluctuations in blood sugar level. Other minor modifying factors will exert some influence on voluntary food consumption and Grossman (1955) and Paloheimo & Dickie (1966a) hold the view that the mechanism controlling voluntary intake and energy balance cannot simply be related to the rate of metabolism but must involve both energy input and output. Hence, total energy expenditure results in a certain component, termed active metabolism, which gives rise to activity which in turn acts on food supply to produce a new ration which supplies the metabolism. This is termed a 'circular causal system' (Frank, 1948; Hutchinson, 1948). This works to maintain a dynamic balance in the animal body. Such a system would lead to new equilibrium states following a change in any part of the system.

Incorporating the theories of the control of voluntary food intake in fish with some considerations of the effects of salinity on metabolic rate is difficult. Rao (1968, 1971) found that both active and standard metabolic rates of rainbow trout were lower in 7.5 ppt sea water than in fresh water. The rates were equal in fresh and 20 ppt sea water and higher in 30 ppt than fresh water. If these results are relevant to the results of the present experiment then theoretically, trout held in 10 ppt should have a lower food consumption than trout in fresh water and trout held in 28/30 ppt should have a higher food consumption. This presupposes that metabolic rate and appetite are directly proportional. As no metabolic rate determinations were carried out in this experiment it is not possible to correlate food consumption with metabolic rate. One possibility is that salinity could have affected the rates of gastric absorption and evacuation to a greater extent than its

possible effect on metabolic rate. This theory is subscribed to by Wallace (1973) who suspected that feeding rate was more directly related to rate of gastric digestion than the rate of oxygen consumption.

The regulation of solute uptake by the gut is an active process and the concentration of sodium in the intestinal lumen has been shown to affect mucosal transfer of non-electrolytes in mammals (Curran, Schultz, Chez and Fuiz, 1967; Curran, Shultz & Goldner, 1971). The explanation for this is that trans-serosal sodium transport regulates transmural transport of non-electrolytes e.g. amino acids (Mepham & Smith, 1966). Factors such as transmural sodium fluxes, sugar fluxes and active transport of solutes in the gut are intimately associated and it is possible that fluctuations in intestinal sodium levels caused by sea water ingestion affect the rate of solute transport across the gut wall. If a change in solute transport occurred and the rate of gastric evacuation was affected then a change in the appetite drive might well have ensued. Confirmation of this hypothesis was attempted indirectly by measuring both the food intake and the metabolic rate of trout in sea water in the following experiment.

The most plausible explanation for the depression of the appetite of trout in 28/30 ppt sea water is that the trout were still suffering from some form of stress incurred during the earlier transition into 30 ppt. It is well documented that one of the first signs of stressful conditions is loss of appetite (Roberts & Shepherd, 1974). It is possible that the dissolved oxygen levels in this salinity were such that a depression in appetite ensued. In fresh water dissolved oxygen concentrations averaged 9.8 mg/l and in 28 ppt sea water averaged 8.0mg/l at those temperatures ($15 \pm 1^{o}C$). Several authors have shown that reducing the

dissolved oxygen content of water containing fish may lower the voluntary food consumption (Dahlberg, Shumway & Doudoroff, 1968; Stewart, Shumway & Doudoroff, 1967; Hermann, Warren & Doudoroff, 1962).

The results obtained from the experiment with the exception of the lowered appetite of fish held in 28/30 ppt compared with fresh water tend to agree with results of Kinne (1960) who found an increase in food consumption with increase in salinity up to 30 ppt for the desert pupfish, Cyprinodon macularius MacLeod (pers. comm.) also found an increase in food consumption with salinity increase up to 28 ppt in the rainbow trout. Otto (1971) found that salinity markedly influenced the food consumption rates of pre-smolt coho salmon. For the largest fish he used, food consumption was highest in 10 ppt and lowest in 20 ppt. This decline in consumption in the latter salinity might have been due to the fact that his fish were all less than 10 cm in length and so may have been under some osmotic stress in the upper salinities. Thus, there is general agreement between the present results and the results of the above authors.

Comparison of food consumption rates between weeks is difficult as fish size influences voluntary feeding rate (Wallace, 1973). Examination of Figure 14 reveals that fish in fresh water which had constant conditions over the whole experimental period, had declining weight-specific feeding rates with time even though the absolute food consumption rates increased with time. This is due to the fact that as fish grow there is an increasing proportion of metabolically 'inert' tissue present in the body and if the fish is to maintain a constant metabolic level it must gradually decrease its weight-specific food consumption rate. This applies only to

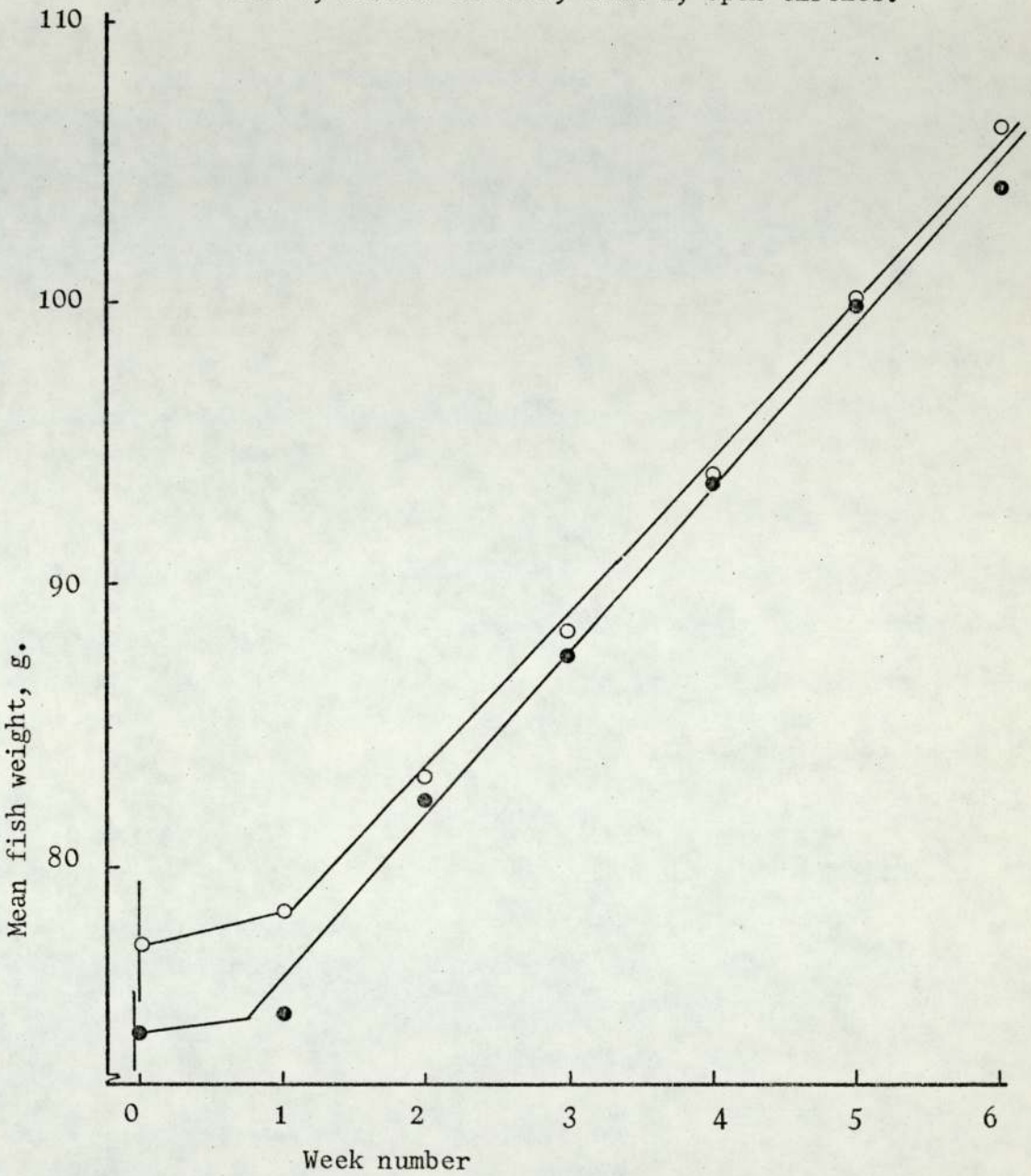
the growing fish with access to unlimited rations as in the present experiment.

Examination of the data on conversion ratios (Table 4) reveals that at low feeding rates during week 1 the conversion ratios were very low being 4.05 for tank 1 and 6.71 for tank 2. As the food consumption increased, the conversion ratios increased to levels ranging from 1.34 - 1.91. The explanation for this is that at low levels of food intake, a large proportion of the ingested food energy is metabolized for maintenance and activity purposes. Relatively little is available for growth. As food intake increases, so proportionately more of the ingested calories are available for conversion into fish tissue and the conversion ratio decreases.

A comparison of the mean weekly increases in the weight of fish between the two tanks is made in Figure 15. Lines of best fit have been drawn on the graph by using the method of Least Squares. If weeks 1 to 6 are examined it is evident that the increase in the mean fish weights every week followed a linear relationship. This is an acceptable result as the duration of the experiment was relatively short. Use of the method outlined in Wetherill (1972) for comparing gradients of lines revealed no significant difference in the gradients of the two lines. Thus, fish held in fresh water had a very similar overall rate of growth to fish held in waters of increasing salinity.

The period of time required for the two populations of trout to train themselves to demand feed was 9 - 10 days. This was in good agreement with the results of Adron et al. (1973) who also found 10 days to be the average training time for trout. In contrast, Landless (1976a)

Fig.15. Weekly changes in mean fish weight over the course of the experiment. At time 0, one standard error either side of the mean is shown.
Tank 1, closed circles; tank 2, open circles.



found that groups of trout took only 2 days to self-train themselves to a demand feeding routine. One possible explanation for this difference might be that Landless' fish were able to feed over a complete 24-hr period. In the present experiment a period of only 13.5 hr/day was used for demand feeding and in Adron's experiment, only 7 hr/day was used. Hence it appears that as the demand feeding period increases the time taken for a population of trout to self-train decreases.

In conclusion, a demand feeding system was successfully employed in the determination of the voluntary food consumption rates of trout in fresh water, 10 ppt, 20 ppt and 28/30 ppt. sea water. Salinity was found to have a pronounced effect on food consumption. High food consumption rates were apparent in 10 ppt and 20 ppt sea water. The reason for this was not determined but it was postulated that either a change in the metabolic rate or an alteration in the absorption rate of non-electrolytes in the gut was responsible. Voluntary food consumption rates were lower for trout in 28/30 ppt sea water than in fresh water. This was probably caused by relatively low dissolved oxygen concentrations in sea water together with the fact that fish may have been suffering mild osmotic stress. Overall growth rates were similar for trout in fresh water and in water of gradually increasing salinity. It was intended that further investigation of the effects of salinity on voluntary food consumption together with studies on metabolic rates should be made in the next experiment.

CHAPTER 4 - Experiment 2 - The Energetics of Growth and Metabolism of Trout in Fresh and Salt Waters

4.1. Introduction

The objective of this experiment was to correlate the growth of trout in waters of different salinity with the turnover of energy within the fish. A hypothesis was put forward which stated that any change in the metabolic rate of trout caused by the environmental salinity is reflected in a change in growth rate. In theory the environmental salinity may affect the metabolic rate of fishes in several ways, mostly through hormonal mediation.

a. Salinity and specific gravity of the water are directly proportional. This might lead to changes in the swimming effort and activity of fish. Salinity may affect the activity of fish by other means. Houston (1957, 1959b) found that the activity of Pacific salmon fry was immediately depressed following transfer to sea water of 22 p.p.t salinity. Although the level of activity recovered with time it never attained that found in fresh water. Similar results have been found by Muir and Niimi (1972). However contrasting results have been found with Sarotherodon (=Tilapia) nilotica (Farmer & Beamish, 1969) and rainbow trout (Rao, 1968); both findings have shown that salinity had no effect on swimming activity. In the present experiment the design of the metabolism tank took this factor into account. This will be discussed later.

b. Salinity may affect metabolic rate through a decreased dissolved oxygen content of the water.

c. A general physiological change may be induced by sea water following alteration in levels of circulating hormones. Several hormones have been implicated, for example thyroxine (Hoar, Black & Black, 1951; Smith, 1956; Hickman, 1959; Baggerman, 1960a and others). In their review of the literature on adrenocortical steroids, Chester Jones, Chan, Henderson and Ball (1969) show that these hormones are involved in regulating osmotic and metabolic levels in teleosts. Changes in the circulating levels of one or more hormones following sea water transfer may result in a change in metabolic activity. Brett (1965) has suggested that a change in metabolic slope (c.f. Appendix 1) may accompany the sea water phase presumably after smoltification.

d. Different salinities will affect the work performed by the organs of osmoregulation. This will result in an alteration in the quantity of energy devoted to osmoregulation which may be significant. If the energy demands for osmotic and ionic regulation are mitigated or intensified by ambient salinity then there may be a change in the total metabolic rate. It was this hypothesis which was tested in the present experiment.

According to Prosser (1955) independence by poikilotherms from any environmental factor is achieved either by adjustment of metabolism or regulation of the internal milieu. With regard to the first factor there is some controversy about the relationship between osmoregulation and metabolism, i.e. whether or not the cost of osmoregulation

is reflected by changes in the metabolic rate. Very often, contradictory results by different researchers have been obtained. The processes involved are probably more complex than envisaged at present and adaptation to different salinities may involve both changes in metabolism and in the internal milieu. Many factors may work to confuse the general picture and most of these have been referred to in Chapter 1 (1.2). For example passive adaptatory mechanisms in sea water may reduce the extent of alterations in the ionic and osmotic composition of body fluids following sea water transfer. Hence any proportionality between salinity and osmotic costs will be masked. Rao (1968) showed that the increase in the cost of regulation in sea water is not proportional to the increase in osmotic gradient. In addition, the size of the fish is an important factor when considering the energy devoted to osmotic regulation. It has long been realised that the larger the fish, the better its tolerance of sea water (c.f. Chapter 1). Smaller euryhaline fish are less capable of sea water tolerance as is supported by Rao (1971) who found smaller fish (25g) have a relatively greater standard oxygen consumption in 30 p.p.t. sea water than large fish (100 - 200 g). Job (1959) suggests that the advantage of an isosmotic medium conferred on a small fish is much larger than that conferred on a large fish which, by virtue of its size, is more capable of regulation. Finally, tolerance of changes in the internal milieu may reduce the need for metabolic adjustments. For example Rao (1969) found that the plasma osmotic concentration of rainbow trout was higher at low temperatures than at high. Both Rao (1969) and Gordon (1963) found an increase in plasma osmotic concentration with increase in external salinity.

Much research has been carried out on the changes in metabolic rate caused by salinity and the metabolic involvement in osmotic regulation. The following authors have investigated the oxygen consumption of various fish in different conditions. Those finding no effect of salinity on oxygen consumption include the following: Raffy (1932, 1933) with Anguilla anguilla, Bullivant (1960) with O. tschawytscha and Gordon et al. (1965) with Periophthalmus sobrinus. Muir and Niimi (1972) with Kuhlia sandvicensis found no significant difference in either active or standard rates of oxygen consumption in fresh water and 30 ppt sea water. Chittenden (1971) found no significant difference in total oxygen consumption of Morone saxatilis held in fresh water or 10 ppt sea water.

In contrast many authors have shown that salinity influences the total oxygen consumption of fish and their results generally can be divided into two categories. Category one includes those results which support the theory that the costs of osmotic and ionic regulation are in direct proportion to the osmotic gradient between the external medium and the body fluids of the fish. This would result in a minimum cost of osmotic regulation at the isosmotic point together with a gradual increase in costs on either side of the isosmotic point (Figure 16). The isosmotic point is taken as that value of the external salinity where the osmotic pressures of external and internal fluids are equal (Leivestad, 1965). Values for salinities which are isosmotic with the body fluids of various fish are given in Table 5. Thus the following work is included in category one. Farmer and Beamish (1969)

Fig.16. Diagram of the theoretical costs of osmotic and ionic regulation in different salinities
Solid line - results belonging to Category One
Broken line - results belonging to Category Two

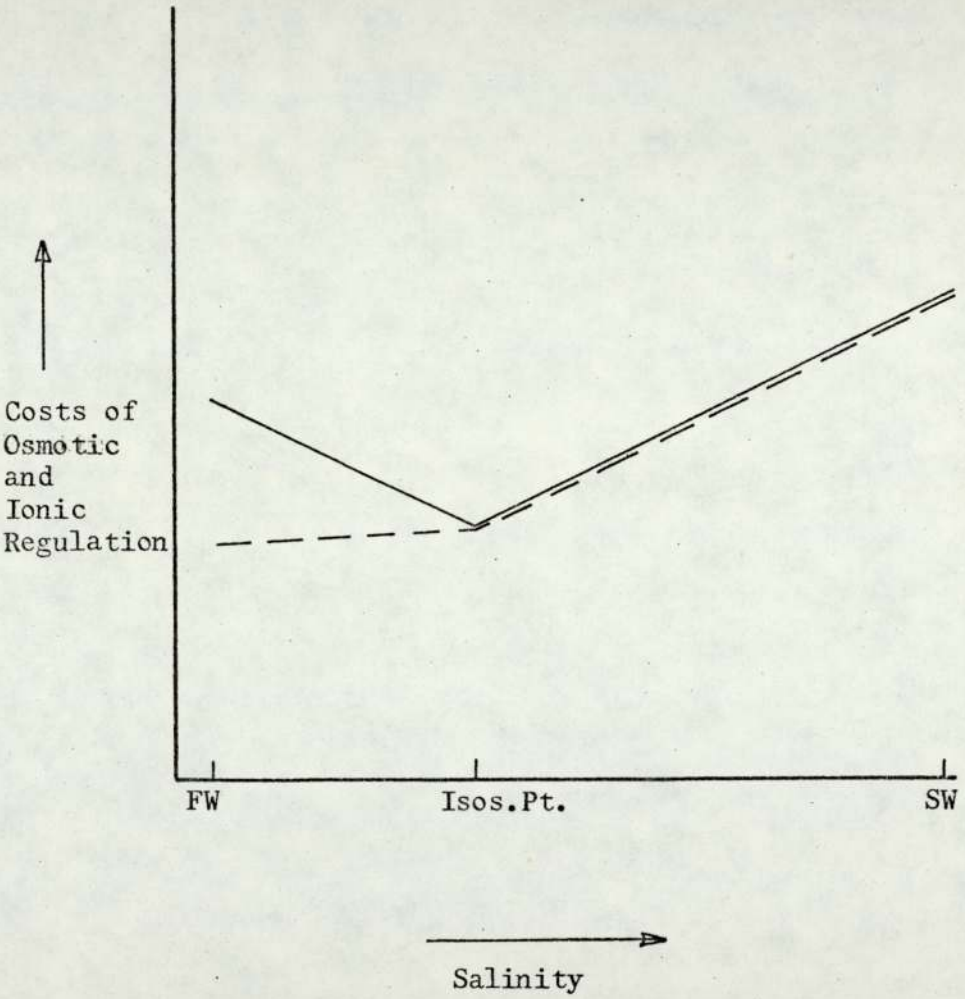


TABLE 5. Values of external salinity which are isosmotic with the body fluids of fish.

Species	Author	Value p.p.t.
Salmonids		
Salmo gairdneri	Rao (1968, 1969, 1971)	7.5
Salmo salar	Byrne et al. (1972)	10.0
Salmo gairdneri	Stokes et al. (1964)	10.1 (295 mOsm/l)
Oncorhynchus tschawytsha	Coche (1967)	10 - 13
Salmo gairdneri	Gordon (1963)	9.2 (270 mOsm/l)
Other species		
General	Brett (1962)	9 - 14
Poecilia reticulata	Gibson et al. (1955)	8.6
Plotosus anguillaris	Job (1959)	12.5
S. mossambica	Job (1969a.b)	12.5

found the oxygen consumption of Sarotherodon nilotica lowest at 11.6 p.p.t., identical in fresh water, 7.5 and 22.5 p.p.t. and highest in 30 p.p.t. sea water. They concluded that the magnitude and not the direction of the osmotic gradient between the body fluids of the fish and the external medium was important in determining energy requirements for osmotic regulation. Beamish (1970) found that the oxygen consumption of Sarotherodon nilotica was higher in both fresh water and 30 p.p.t. sea water than at the isosmotic salinity, 11.6 p.p.t.. Veselov (1949: from Black, 1951) recorded a 60% reduction in the uptake of oxygen of goldfish in 10 - 15 p.p.t. sea water over fresh water. Job (1959) found a substantial reduction in the standard metabolism of Plotosus anguillaris in sea water of 12.5 p.p.t., the isosmotic value for this salinity. His figures show that the cost of osmotic regulation is significantly manifested in metabolic rate and that changes in oxygen consumption in different salinities are positively related to changes in standard metabolism. With reference to the rainbow trout, Busnel, Drilhon & Raffy (1946: from Black, 1951) demonstrated that alevins and fry had an oxygen consumption 8% lower in a 5 p.p.t. NaCl solution than in fresh water and a 35% lower consumption in 10 p.p.t. NaCl solution. Rao (1968, 1971) has undertaken a comprehensive study of the interactions of activity, temperature and salinity on the oxygen consumption of rainbow trout. He found that at all levels of activity the lowest rates of oxygen consumption were found at 7.5 p.p.t and these rates increased in salinities on either side of this isosmotic point. He concluded that the differences in metabolic rate at a given level of activity in various salinities could be attributed to the cost of osmotic regulation.

Category two of results of the interaction of salinity and metabolic rates involve those where the costs of osmotic regulation are least in fresh water and greatest in sea water. The theory behind these findings is that osmoregulatory mechanisms function very efficiently and the energy expended on osmotic regulation is a small proportion of the total energy consumed (Potts, 1954). Thus the energy expended on osmotic and ionic regulation in fresh and brackish waters is so small that changes caused by alterations in external salinity will be masked by the overall metabolic expenditure. Nordlie and Leffler (1975) believe that the energy expended on ionic and osmotic regulation starts to increase once ingestion of sea water is started. In this case much salt will be absorbed through the gut and more energy will have to be expended to remove this load. Hence, past a certain point (10 - 15 p.p.t) the energetic costs of regulation increase with increasing salinity (Figure 16).

In support of this theory are results of Nordlie et al (1975) and and Hickman (1959), the latter author finding that the standard metabolic rate of Platichthys stellatus was least when acclimated to fresh water and greatest in sea water. He also relates the increased rate of metabolism in sea water to increased thyroid activity. The results of Shaw et al. (1975) indirectly support the theory in that changes in ambient salinity between 0 and 20 p.p.t. caused little difference in the growth rate of S. salar which may have shown that costs of regulation did not vary much in these salinities. This latter theory may not have such widespread application as might at first be supposed in that some authors have demonstrated that the costs of osmotic regulation and hence standard metabolism are not similar in fresh and brackish waters.

Farmer et al. (1969) showed with S. nilotica that if the energy requirement for osmotic regulation was zero at the isosmotic salinity (11.6 p.p.t) then 29% of the total oxygen consumption was required for osmotic regulation in 30 p.p.t. and 19% at 0, 7.5 and 22.5 p.p.t. The values for the proportion of the oxygen consumption required for osmotic regulation will be higher than these figures as even at the isosmotic salinity, energy expended on ionic and osmotic regulation will be measurable. Hence up to 30% of the total oxygen consumption can be attributed to ionic and osmotic regulation according to salinity. Rao (1968, 1971) calculated that in fresh water for the rainbow trout, 20% of the metabolism is involved in osmotic regulation while 27% is involved in 30 p.p.t. sea water. These figures are very similar to those of Farmer et al. (1969).

Despite the difference between the two theories, they have the similarity that the cost of osmotic regulation is lower at about 10 p.p.t. than in full sea water. In marked contrast to this are the results of Job (1969a, b) who found with S. mossambica that the oxygen uptake was greatest at the isosmotic salinity (12.5 p.p.t). He attributed this to the fact that the standard metabolic rate was lowest at this point which led to a greater scope for activity, this being the difference between active and standard metabolism. This, he theorised, would lead to a greater level of activity at the isosmotic salinity which would result in an overall higher total oxygen consumption. However, this was conjectural as he apparently did not measure the activity level of his fish. Contrasting results were found by Rao (1971) who found that the scope for activity of rainbow trout was reduced at intermediate salinities.

Thus it is apparent that there are marked differences in the re-

sponses of fish to different external salinities and as a result of this a number of theories have been advanced concerning the extent of the metabolic involvement in ionic and osmotic regulation. It was the aim of the present experiment to measure the total oxygen consumption of the rainbow trout in waters of different salinity and to attempt to correlate any changes in metabolic rate with changes in growth rate. This was attempted by constructing comprehensive energy-turnover equations for fish in different salinities at three different ration levels. The energy-turnover equation used was similar to that used by Solomon and Brafield (1972).

$$I = F + U + R + \Delta B$$

where I = Caloric intake

F = Calories voided in faeces

U = Calories excreted as non-faecal waste

R = Calories respired

ΔB = Calories deposited as growth.

4.2. Basic Considerations

Oxygen Consumption

According to the second law of thermodynamics the liberation of heat is a necessary accompaniment of any transformation of energy even when performed at maximum efficiency. Therefore, the transformation of energy involved in metabolism is also accompanied by heat liberation. Thus, metabolism may be measured by direct calorimetry in which the amount of heat liberated is determined. Direct calorimetry is very difficult to perform with fish because of the high specific heat of water and the low metabolic rate of poikilotherms.

Rubner (from Brody, 1945) introduced the method of indirect calorimetry whereby oxygen uptake is used as a measure of metabolic rate. This method is valid as long as oxygen debt does not occur and the proportion of metabolic substrates oxidised can be estimated as different substrates require varying quantities of oxygen for their oxidation. With regard to the first point, Kutty (1968) showed that when dissolved oxygen concentrations were adequate (>50%) rainbow trout were aerobic even when performing intensive work. Thus, indirect calorimetry provides a measure of metabolic rate in terms of the quantity of oxygen consumed by a given weight of fish per unit time.

Respiratory metabolism is usually reported as standard, routine and active rates depending on the level of activity of the fish (Fry, 1957). Standard metabolism is the minimum rate of oxygen consumption in the absence of activity and is usually measured after 24-hr of fasting. Routine metabolism is usually taken as the oxygen consumption of fish during 'free' or spontaneous activity in the respirometer. Finally, active metabolism is the maximum rate of oxygen consumption consist-

ent with the highest sustained rate of activity and it has a characteristic level for any temperature. Often there have been problems in defining this maximum level of sustained activity which has led to slightly different experimental approaches among researchers.

None of the above definitions of metabolic rate was used in the present experiment. Usually, when determining active and standard metabolic rates fish are starved prior to the test. When determining routine metabolic rates the activity of the fish is uncontrolled even though it is usually measured. In the present experiment it was decided to adopt the methods and respirometer design of Brett, Sutherland and Heritage (1971) where the fish tank in which the fish feeds and grows may be used as a respirometer. Activity was maintained at a constant low level by the response of the fish to a circular water current and feeding was practised as normal. Hence, fish were not disturbed by removing them to a respirometer and back, and increases in oxygen consumption as a result of feeding could be measured. This approach is of great value when examining long-term energy budgets. Hence, six of the twelve available tanks were modified and used as combined metabolism/growth tanks as described in Chapter 2. The tanks were used intermittently to determine the oxygen consumption of a number of fish swimming and feeding normally and the values obtained converted into the calories respired by using an oxy-calorific coefficient.

The Oxy-calorific coefficient

Accurate conversion of the quantity of oxygen consumed by an animal into the amount of calories oxidised and lost as heat is possible if an accurate oxy-calorific coefficient (Q_{ox}) is used as a multiplying

factor. In theory, the value of the Q_{ox} will depend on the proportions of the three respiratory substrates (protein, fat, carbohydrate) respired and the end-product of biological oxidation. Brafield & Solomon (1972) give Q_{ox} values of 3.2 cal/mg oxygen respired for protein respiration, 3.28 for fat and 3.53 for carbohydrate. With regard to the latter point, an animal which respires protein may excrete its nitrogenous waste in several different forms, the main three being uric acid, urea and ammonia. The proportion of these depends on species and food quality and quantity together with environmental condition. For example, if ammonia is produced from the oxidation of protein, 4.913 Kcal/g protein will be lost as heat. If urea is produced, 4.834 Kcal/g.protein are lost as heat (Brafield & Solomon, 1972). This is due to the greater molecular complexity of urea with a resultant greater bond energy. This complication is probably trivial in the case of fish as Brett (1962) has suggested that 98% of protein nitrogen is excreted as ammonia. Hence the amount of urea and other nitrogenous waste products will probably be small. Uric acid, creatine and creatinine are listed by Black (1957) on nitrogenous waste products occurring in small quantities.

The accurate determination of the proportions of substrates respired is a difficult technique which can involve simultaneous measurements of oxygen consumption and ammonia and carbon dioxide liberation. Therefore certain assumptions are often made. For example, Solomon and Brafield (1972) assumed that in well fed fish substrates will be respired in the same proportions as occur in the food. This will result in a reasonable estimation of the true Q_{ox}. In the present case, if substrates are respired in the same proportions as occur in trout pellets

a Q_{ox} of 3.35 cal/mg oxygen is produced. In the present experiments it was decided that no such assumptions would be made and a Q_{ox} would be used which had previously received support from several authors. Therefore in all experiments when converting mg of oxygen consumed into calories respired a Q_{ox} of 3.42 was used. This value has been cited by Brody (1945) and Kleiber (1961) and has been deemed valid for fish by Winberg (1956) and Averett (1969). Brody (1945) has also mentioned that there is comparatively little variation in this value with changes in the biochemical composition of the respiratory substrate.

Values obtained for the oxygen consumption of fish in mg/hr can be used also to estimate the energy of excreted materials according to Brafield and Solomon (1972). They state that where ammonia is the principal nitrogenous excretory material a conversion factor of 0.51 cal/mg O_2 consumed will give an accurate estimate of the calories excreted as ammonia. Therefore this method was used in the formation of energy budgets for the following experiments.

The Energy Balance Equation

An equation was constructed which related the energy ingested as food to the energy expended as metabolism and retained as growth. The balanced equation of Winberg (1956) was considered to be too assumptive for the specific needs of this experiment. Winberg's equation is:-

$$Qc = 1.25 (Qr + Qg)$$

where

Qc = Energy of the ration

Qr = Energy of metabolism

Qg = Energy of weight increase.

This equation assumes that 0.8 of the gross energy of the ration is metabolisable where the metabolisable energy is taken as the food consumed minus the energy lost through faeces and excreted waste (Brody, 1945; Kleiber, 1961). The metabolisable energy is that portion of the gross energy which is capable of transformation in the body (Maynard & Loosli, 1969). As environmental salinity might have influenced the digestibility of the ration or the nitrogen-balance of the fish so altering the factor of 0.8 it was decided that a more comprehensive equation would be needed.

Accordingly an adaptation of the equation described by Warren and Davis (1967) was used which made no assumption as to the metabolisable energy of the food. Warren and Davis's equation is:-

$$Qc - Qw = Qg + Qr$$

where

Qc = Energy of the ration

Qw = Energy of faeces and non-faecal waste

Qg = Energy of weight increase

Qr = Energy metabolically utilised.

The equation used in the present experiment divides 'Qw' into two components; the energy of the faeces (Qf) and the non-faecal energy, Qe, which comprises the energy of ammonia, urea, and other nitrogenous excretory waste. Hence:-

$$Qc = Qg + Qr + Qf + Qe$$

This equation is similar to that used by Solomon and Brafield (1972).

All components of the above equation were determined directly except for 'Qe' which was estimated. Accordingly, provision was made in the construction of the experimental system for adequate measurements of faecal output and respiratory rates (Chapter 2, Section 4). In addition, growth and food intake was accurately measured and energy-balance equations were drawn up for fish in different salinities fed at three different ration levels.

4.3. Materials and Methods

The experimental system used in this experiment together with the design of the metabolism/growth tank have been described in full in Chapter 2. Briefly, each of the two recirculating systems included six, two-foot diameter fish tanks; three of each six had been adapted for use as metabolism/growth tanks and the remaining tanks used only for growth rate determinations. The water temperature in both systems was 12 $\pm$0.5^{o}C and photoperiod was set at 14 hrs. on, 10 hrs. off. As in the previous experiment, one system held fresh water throughout the experiment and was used as the control. The other system held in turn three different concentrations of sea water depending on the phase of the experiment. All fish were fed on 'Beta' floating trout pellets, grade number 4.

An experiment was designed to determine the growth and metabolism of rainbow trout at three different feeding rates when held in fresh water, 10 p.p.t., 20 p.p.t and 28 p.p.t sea water. This undertaking was divided into three phases; experiment 1 which compared growth and metabolism of fish held in fresh water and 10 p.p.t. sea water; experiment 2 which compared fresh water and 20 p.p.t. sea water; experiment 3 which compared fresh water and 28 p.p.t. sea water. It will be helpful if Tables 6, 7 and 8 are consulted. These show most of the detail which is now elaborated.

Three ration levels were chosen as providing a good range of food intake. These were 1.0% and 1.5% of the live body weight per day and repletion feeding. It was planned that the results of feeding fish to repletion would be valuable for comparison with the results of the previous experiment. Examination of Tables 6, 7 and 8 reveals that

Experiment 1

Salinity	Type of Tank	Ration Level	Number of Metabolism Tests		
			Fortnight		
			1	2	3
Fresh	Growth/Metab.	1%	12	12	12
	Growth/Metab.	1.5%	12	12	12
	Growth/Metab.	Repletion	12	12	12
	Growth	1%	-	-	-
	Growth	1.5%	-	-	-
	Growth	Repletion	-	-	-
10 p.p.t	Growth/Metab.	1%	12	12	12
	Growth/Metab.	1.5%	12	12	12
	Growth/Metab.	Repletion	12	12	12
	Growth	1%	-	-	-
	Growth	1.5%	-	-	-
	Growth	Repletion	-	-	-

Table 6. Experiment 1. Duration 42 days. The allocation of feeding rates among the 12 tanks of fish together with the distribution of metabolism tests between fortnights.

Experiment 2

Salinity	Type of Tank	Ration Level	Number of Metabolism Tests		
			Fortnight		
			1	2	3
Fresh	Growth/Metab.	1%	12	12	12
	Growth/Metab.	1.5%	12	12	12
	Growth/Metab.	Repletion	12	12	12
	Growth	1%	-	-	-
	Growth	1.5%	-	-	-
	Growth	Repletion	-	-	-
20 p.p.t	Growth/Metab.	1%	12	12	12
	Growth/Metab.	1.5%	12	12	12
	Growth/Metab.	Repletion	12	12	12
	Growth	1%	-	-	-
	Growth	1.5%	-	-	-
	Growth	Repletion	-	-	-

Table 7. Experiment 2. Duration 40 days. The allocation of feeding rates among the 12 tanks of fish together with the distribution of metabolism tests between fortnights.

Experiment 3

Salinity	Type of Tank	Ration Level	Number of Metabolism Tests	
			Fortnight	
			1	2
Fresh	Growth/Metab.	1%	12	12
	Growth/Metab.	1.5%	12	12
	Growth/Metab.	Repletion	12	12
	Growth	1%	-	-
	Growth	1.5%	-	-
	Growth	Repletion	-	-
28 p.p.t	Growth/Metab.	1%	12	12
	Growth/Metab.	1.5%	12	12
	Growth/Metab.	Repletion	12	12
	Growth	1%	-	-
	Growth	1.5%	-	-
	Growth	Repletion	-	-

Table 8. Experiment 3. Duration 28 days. The allocation of feeding rates among the 12 tanks of fish together with the distribution of metabolism tests between fortnights.

each ration level was fed in duplicate within one salinity, in other words at one salinity the 1% ration was fed to fish in a growth tank and a metabolism/growth tank. Similarly with the 1.5% and repletion ration levels. The three feeding levels were allocated within each 'block' of three tanks by using tables of random numbers.

Each experiment was divided into fortnightly periods for statistical purposes. Thus experiment 1 (fresh water:10 p.p.t) was divided into three fortnights, experiment 2 (fresh water:20 p.p.t) also into three fortnights and experiment 3 (fresh water:28 p.p.t) into two fortnights (Tables 6, 7 and 8). The weight of fish in every tank was determined after each fortnight. This will be described in more detail in the experimental procedure. In each experiment, twelve separate metabolic rate determinations were made every fortnight on each of the metabolism/growth tanks. This will be described in more detail in the experimental procedure. Finally, faeces were collected daily from the faecal traps beneath each of the metabolism/growth tanks.

The four major components of the energy-balance equation, i.e. caloric intake, calories retained as growth, calories respired, and calories lost as faeces, enabled complete energy budgets to be drawn up for each population of trout in each of the metabolism/growth tanks.

Experimental Procedure

The experimental procedure was the same for all three experiments and the execution of experiment 1 will be described as an illustration. Reference should be made to Table 9.

At the commencement of the first experiment 264 trout of approximately the same weight, 85 g each, were weighed by the 'batch'

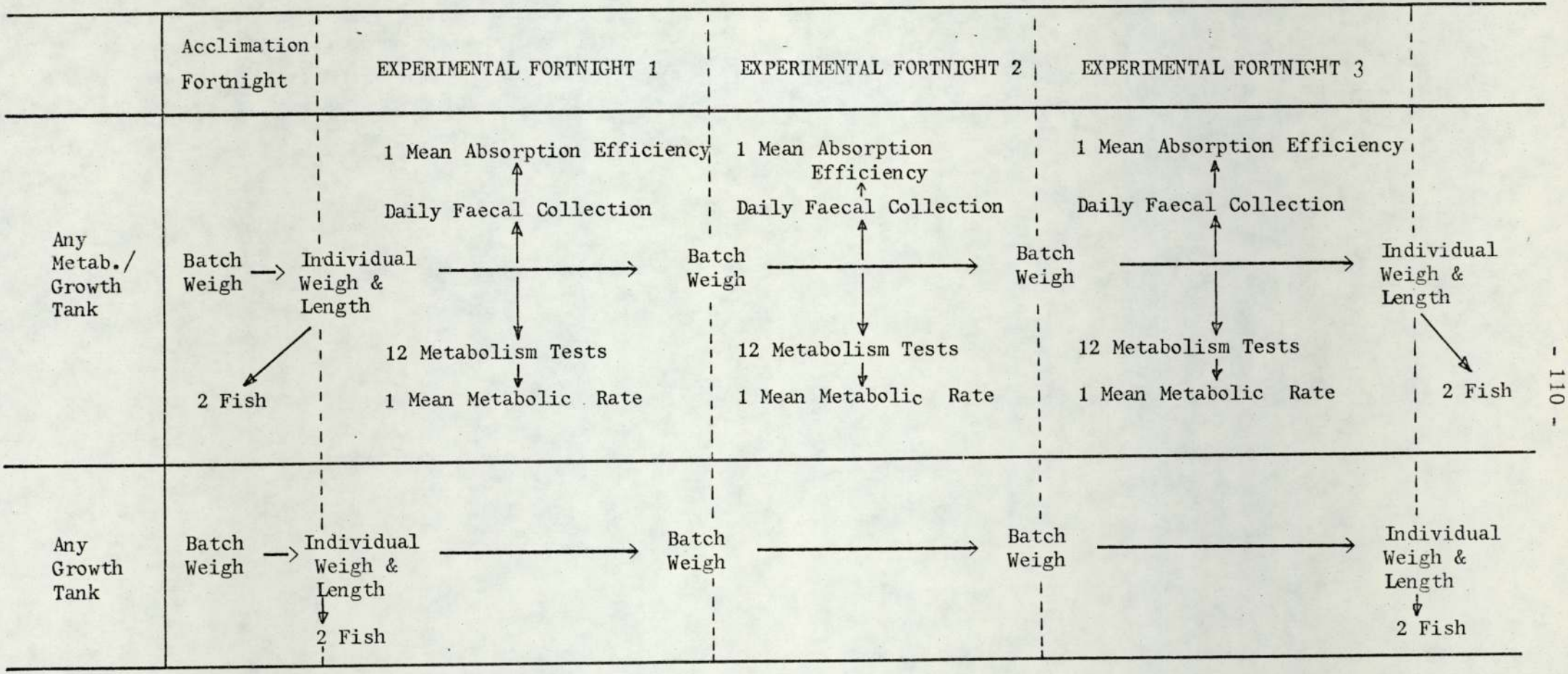


Table 9. Experimental procedure for any one metabolism/growth tank and any one growth tank during an experiment.

Note - There were only 2 Experimental Fortnights during Experiment 3.

method described in Chapter 2. Twenty-two were allocated to each fish tank. One system was holding fresh water and the other 10 p.p.t sea water. Trout were put directly into this latter salinity without any apparent ill effects. The fish were then acclimated to the general conditions over one fortnight and fish were fed at the ration level previously allocated. All fish were fed three times per day at the following times: 9.30 a.m., 2.00 p.m. and 5.30 p.m. The four tanks of fish which were fed to repletion were treated as follows. At every feed, pellets were offered until the appetites of the fish had visibly decreased. The appetites were considered to be satiated when two to four pellets were left floating on the surface of the water. These pellets were not recovered as they were invariably eaten within 15 min. of cessation of feeding. It was anticipated that feeding each tank of fish to repletion in the same way would yield results valid for comparative purposes.

Following the preliminary acclimation fortnight the experiment was started. Fish were starved for 12-hr and then weighed individually under anaesthetic using the method described in Chapter 2. At the same time their fork lengths were determined to the nearest 1 mm on a measuring board. Two fish from each tank were retained, killed by a blow on the head and deep-frozen for future proximate carcass analysis. The weight of food fed per tank per day was increased after each fortnightly weighing in order to keep to the feeding rates allocated previously to each tank. All fortnightly weighings with the exception of the first (at the start of experimental fortnight 1) and the last were conducted on a 'batch basis'. Individual weighing even when performed under anaesthetic tends to be stressful to fish. This was to be

particularly avoided for the sea water fish where slime can be removed by handling. This mucus is important in reducing skin permeability to ions and water. Individual handling has also been shown to induce 'laboratory diuresis' in fish (Holmes, 1961) and again this might be detrimental to the performance of sea water fish. After the third fortnight, it was necessary to weigh and measure fish individually. Again two fish from each tank were retained, killed and deep frozen for future proximate carcass analysis. At this point, experiment 1 was terminated and the fish were removed from the experimental systems. Examination of Table 9 should clarify any further description of experimental procedure. This shows the execution of various determinations on any one metabolism/growth tank and any one growth tank during the three experiments.

During the course of each experiment faeces were collected daily from the faecal traps of the metabolism/growth tanks. They were siphoned into glass beakers and then filtered through a 21 cm Buchner filter funnel, air-dried for 24 hr. and then weighed. After weighing, aliquots from each sample were collected and ground with a pestle and mortar and the calorific content determined in a Ballistic Bomb Calorimeter (A. Gallenkamp & Co. Ltd., Christopher St., London) using benzoic acid as standard.

During the course of each experiment metabolic rate determinations were performed on fish in the metabolism/growth tanks. The specific procedure has been detailed in Chapter 2. Simultaneous determinations were made on two tanks, one fresh water, the other sea water and, as each determination lasted one hour, four determinations could be made per day on each tank. During every fortnight, three days of tests

were allocated to each of the six metabolism/growth tanks. Thus, a total of twelve metabolic rate determinations was performed every fortnight on each of the metabolism/growth tanks of fish (Tables 6 and 9).

It had been found previously that a slight fall occurred in the total metabolic rate of fish during the hours of darkness. Therefore it was considered necessary to adjust the experimental daytime metabolic rate results by a suitable correction factor. A correction factor for each ration level was determined in Chapter 6. An improvement in the design of the metabolism chamber permitted greater accuracy of measurement in this later experiment. Accordingly all the daily metabolic rate values were reduced by a factor of 6.15%. This was the average percentage reduction in total metabolic rate during the hours of darkness. There was little difference in this reduction at different feeding rates.

In between experiments, the performance of the metabolism/growth tanks was checked by performing 'blank' determinations with no fish in the chamber. On all occasions it was shown that no change occurred in the dissolved oxygen content of the circulating water. Thus no errors were introduced by the experimental equipment.

The other experiments of the series, i.e. fresh water ; 20 p.p.t and fresh water : 28 p.p.t. were undertaken in the same manner. Different fish were used in each experiment and careful acclimation of trout to higher salinities was practised. It was found that trout of approximately 80 g each could be transferred directly from fresh water to 10 p.p.t sea water with no ill effects. Acclimation to higher salinities involved increasing the salinity by increments of 5 p.p.t.

over two to three days. Despite care being taken in acclimating trout to 28 p.p.t. sea water before the experiment began, several mortalities occurred after four weeks of the experiment and it was decided to terminate this experiment after the fourth weighing (Tables 8 and 9).

Proximate Carcass Analysis

Proximate carcass analysis was carried out on deep frozen fish using the following procedures. Firstly, lipid extraction was carried out using the method of Bligh and Dyer (1959) which involves homogenising approximately 100 g wet fish with chloroform, methanol and water. Filtering the resulting homogenate through a 14 cm Buchner filter gave a filtrate which contained approximately 95% of the total lipid. Aliquots of the filtrate were taken and the solvent evaporated off in a rotary evaporator. The weight of the lipid in the aliquots was determined and by knowing the total volume of the filtrate, the total lipid weight extracted was found. The residue left in the filter was then carefully dried in an oven at 60°C for 12 hr and the dry weight determined by weighing on a balance accurate to 0.1 g. This material was then ground finely with mortar and pestle and the remaining lipid was extracted by subjecting the whole of the residue to the Soxhlet method of lipid extraction using di-ethyl ether as the solvent. This weight of lipid was determined and added to the previous lipid weight. The weight of the lipid free residue was then measured.

Summing the lipid/moisture free carcass weight and the total lipid weight and subtracting the total from the initial wet weight of the fish gave the moisture content of the fish indirectly. It was anticipated that this method for estimating moisture content was more reliable than a

direct method involving oven-drying of the whole fish. This method may involve the loss of lipid from the fish tissue.

After air drying the lipid/moisture free residue to remove all the di-ethyl ether aliquots were taken for protein and ash determinations. The latter determinations were made by heating samples in triplicate in a muffle furnace (FR 610 A. Gallenkamp & Co. Ltd., Christopher Street, London) at 580^{o}C for four hours. Protein determinations were made by measuring the total nitrogen content of triplicate samples using the micro-Kjeldahl technique (A.O.A.C. Methods, 1970) and applying the factor of 6.25 to the results to convert total nitrogen weights to protein.

4.4. Results

In order to facilitate comparison, separate examination of the results of growth, metabolism and faeces will be made before the energy-balance equations are constructed.

Growth

The food consumption, growth rates and conversion ratios of fish in all three experiments are shown in Tables 10, 11 and 12. The formulae used in calculating specific growth rates and conversion ratios have been detailed in Chapter 3. These parameters were calculated for each tank of fish in each experiment over the duration of each experiment. As each ration level was duplicated within each salinity, statistical comparisons of both specific growth rates and conversion ratios between salinities could be made. Analysis of variance followed by Students 't' tests were used in the analysis.

Examination of the results of fresh water and 10 p.p.t. sea water, Table 10, reveal that conversion ratios were lower for fish in 10 p.p.t. sea water than for fish in fresh water at all ration levels. At the repletion ration levels this difference was significant ($p < 0.01$). Specific growth rates were slightly higher for fish in 10 p.p.t. sea water than fish in fresh water at all ration levels. This difference was significant at the repletion feeding level ($p < 0.05$). The lack of significance between growth rates in fresh water and 10 p.p.t sea water at the 1% and 1.5% ration levels is probably caused by the logarithmic nature of the specific growth rate equation reducing the possible differences.

Examination of the results of fresh water and 20 p.p.t sea water, Table 11, reveals that the average growth rates and conversion ratios

Table 10. Experiment 1. Fresh water:10 p.p.t sea water.
Duration of experiment - 42 days

Food consumption, growth rates and conversion ratios of fish fed at three feeding rates in two salinities

Salinity	Feeding Rate % Body Weight/day	Total Initial Weight of fish per tank, g.	Total Final Weight of fish per tank, g.	Total Food consumed, g./tank	Mean conversion Ratio n = 2	Probability	Mean Specific Growth Rate %/day. n = 2	Probability
Fresh	1.0	1761	2294	809.7	1.51	Not sig.	0.635	Not sig.
	1.0	1796	2347	826.9				
10 p.p.t	1.0	1792	2350	826.1	1.47		0.645	
	1.0	1776	2337	819.7				
Fresh	1.5	1831	2769	1332.5	1.41	Not sig.	0.99	Not sig.
	1.5	1798	2734	1311.1				
10 p.p.t	1.5	1789	2721	1304.5	1.385		1.01	
	1.5	1781	2732	1302.7				
Fresh	R[a]	1752	2663	1703.0	1.86	<0.01	1.025	<0.05
	R	1769	2753	1821.3				
10 p.p.t	R	1753	2717	1716.2	1.74		1.09	
	R	1759	2838	1835.2				

a R = Repletion feeding level

were very similar between salinities when comparing equivalent feeding rates. No significant differences were found except between the means of the specific growth rates at the repletion level of feeding. Specific growth rate was significantly higher ($p < 0.05$) for fish in 20 p.p.t sea water than for fish in fresh water. This was probably due to a slightly higher feeding rate by fish in one of the 20 p.p.t sea water tanks.

Examination of the results of fresh water and 28 p.p.t sea water, Table 12 reveals that at the 1.0% and 1.5% feeding rates the mean conversion ratios were lower in fresh water than in sea water. These differences were significant ($p < 0.001$). At the repletion feeding level the mean conversion ratios were lower in sea water than in fresh. This difference was significant ($p < 0.01$) and was probably a result of the lower food consumption rates of fish in 28 p.p.t sea water. Specific growth rates were higher for fish in fresh water than in 28 p.p.t sea water at all equivalent ration levels. These differences were significant ($p < 0.01$ and $p < 0.001$) at the 1.0% and 1.5% feeding rates respectively and significant ($p < 0.001$) at the repletion feeding level. No significant difference was found between growth rates at the 1.5% and repletion feeding levels in 28 p.p.t sea water.

As feeding rate increased, the specific growth rate of fish increased in all salinities. Differences in specific growth rates between adjacent feeding rates were always significant ($p < 0.001$) with two exceptions:

a. the growth rates at the 1.5% and repletion feeding levels in 28 p.p.t sea water.

b. the growth rates at the 1.5% and repletion feeding levels in fresh water, experiment 1. In all salinities the conversion ratios were

Table 11. Experiment 2. Fresh water:20 p.p.t sea water.
Duration of Experiment - 40 days.
Food consumption, growth rates and conversion ratios of fish fed at three feeding rates in two salinities

Salinity	Feeding Rate % Body weight/day	Total Initial Weight of fish per tank, g.	Total Final Weight of fish per tank, g.	Total Food consumed, g./tank	Mean Conversion Ratio n = 2	Probability	Mean Specific Growth Rate % /day n = 2	Probability
Fresh	1.0	1852	2372	806.2	1.535	Not sig.	0.625	Not sig.
	1.0	1862	2396	811.9				
20 p.p.t	1.0	1882	2430	821.5	1.53		0.625	
	1.0	1881	2405	818.3				
Fresh	1.5	1873	2739	1282.0	1.44	Not sig.	0.975	Not sig.
	1.5	1870	2791	1289.4				
20 p.p.t	1.5	1878	2760	1287.8	1.435		0.975	
	1.5	1865	2776	1284.7				
Fresh	R [a]	1879	2849	1824.4	1.85	Not sig.	1.03	<0.05
	R	1861	2804	1716.9				
20 p.p.t	R	1869	2900	1916.9	1.845		1.06	
	R	1877	2822	1730.5				

a. R = Repletion feeding level

Table 12. Experiment 3. Fresh water: 28 p.p.t sea water.
Duration of experiment - 28 days.
Food consumption, growth rates and conversion ratios of fish fed at three feeding rates in two salinities

Salinity	Feeding Rate % Body Weight/day	Total Initial Weight of fish per tank,g.	Total Final Weight of Fish per tank,g.	Total Food consumed, g./tank	Mean Conversion Ratio. n = 2	Probability	Mean Specific Growth Rate % /day. n = 2	Probability
Fresh	1.0	1873	2234	548.6	1.535	<0.001	0.625	<0.01
	1.0	1795	2134	525.3				
28 p.p.t	1.0	1865	2170	542.7	1.785		0.55	
	1.0	1871	2175	544.3				
Fresh	1.5	1896	2481	853.6	1.47	<0.001	0.955	<0.001
	1.5	1846	2407	830.3				
28 p.p.t	1.5	1872	2389	837.2	1.65		0.855	
	1.5	1868	2364	833.6				
Fresh	R[a]	1885	2535	1253.8	1.91	<0.01	1.045	<0.001
	R	1886	2518	1195.2				
28 p.p.t	R	1866	2383	946.0	1.85		0.875	
	R	1883	2409	983.1				

a R = Repletion feeding level

lowest at the 1.5% ration level and highest at the repletion ration level.

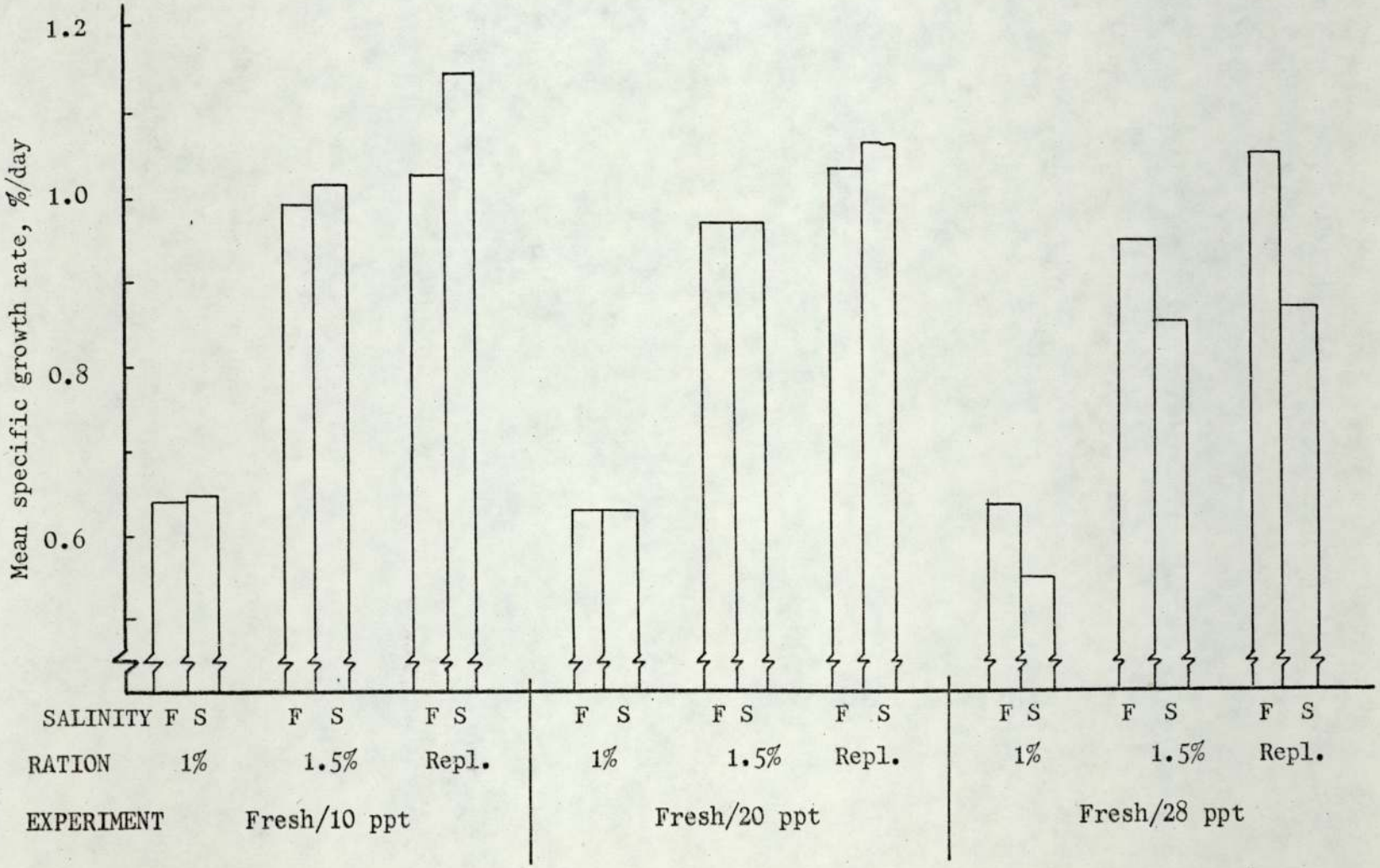
These results are shown in histogram form in figures 17 and 18. Figure 17 shows the mean specific growth rates of fish at different feeding rates in all three experiments. Figure 18 shows the mean conversion ratios of fish at different feeding rates in all three experiments.

Some problems were encountered in the sea water system in the fifth week of experiment 3, fresh water and 28 ppt sea water. At the beginning of the experiment it was noticed that fish in the sea water system had slightly lower appetites than was anticipated even though fish had been acclimated carefully to that salinity. At the start of the fifth experimental week the appetites became even more depressed and several of the fish turned a dark colour. A few days later, several fish died and it was then decided to terminate the experiment. Immediate individual weighing and length determination were undertaken.

Food consumption

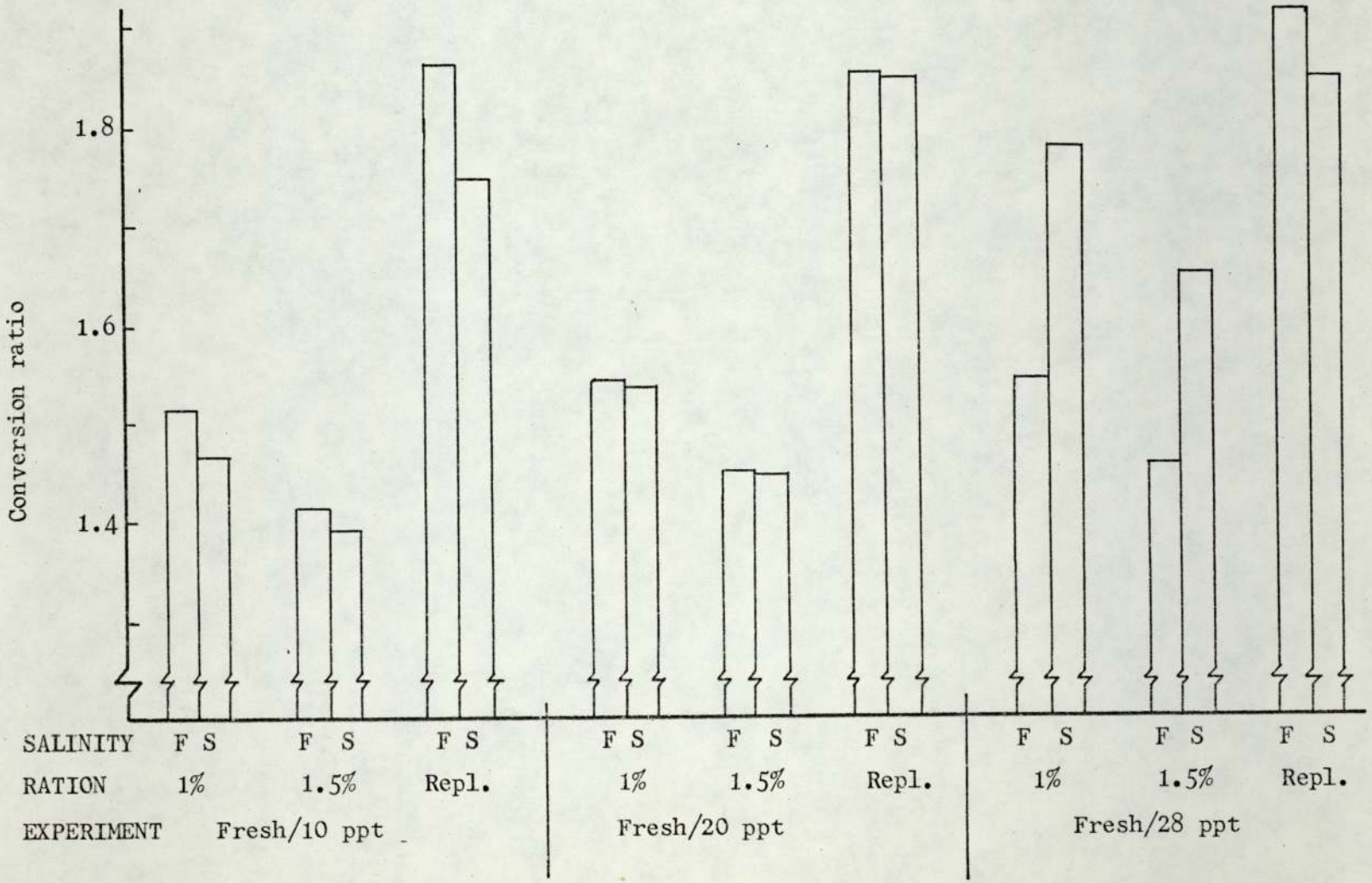
Mean daily food consumption rates were determined for each salinity by summing the food consumed by two repletion tanks over an experiment and then dividing by the number of days (Table 13). Comparison between feeding rates within each experiment was accomplished by using either Student's 't' test or the Fisher-Behren test depending upon the significance of the variance ratios. No significant differences were found between the mean food consumption rates in fresh water and 10 ppt sea water. Similarly, no significant differences were found between fresh water and 20 ppt sea water. However, mean food consumption rates were significantly lower ($p < 0.001$) for fish in 28 ppt sea water than for fish in fresh water.

Fig.17. The specific growth rates of fish fed at three feeding rates in the three phases of Experiment 1. Each value shown is a mean of two replicates



Note - F = Fresh water; S = Sea water

Fig. 18. The conversion ratios of fish fed at three feeding rates in three phases of Experiment 1. Each value shown is a mean of two replicates



Note - F = Fresh water; S = Sea water

Table 13. Mean daily food consumption of fish fed to repletion in different salinities*

Salinity	Mean Food Consumption of two tanks, g/day	Standard Error of ± Mean	Probability	Weight-adjusted mean food consumption g/kg fish/day
Fresh	83.90[a]	± 0.571	Not significant	18.78
10 ppt	84.54[a]	± 0.704		18.65
Fresh	88.52[b]	± 0.606	Not significant	18.85
20 ppt	91.18[b]	± 0.642		19.26
Fresh	87.46[c]	± 0.571	p <0.001	19.82
28 ppt	68.90[c]	± 0.672		16.13

a n = 84 (42 x 2)

b n = 80 (40 x 2)

c n = 56 (28 x 2)

*Each mean food consumption figure is the daily average of the total summed food consumption of two repletion tanks over the course of each experiment.

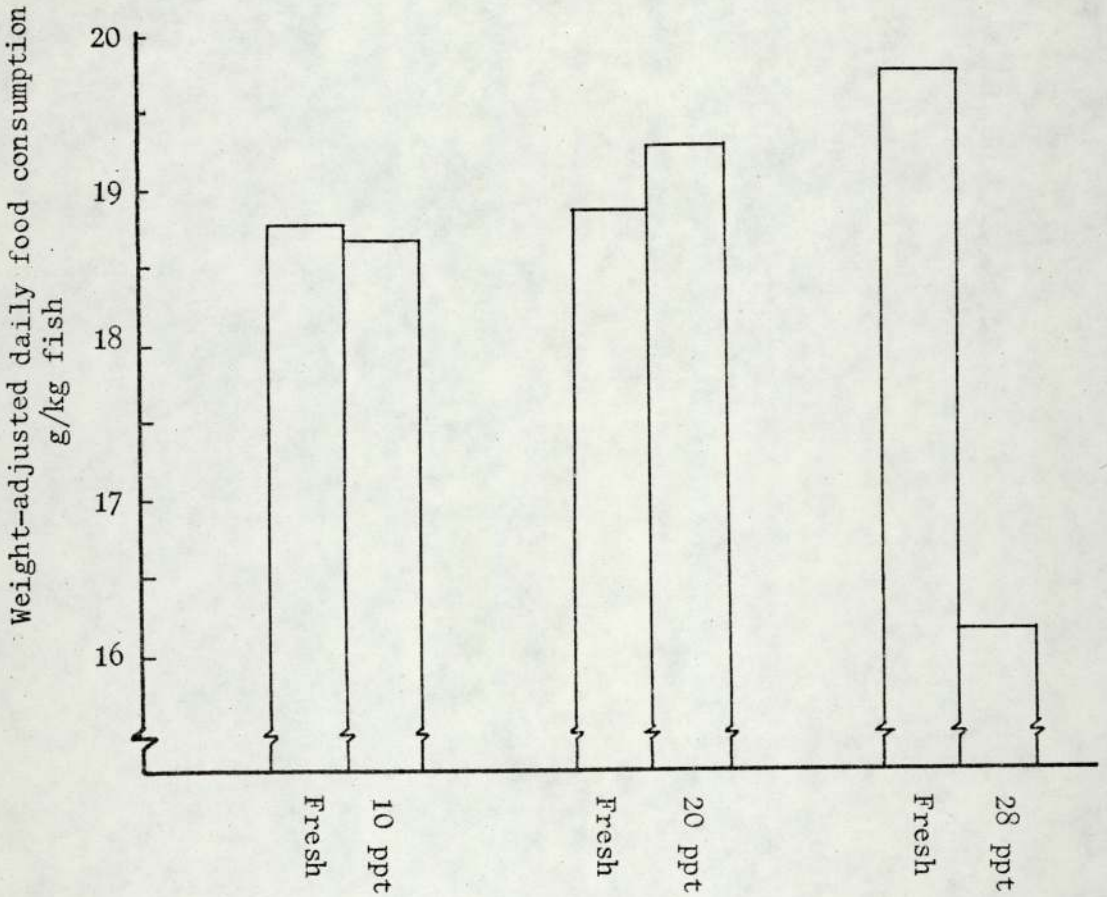


Fig. 19. Weight-adjusted mean daily food consumption of fish in different salinities fed to repletion. Each value shown is a mean of 2 replicates

The weight-adjusted rates of voluntary food intake are shown in Table 13 and also in histogram form in Figure 19.

Condition Factor and Proximate Carcass Analysis

Condition factor is given by the following equation (Brown, 1957):-

$$\text{Condition Factor} = K = \frac{\text{Fish Weight, g} \times 100}{(\text{Length, cm})^3}$$

Condition factor provides an index of the 'fatness' of fish and the values obtained usually range about a mean of 1.0.

The mean condition factor for each tank of fish was determined from the individual fish weighings before and after each experiment (cf. Table 9). These values were compared taking each experiment in turn, by analysis of variance and subsequent Student's 't' test. Mean condition factors of two tanks of fish on the same ration determined before and after each experiment are shown in Table 14. At each of the three ration levels, condition factors were significantly higher ($p < 0.001$) after each experiment than before. At the commencement of each experiment no significant differences were found between any mean condition factor either between salinities or between ration level. The average condition factor was 1.18. At the end of all three experiments it was found that there was a significant difference between the mean condition factors of fish fed at a 1.0% level and at a repletion level ($p < 0.05$). Also at the end of all experiments there were no significant differences between salinities at different ration levels with one exception. There was a significant difference ($p < 0.05$) between the mean condition factors of fish fed to repletion after experiment 3 had finished. Fish in 28 ppt sea water had lower condition factors than fish in fresh water probably as a result of the relatively lower feeding rate in that former salinity. These results are shown in histogram

Table 14. Condition factors of fish before and after Experiments 1, 2 and 3

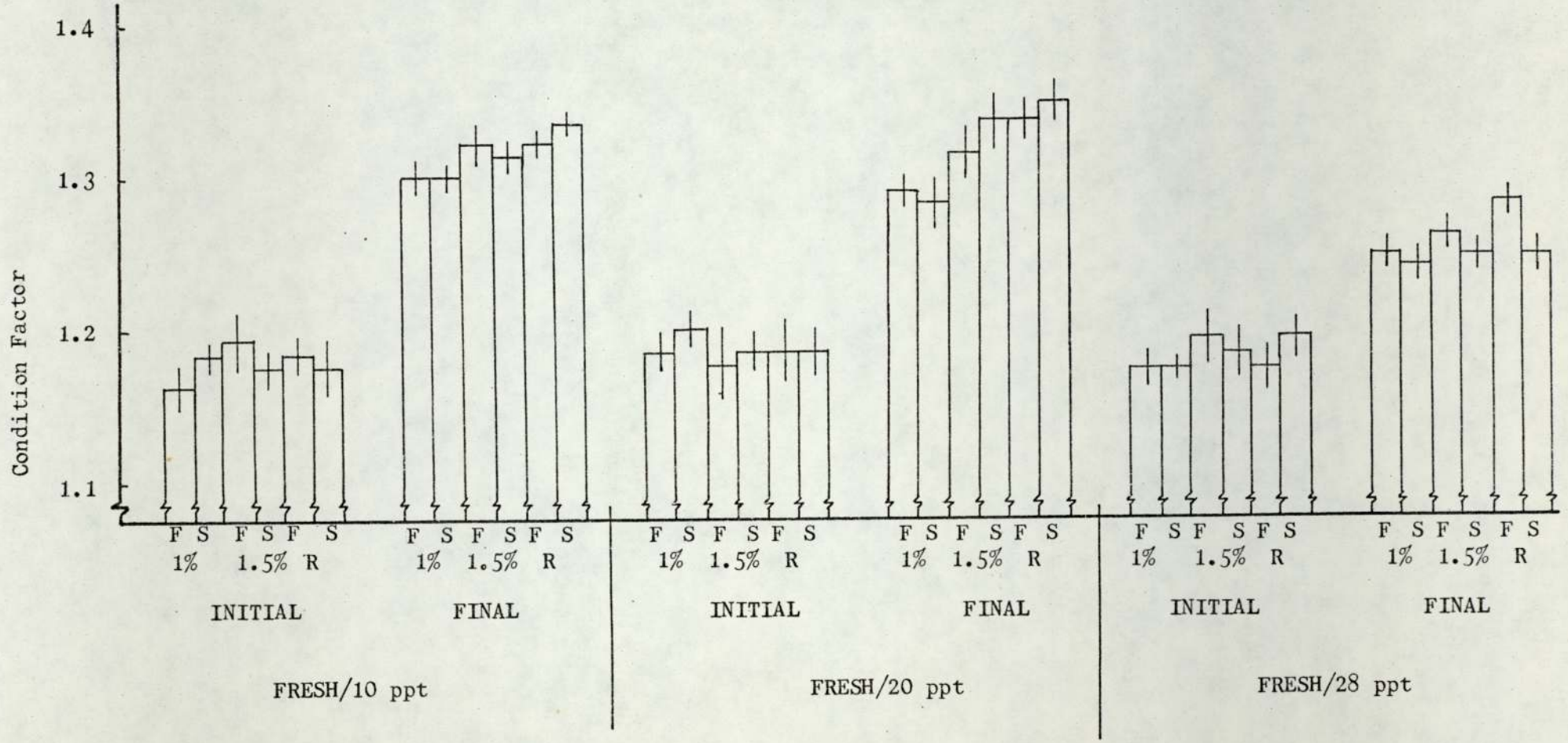
Ration	EXPERIMENT 1				EXPERIMENT 2				EXPERIMENT 3			
	Initial		Final		Initial		Final		Initial		Final	
	Fresh	10 ppt	Fresh	10 ppt	Fresh	20 ppt	Fresh	20 ppt	Fresh	28ppt	Fresh	28 ppt
1.0%	1.16[b]	1.18	1.30	1.30	1.18	1.20	1.29	1.28	1.17	1.17	1.25	1.24
1.5%	1.19	1.17	1.32	1.31	1.17	1.18	1.31	1.33	1.19	1.18	1.26	1.25
R[a]	1.18	1.17	1.32	1.33	1.18	1.18	1.33	1.34	1.17	1.19	1.28	1.25

a. R = Repletion feeding

Means joined by the same line are not significantly different at the 5% probability level

b. Each value is a mean of two values from tanks of fish fed identical rations in the same salinity

Fig. 20. Mean condition factors of fish fed at different feeding rates before and after each experiment. Each value is the mean of two tanks of fish. One standard error each side of means is shown.



form in Figure 20.

A procedure of selectivity was adopted before analysing the carcasses retained from the beginning and end of each experiment. Due to the mechanical failure of a deep-freeze unit some carcasses had become damaged and it was thought unwise to include these carcasses for analysis. Hence, proximate body composition was determined for carcasses retained from the end of the fresh water:10 ppt sea water experiment and from the end of the fresh water:20 ppt sea water experiment. In this latter case only the sea water group were analysed. Triplicate samples at each ration level and salinity were analysed for moisture, protein, oil and ash. The means of each triplicate were compared by analysis of variance and subsequent Students 't' test and the results are shown in Table 15. At all three salinities the moisture and protein content of the carcasses decreased as feeding rate increased. Also, oil content increased as feeding rate increased. Examining each salinity independently revealed that the increases in percentage dry material (inverse of moisture content in Table 15) with feeding rate were not significant. This was attributed to the variability in values obtained for moisture content. The decreases in protein content with feeding rate were significant ($p < 0.05$) between the 1.0% and 1.5% feeding rates in both fresh water and 20 ppt sea water, and between the 1.5% and repletion feeding rates in 10 ppt sea water. The increases in oil content with feeding rate were significant between the 1.0% and the 1.5% feeding rates in fresh water, 10 ppt and 20 ppt sea water ($p < 0.05$, $p < 0.05$, $p < 0.01$ respectively). Differences in ash content between feeding rates were generally insignificant except between the 1.0% and the 1.5% feeding rates in 10 ppt sea water ($p < 0.05$) and between the 1.5% and the repletion feeding rates in 20 ppt sea water

Table 15. Proximate carcass analysis values of fish retained from the termination of experiments 1 and 2

Salinity	Ration %	Dry Matter[a] % Live weight	± Standard error of mean	Probability	Protein[a] Content % Dry weight	± Standard error of mean	Probability	Oil content[a] % Dry weight	± Standard error of mean	Probability	Ash content[a] % Dry weight	± Standard error of mean	Probability	Mean calorific Value[a,b] Kcal/g dry matter
Fresh Water	1.0	22.9 ± 0.656		N.S.	68.9 ± 0.418		<0.05	22.5 ± 0.417		<0.05	8.5 ± 0.083		N.S.	6.0191
	1.5	24.5 ± 0.502			67.5 ± 0.389			24.0 ± 0.383			8.4 ± 0.092			6.0817
	R	25.7 ± 1.888		N.S.	66.9 ± 0.564		N.S.	24.7 ± 0.418		N.S.	8.5 ± 0.071		N.S.	6.1140
10 ppt Sea Water	1.0	23.3 ± 0.555		N.S.	68.0 ± 0.415		N.S.	22.5 ± 0.444		<0.05	8.5 ± 0.092		<0.05	5.9682
	1.5	24.1 ± 0.540			67.8 ± 0.386			23.8 ± 0.302			8.2 ± 0.084			6.0798
	R	25.1 ± 1.041		N.S.	66.1 ± 0.452		<0.05	23.9 ± 0.387		N.S.	8.3 ± 0.096		N.S.	5.9932
20 ppt Sea Water	1.0	22.2 ± 0.831		N.S.	68.5 ± 0.302		<0.05	22.0 ± 0.316		<0.01	8.5 ± 0.079		N.S.	5.9492
	1.5	24.3 ± 0.890			67.4 ± 0.316			23.9 ± 0.355			8.4 ± 0.065			6.0666
	R	25.1 ± 0.961		N.S.	66.5 ± 0.265		N.S.	24.6 ± 0.341		N.S.	8.2 ± 0.057		<0.05	6.0819

R = Repletion feeding

a = Each value is the mean of 3 replicates

b = Calorific Value is estimated by using the following equivalents from Brody (1945)- Protein = 5.65 Kcal/g dry matter
Oil = 9.45 Kcal/g

N.S = Means not significantly different

($p < 0.05$).

Comparison of mean constituent values at each feeding rate between salinities revealed no significant differences. Hence, salinity appeared not to affect body composition at any ration level. However, feeding rate did appear to exert an influence on the general body composition.

Metabolic Rates

It has been described previously that twelve, hour-long metabolic rate determinations were undertaken during each fortnight of each experiment on each of the metabolism/growth tanks (Table 9). A mean value was determined of each of the twelve determinations. Thus three fortnightly means for each metabolism/growth tank were obtained for experiments 1 and 2 and two means were obtained for experiment 3. Comparison of these mean values was undertaken for each experiment in turn using analysis of variance and subsequent Student's 't' test. Differences in metabolic rates between salinities and between feeding rates were examined. Table 16 and Figure 21 show mean metabolic rate values for each feeding rate over the entire duration of each experiment i.e. each value shown for experiments 1 and 2 is a mean of 3 fortnightly means while each value shown for experiment 3 is a mean of 2 fortnightly means.

Mean metabolic rates of fish were lowest at the 1% feeding rate and highest at the repletion feeding rate ranging from 187 - 206 mg O_2/kg. fish/hr to 326 - 409 mg O_2/kg fish/hr. In all three experiments and at all salinities the differences between mean metabolic rates at two adjacent feeding rates were highly significant ($p < 0.001$). Examination of differences in metabolic rates between salinities was made with each experiment in turn using the fresh water values as control readings in all cases. Metabolic rates were significantly lower for fish in

Table 16. Mean values of metabolic rates of fish fed at different feeding rates in experiments 1, 2 and 3.

Experiment		1% Ration M.R.[a] ± S.E.	Probability	1.5% Ration M.R. ± S.E.	Probability	Repletion Feeding M.R. ± S.E.	Probability
1	Fresh	207 ± 2.91	<0.01	274 ± 2.91	<0.01	409 ± 4.06	<0.001
	10 p.p.t	189 ± 3.71		257 ± 2.51		374 ± 3.11	
2	Fresh	206 ± 3.60	N.S.	262 ± 2.68	N.S.	403 ± 4.66	N.S.
	20 p.p.t	206 ± 4.52		266 ± 3.78		396 ± 8.66	
3	Fresh	187 ± 2.00	<0.05	260.5 ± 1.50	<0.02	387 ± 5.00	<0.001
	28 p.p.t	201 ± 3.00		276 ± 2.00		326 ± 4.00	

Each metabolic rate value for experiments 1 and 2 is a mean of 3 values

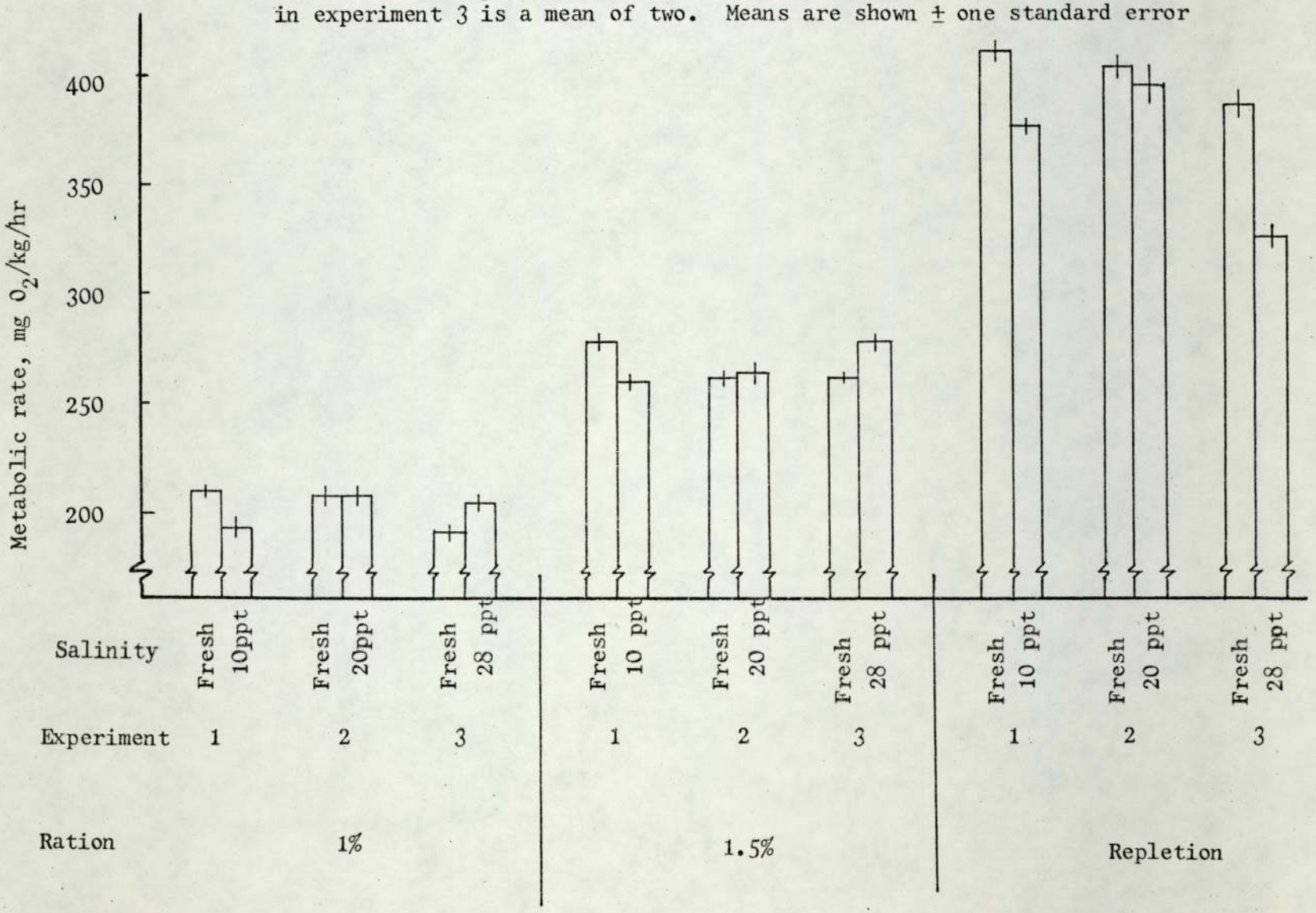
Each metabolic rate value for experiment 3 is a mean of 2 values

N.S. = Means not significantly different

a M.R. Metabolic rate in mg.O_2/kg.fish/hr.

b S.E. Standard Error

Fig.21. Mean metabolic rates at each feeding rate in different salinities. Each value in experiments 1 and 2 is a mean of three. Each value in experiment 3 is a mean of two. Means are shown ± one standard error



10 ppt sea water than for fish in fresh water ($p < 0.01$ at the 1% and 1.5% feeding rates; $p < 0.001$ at the repletion feeding rate). No significant differences were found between metabolic rates of fish in 20 ppt sea water and fresh water at any ration level. Metabolic rates were significantly higher for fish in 28 ppt sea water than for fish in fresh water at the 1% and 1.5% feeding rates ($p < 0.05$ for the 1% feeding rate- $p < 0.02$ for the 1.5% feeding rate). Conversely, at the repletion feeding level the metabolic rates were significantly lower ($p < 0.001$) for fish in 28 ppt sea water than for fish in fresh water. This undoubtedly was a reflection of the lower feeding rate by fish in 28 ppt sea water compared with fish in fresh water.

Faeces

Faeces were collected daily from each of the six faecal traps attached to the metabolism/growth tanks during every experiment. After drying, weighing and grinding, samples from each faecal trap were collected into six 'pools' for each tank of fish. Five samples from each pool were used in determining the caloric density of the faeces obtained from fish fed at different feeding rates in different salinities. These mean values are shown in Table 17 and Figure 22. Comparison of these results by analysis of variance and subsequent Student's 't' test attempted to clarify the differences between ration levels and between salinities. In all three experiments no significant differences in faecal caloric densities were found between the 1% and the 1.5% ration levels. In all three experiments the faecal caloric density was significantly higher (Table 17) at the repletion feeding level than at the other two feeding rates with the exception of the faeces obtained from the fish in 28 ppt sea water in experiment 3. No sig-

Table 17. Caloric density[a] of faeces from fish fed at different feeding rates in different salinities.

Comparison between feeding rates.

Experiment	Salinity	Ration 1%	1.5%	R
1	Fresh	3.7925	3.7975	3.9325
	Probability	N.S.		<0.02
	10 ppt	3.7825	3.8100	3.9875
	Probability	N.S.		<0.001
2	Fresh	3.7925	3.8100	3.9150
	Probability	N.S.		<0.05
	20 ppt	3.7975	3.8050	3.9400
	Probability	N.S.		<0.001
3	Fresh	3.7875	3.8050	3.9175
	Probability	N.S.		<0.001
	28 ppt	3.7975	3.7975	3.8250
	Probability	N.S.		N.S.

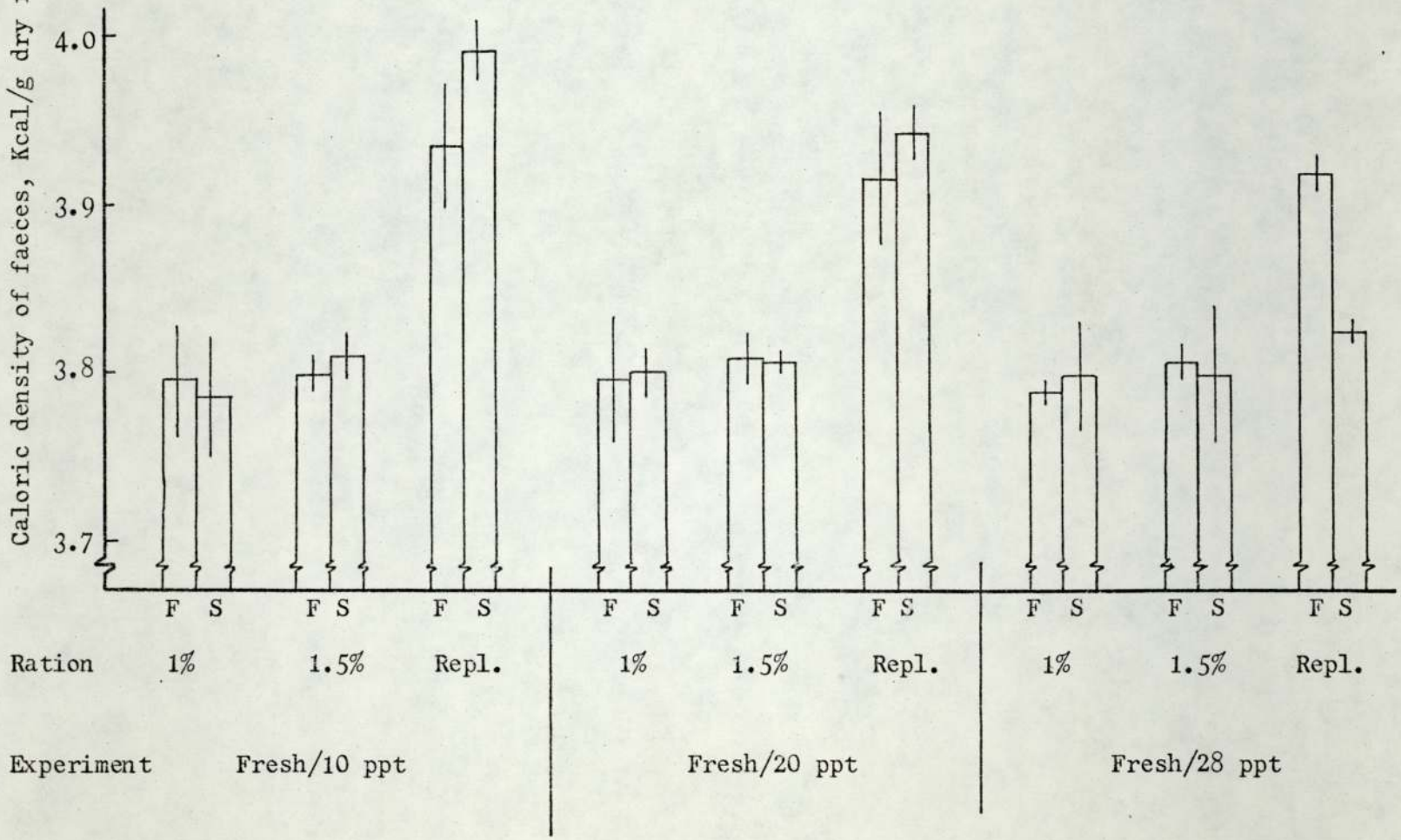
a Caloric density = Kcal/g dry faeces

All values are means of 5 replicates

N.S. = Means not significantly different

R = Repletion feeding

Fig.22. Faecal caloric density at three feeding rates in different salinities. Each value is a mean of five replicates. One standard error either side of each mean is shown.



nificant differences were observed in faecal caloric density between fresh water and sea water at any ration level with one exception (Table 18). Faeces obtained from fish in 28 ppt sea water were significantly lower ($p < 0.001$) than those obtained from fish in freshwater at the repletion feeding level. This was undoubtedly due to the lower food consumption of fish in 28 ppt sea water.

Thus it is evident that no basic difference in faecal caloric density is caused by environmental salinity. However at the highest feeding rates faecal caloric density is significantly higher than that found at lower feeding rates.

Multiplying the dry weight of faeces obtained every fortnight by its appropriate caloric density gave the quantity of calories voided in the faeces by each tank of fish during each experiment. As this rose considerably from fortnight to fortnight the values were rendered comparative by relating them to the food calories ingested per fortnight. Thus the following equation was used which gave a measure of absorption efficiency of ingested calories per fortnight.

$$\text{Apparent Absorption Efficiency} = \frac{\text{Caloric Intake - Faecal calories}}{\text{Caloric Intake}} \times 100$$

The term apparent absorption efficiency is used here because no account is taken of the metabolic faecal nitrogen or lipid which is of endogenous origin. Mean absorption efficiencies each being a mean of a fortnightly estimate (cf Table 9) are given in Tables 19 and 20. Comparison of these results was undertaken by analysis of variance and subsequent Student's 't' test in order to reveal differences caused by salinity and by feeding rate. In Table 19 it can be seen that no significant differences were seen between the absorption efficiencies of

Table 18. Caloric density of faeces from fish fed at different feeding rates in different salinities. Comparison between salinities.

Exp.	Salinity	1% Ration Caloric Density[a] ± S.E.	Probability	1.5% Ration Caloric Density ± S.E.	Probability	Repletion Feeding Caloric Density ± S.E.	Probability
1	Fresh	3.7925 ± 0.031	N.S.	3.7975 ± 0.011	N.S.	3.9325 ± 0.036	N.S.
	10 ppt	3.7825 ± 0.036		3.8100 ± 0.015		3.9875 ± 0.018	
2	Fresh	3.7925 ± 0.038	N.S.	3.8100 ± 0.014	N.S.	3.9150 ± 0.040	N.S.
	20 ppt	3.7975 ± 0.014		3.8050 ± 0.007		3.9400 ± 0.016	
3	Fresh	3.7875 ± 0.007	N.S.	3.8050 ± 0.010	N.S.	3.9175 ± 0.009	<0.001
	28 ppt	3.7975 ± 0.032		3.7975 ± 0.040		3.8250 ± 0.006	

a Figures are means of 5 values
All in Kcal/g dry faeces

N.S. = Means not significantly different
R = Repletion feeding
S.E. = Standard error of Mean

Table 19. Mean absorption efficiences of ingested calories at different feeding rates in Experiments 1, 2 and 3. Comparison between salinities.

EXPERIMENT		1% Ration				1.5% Ration				Repletion Ration			
		Apparent Absorption Efficiency %	±	S.E.	Probability	Apparent Absorption Efficiency %	±	S.E.	Probability	Apparent Absorption Efficiency %	±	S.E.	Probability
1	Fresh	82.1	±	0.24	N.S.	80.8	±	0.23	N.S.	78.2	±	0.12	N.S.
	10 ppt	82.3	±	0.18		80.7	±	0.12		78.8	±	0.18	
2	Fresh	82.9	±	0.12	N.S.	81.2	±	0.07	N.S.	79.3	±	0.07	N.S.
	20 ppt	83.0	±	0.19		81.0	±	0.12		79.2	±	0.18	
3	Fresh	82.6	±	0.07	<0.02	81.0	±	0.08	<0.01	78.3	±	0.19	N.S.
	28 ppt	81.2	±	0.15		79.6	±	0.09		78.6	±	0.08	

N.S. = Means not significantly different

S.E. = Standard Error

The absorption efficiency values for experiments 1 and 2 are means of 3 values while those for experiment 3 are means of 2 values

fish in fresh water, 10 ppt and 20 ppt sea water taking each feeding rate independently. However, absorption efficiencies were significantly lower for fish in 28 ppt than for fish in freshwater at the 1% and 1.5% feeding rates. No such difference was observed at the repletion feeding level where food consumption between fish in the two salinities was very different. In Table 20 it can be seen that examination of each salinity in turn reveals that an increase in feeding rate resulted in a significant decrease in absorption efficiency. Values ranged from 83.0 - 81.2% at the 1% feeding rate to 79.3 - 78.2% at the repletion feeding level. These figures are shown in Figure 23; apparent absorption efficiencies are plotted against the daily food ingested (calories) adjusted to the mean fish weight.

Energy Balance

For the purposes of constructing the energy balance equations the following calculations were used in converting food consumed and weight gained into caloric equivalents.

The Food

The composition of 'Beta' floating trout pellets according to the manufacturers is as follows:-

Oil	=	4.5%
Protein	=	40.0%
Fibre	=	4.5%
Ash	=	12.0%
Moisture	=	10.0%
Nitrogen-free Extractives	=	29.0%

Using caloric equivalents obtained from Brody (1945) of 9.45 Kcal/g for oil, 5.65 for protein and 4.1 for carbohydrate a figure of 4.0587

Table 20. Mean apparent absorption efficiencies of ingested calories at different feeding rates in experiments 1, 2 and 3. Comparison between feeding rates.

Experiment		Ration 1%	1.5%	R
1	Fresh	82.1	80.8	78.2
	Probability	<0.02		<0.001
	10 ppt	82.3	80.7	78.8
	Probability	<0.01		<0.01
2	Fresh	82.9	81.2	79.3
	Probability	<0.001		<0.001
	20 ppt	83.0	81.0	79.2
	Probability	<0.001		<0.01
3	Fresh	82.6	81.0	78.3
	Probability	<0.01		<0.01
	28 ppt	81.2	79.6	78.6
	Probability	<0.02		<0.02

The apparent absorption efficiency values for experiments 1 and 2 are means of 3 values while those for experiment 3 are means for 2 values

R = Repletion feeding

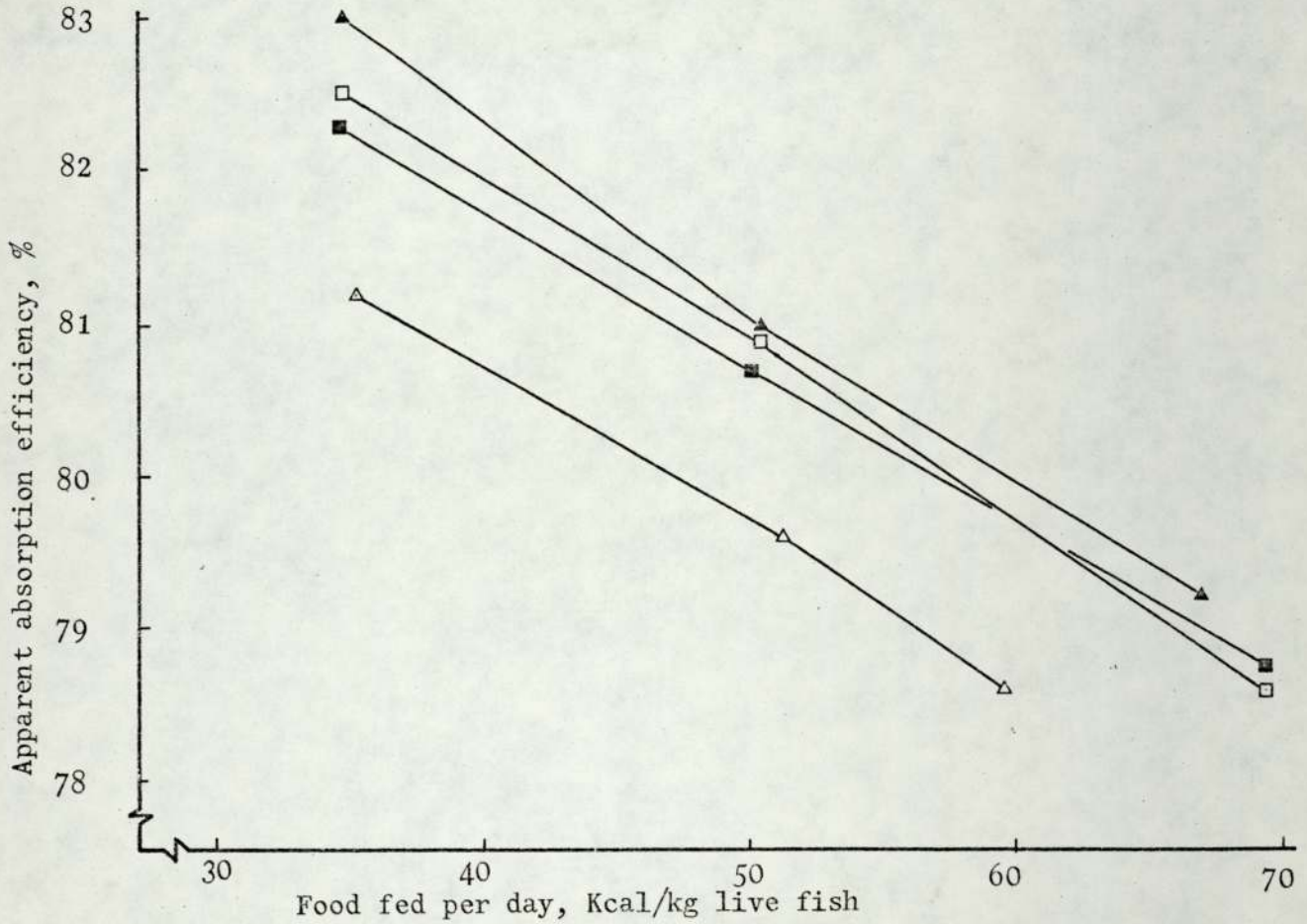


Fig.23. The changes in apparent absorption efficiency with increase in food fed in different salinities. Each point is a mean of three values except if fresh water where each point is a mean of nine (3 x 3). Fresh water, open squares □ ; 10 ppt, closed squares ■ 20 ppt., closed triangles ▲ ; 28 ppt, open triangles △

Kcal/g dry food was obtained and used in all conversions.

Growth

Growth in terms of g wet weight per unit time was converted into Kcal by using figures from Table 15. As no significant differences were found between any of the salinities for each component, average figures for each ration were used. These were:-

	Average Dry Matter % of wet weight	Average Calorific Value Kcal/g
1% ration	22.8	5.9788
1.5% ration	24.3	6.0760
Repletion ration	25.3	6.0630

As described earlier metabolic rates were converted into calories of heat liberated by multiplying the rates of oxygen consumption by 3.42 cals/mg O_2 consumed. Also, the quantity of energy lost through excretion was determined by multiplying the rates of oxygen consumption by 0.51 cals/mg O_2 consumed.

Using the results obtained after each fortnight a complete energy budget was determined for each metabolism/growth tank of fish for each experiment. Thus, three independent energy budgets were drawn up for each metabolism/growth tank for experiments 1 and 2 and two independent energy budgets for each metabolism/growth tank for experiment 3. To illustrate this the energy budgets for the first fortnights in each of experiments 1, 2 and 3 are presented in Tables 21, 22 and 23. Each table shows the absolute quantity of calories ingested per day in different salinities at different feeding rates. The fate of the ingested calories is then divided into absolute calories voided in faeces, lost to excretion, lost to respiration and gained as growth

Table 21. The energy budgets obtained during the first fortnight of experiment 1 in fresh water and 10 p.p.t sea water at 3 feeding rates.

	1% Ration		1.5% Ration		Repletion Ration	
	Fresh	10 ppt	Fresh	10 ppt	Fresh	10 ppt
Food consumed Kcal/day	65.59	64.86	98.50	97.57	135.68	134.95
Faeces Kcal/day	11.74	11.48	18.91	18.83	29.58	28.61
Apparent Absorption Efficiency %	82.1	81.8	80.8	80.1	78.2	78.0
Average Metabolic Rate mgO_2 /kg/hr	202	196	269	254	402	375
Excreted Kcal/day	4.65	4.46	6.36	5.96	9.46	8.77
Respired Kcal/day	31.16	29.94	42.67	39.98	63.45	58.82
Growth Kcal/day	16.32	16.58	28.44	28.79	30.80	33.33
% Balance	97.4	96.3	97.8	95.9	98.2	96.0

$$\% \text{ Balance} = 100 \times \frac{\text{Sum of Calories as Faeces, Respiration, Excreted and Growth}}{\text{Total Ingested Calories}}$$

Table 22. The energy budgets obtained during the first fortnight of experiment 2 in fresh water and 20 p.p.t sea water at 3 feeding rates.

	1% Ration		1.5% Ration		Repletion Ration	
	Fresh	20 ppt	Fresh	20 ppt	Fresh	20 ppt
Food consumed, Kcal/day	68.02	68.71	102.48	102.20	135.97	137.14
Faeces Kcal/day	11.63	11.68	19.27	19.52	28.14	28.52
Apparent Absorption Efficiency %	82.9	83.0	81.2	80.9	79.3	79.2
Average Metabolic Rate mg O_2/kg/hr	199	197	266	267	394	381
Excreted Kcal/day	4.74	4.74	6.54	6.55	9.66	9.42
Respired Kcal/day	31.81	31.78	43.89	43.92	64.81	63.19
Growth Kcal/day	16.70	16.43	29.58	29.30	31.37	31.47
% Balance	95.4	94.1	96.9	97.2	98.5	96.7

Table 23. The energy budgets obtained during the first fortnight of experiment 3 in fresh water and 28 ppt sea water at 3 feeding rates.

	1% Ration		1.5% Ration		Repletion Ration	
	Fresh	28 ppt	Fresh	28 ppt	Fresh	28 ppt
Food consumed Kcal/day	65.57	68.35	101.14	102.36	144.65	120.38
Faeces Kcal/day	11.41	12.78	19.22	20.78	31.39	27.2
Apparent Absorption Efficiency %	82.6	81.3	81.0	79.7	78.3	77.4
Average Metabolic Rate mg O_2/kg/hr	189	198	262	274	382	322
Excreted Kcal/day	4.34	4.71	6.34	6.66	9.50	7.91
Respired Kcal/day	29.10	31.59	42.51	44.63	63.73	53.03
Growth Kcal/day	15.78	14.24	27.62	24.62	32.15	27.03
% Balance	92.5	92.6	94.6	94.5	94.5	95.7

per day. A measure of the accuracy of the entire range of experimental techniques is given by the percentage balance where,

$$\% \text{ Balance} = \frac{\text{Sum of Calories as Faeces, Respiration, Excretion and Growth}}{\text{Total Ingested Calories}} \times 100$$

In all cases, accuracy was acceptable in that the percentage balances ranged from 92.5% minimum to 98.5% maximum. The percentage balances from the other fortnights' results of the three experiments were very similar. In all cases, the balance was less than 100% indicating that some unaccounted caloric loss was a general occurrence.

The deployment of ingested calories as faeces, excretion, respiration and growth at each ration level in all salinities was rendered comparative by converting them into percentages of the daily caloric intake for each fortnight of every experiment. The mean values of these fortnightly replicates are shown in Tables 24 and 25. Between-salinity differences were examined using analysis of variance and subsequent Student's 't' test. Table 24 shows the mean deployment of ingested calories to faeces and respiration in experiments 1, 2 and 3. The percentage of ingested calories voided as faeces varied from 17.0 to 21.8%. No significant difference was found between fresh water, 10 ppt and 20 ppt sea water at any ration level. However, significantly more of the ingested calories were lost as faeces in 28 ppt sea water than in fresh water at the 1.0% and 1.5% feeding rates. The fact that at the repletion feeding rate no difference in faecal calories was seen between fresh water and 28 ppt sea water was attributed to the much lower food consumption rate in the latter salinity.

The percentage of calories ingested lost to respiration varied

Table 24. The mean percentage of ingested calories deployed in faeces and respiration at three feeding levels in experiments 1, 2 and 3.

Note - For experiments 1 and 2, each figure is the mean of 3 fortnightly determinations.
For experiment 3, each figure is the mean of 2 fortnightly determinations.

		EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	Ration	Fresh	10 ppt	Fresh	20 ppt	Fresh	28 ppt
Percentage Ingested Calories as FAECES	1.0%	17.9	17.7	17.1	17.0	17.4	18.8
	Probability	N.S.		N.S.		<0.02	
	1.5%	19.2	19.3	18.8	19.0	19.0	20.4
	Probability	N.S.		N.S.		<0.01	
	R.	21.8	21.2	20.7	20.8	21.7	21.4
	Probability	N.S.		N.S.		N.S.	
Percentage Ingested Calories RESPIRED	1.0%	47.0	45.6	46.8	46.3	44.5	46.1
	Probability	<0.05		N.S.		<0.05	
	1.5%	43.3	40.6	43.0	42.9	42.1	43.7
	Probability	<0.01		N.S.		<0.05	
	R.	46.0	43.9	47.5	46.5	44.8	44.2
	Probability	<0.01		N.S.		N.S.	

N.S.= Means not significantly different
R = Repletion feeding rate

Table 25. The mean percentages of ingested calories deployed in excretion and growth at three feeding levels in experiments 1, 2 and 3.

Note - For experiments 1 and 2 each figure is the mean of 3 fortnightly determinations. For experiment 3, each figure is the mean of 2 fortnightly determinations

	Ration	EXPERIMENT 1 Fresh	EXPERIMENT 1 10 ppt	EXPERIMENT 2 Fresh	EXPERIMENT 2 20 ppt	EXPERIMENT 3 Fresh	EXPERIMENT 3 28 ppt
Percentage Ingested Calories EXCRETED	1.0%	7.1	6.8	6.9	6.9	6.7	6.9
	Probability	<0.01		N.S.		<0.05	
	1.5%	6.5	6.1	6.4	6.4	6.3	6.5
	Probability	<0.001		N.S.		<0.05	
	R	6.8	6.5	7.1	7.0	6.8	6.6
	Probability	<0.01		N.S.		N.S.	
Percentage Ingested Calories as GROWTH	1.0%	25.1	25.8	24.6	23.9	24.0	20.9
	Probability	<0.02		N.S.		<0.01	
	1.5%	28.5	29.7	28.6	28.7	27.5	24.2
	Probability	<0.01		N.S.		<0.01	
	R	23.0	24.9	23.1	22.9	22.2	22.5
	Probability	<0.01		N.S.		N.S.	

R = Repletion feeding rate. N.S. Means are not significantly different

between 42.1% and 47.5% depending upon feeding rate and salinity. At all feeding rates the percentage of ingested calories respired was significantly less for fish in 10 ppt sea water than for fish in fresh water. No significant differences were found between fish in 20 ppt sea water and fresh water. Calories respired were significantly higher by fish in 28 ppt sea water than by fish in fresh water at the 1% and 1.5% ration levels. No significant difference was found between fresh water and 28 ppt sea water at the repletion feeding rate. At each salinity, the percentage of ingested calories respired was significantly lower at the 1.5% than the 1% feeding rate ($p < 0.001$). At each salinity, the percentage of ingested calories respired was significantly lower at the 1.5% than the repletion feeding rate ($p < 0.001$).

Table 25 shows the mean deployment of ingested calories to excretion and growth in experiments 1, 2 and 3. The percentages of ingested calories excreted varied from 6.1 to 7.1% depending upon salinity and feeding rate. As the figures were derived from the same metabolic rate data as the respiration figures in Table 24, the patterns of significant differences between salinities were the same.

The percentages of ingested calories retained as growth varied from 22.2% to 29.7% depending upon salinity and feeding rate. Calories retained were significantly higher by fish in 10 ppt sea water than by fish in fresh water at all feeding rates. No significant differences were observed in calories retained between fish in fresh water and 20 ppt sea water at any feeding rate. Calories retained were significantly lower by fish in 28 ppt sea water than by fish in fresh water at the 1% and 1.5% feeding rates. No significant difference in calories retained was found between fish in fresh water and 28 ppt sea water at the repletion feeding

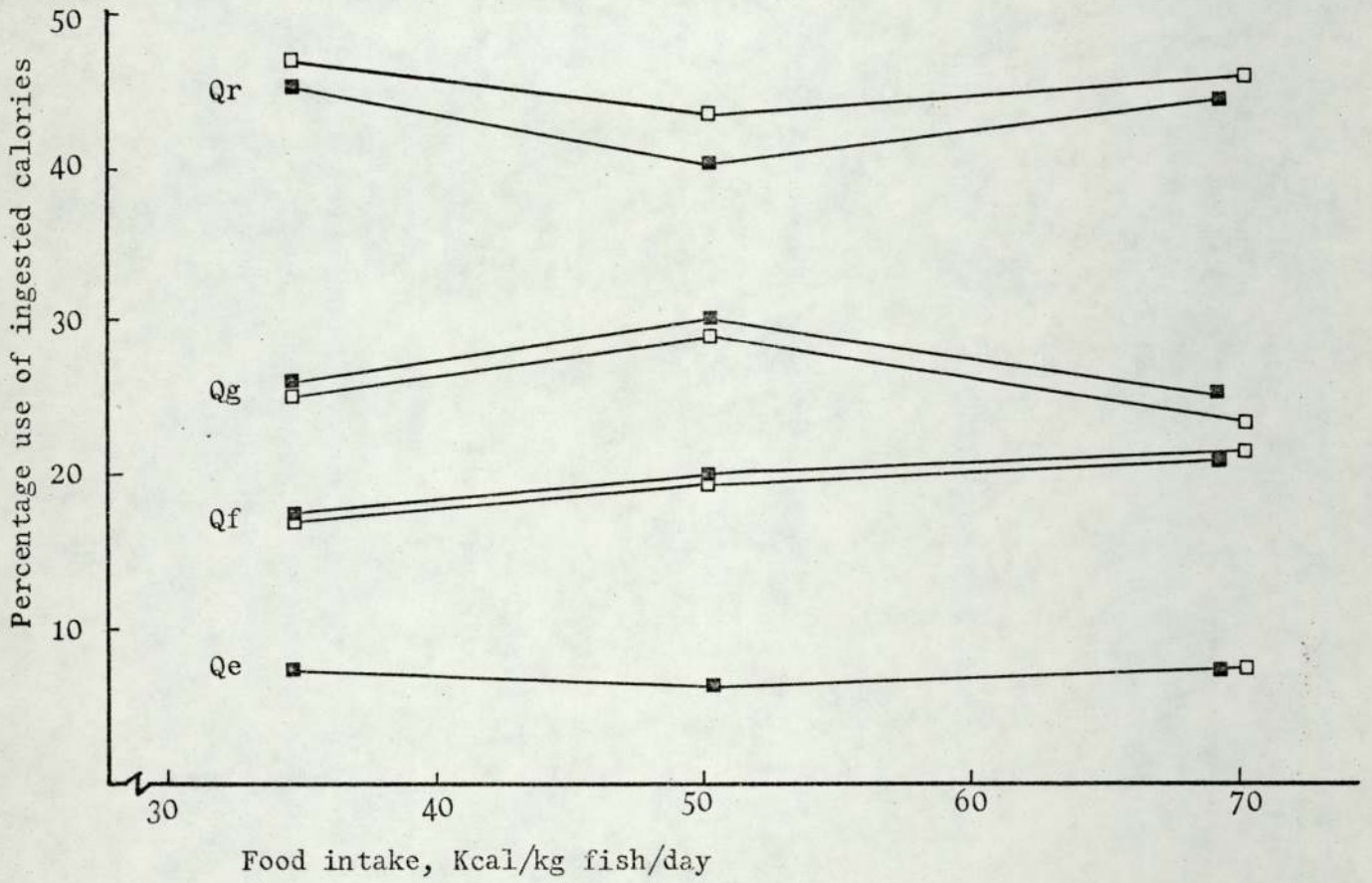


Fig. 24. The fate of ingested food calories at different feeding rates in fresh water and 10 ppt sea water (experiment 1).
Fresh water, open squares □ ; 10 ppt, closed squares ■
Metabolism, Qr; Growth, Qg, Faeces, Qf; Excretion, Qe
Note: Qe lines are superimposed

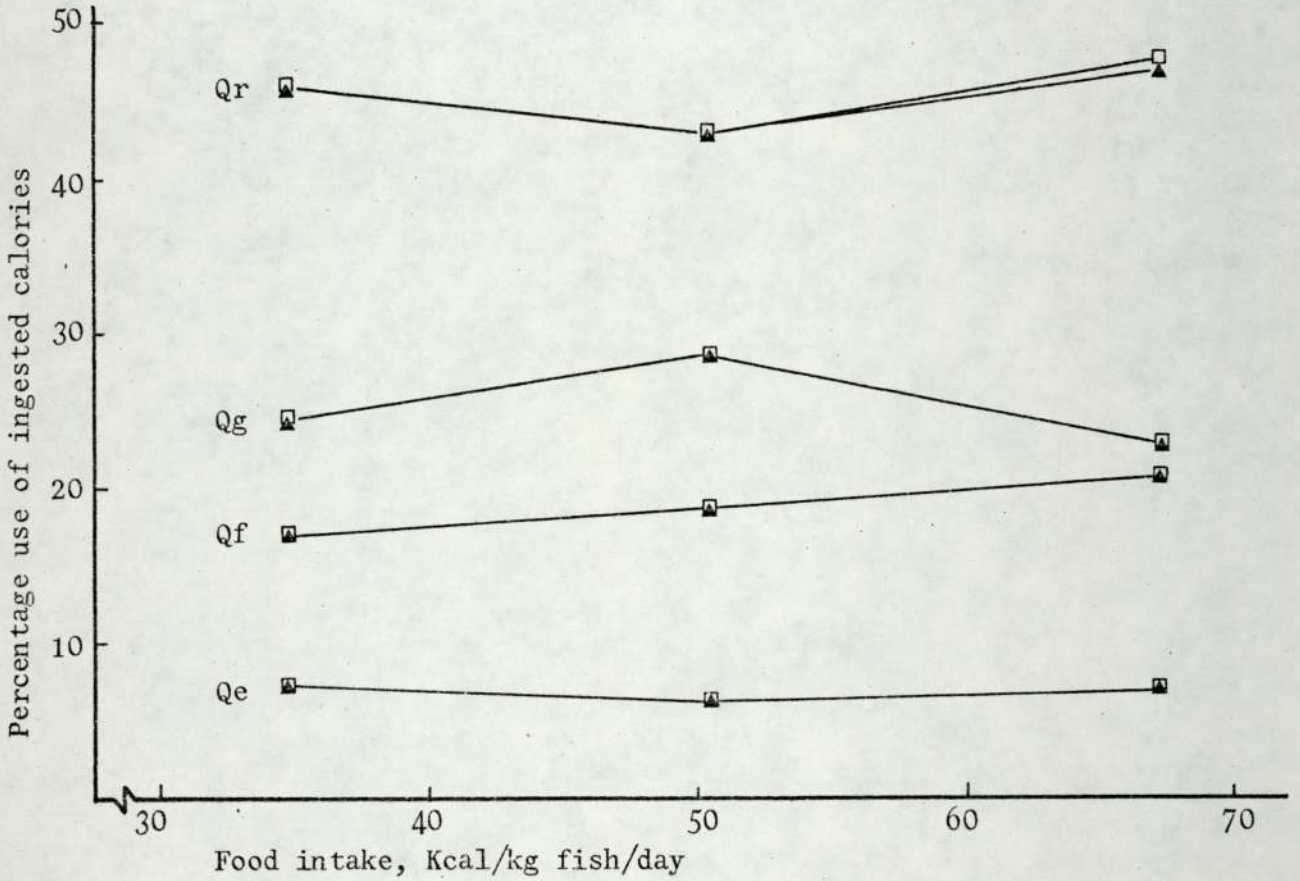


Fig.25. The fate of ingested food calories at different feeding rates in fresh water and 20 ppt sea water (experiment 2).
Fresh water, open squares □ ; 20 ppt, closed triangles ▲

Metabolism, Qr ; Growth, Qg; Faeces, Qf ; Excretion, Qe

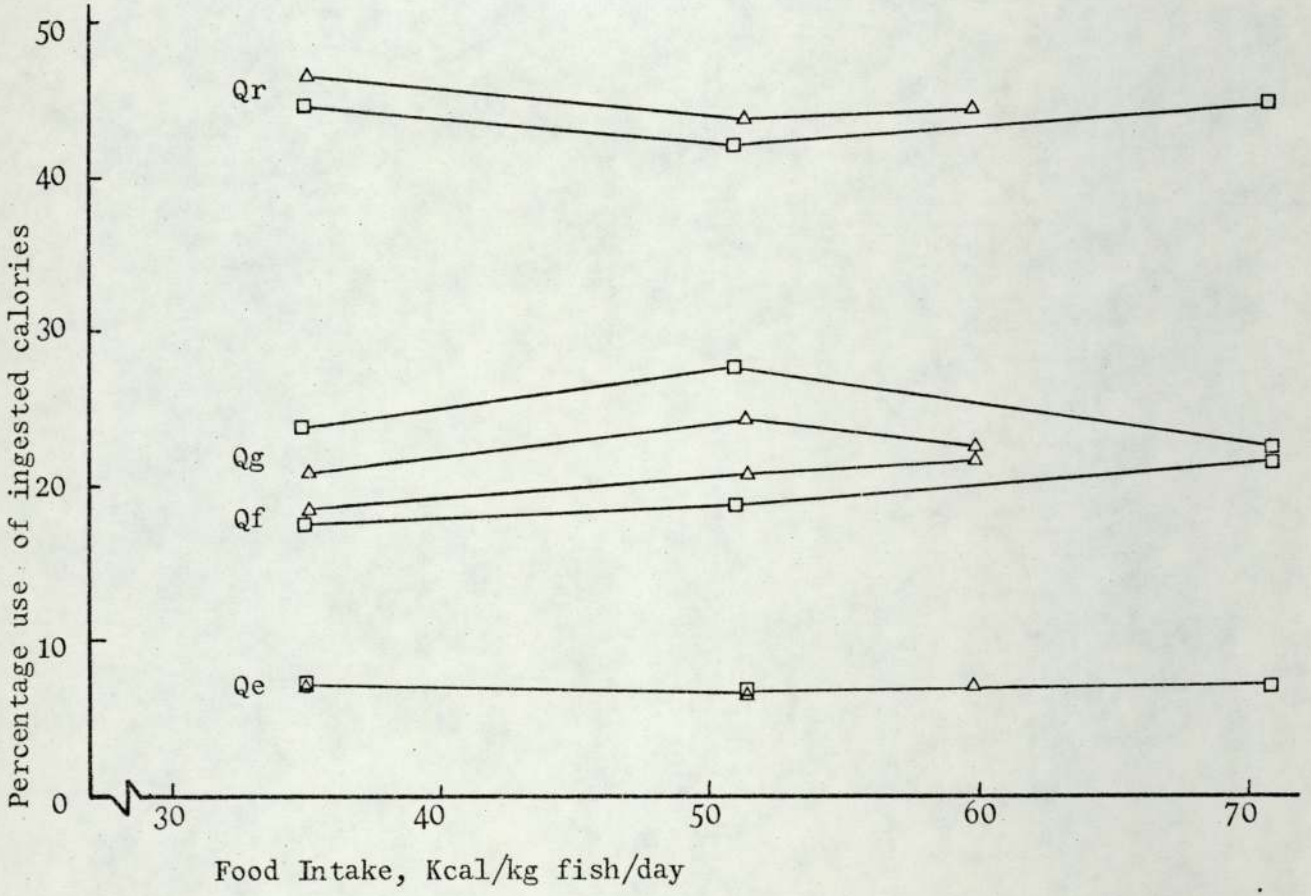


Fig.26. The fate of ingested food calories at different feeding rates in fresh water and 28 ppt sea water (experiment 3).Freshwater, open squares, □ ; 28 ppt, open triangles, △

Metabolism, Qr ; Growth, Qg ; Faeces, Qf ; Excretion, Qe.

level. At each salinity the percentage of ingested calories retained was significantly higher at the 1.5% than the 1% feeding rate ($p < 0.001$). At each salinity, the percentage of ingested calories respired was significantly higher at the 1.5% than the repletion feeding rate ($p < 0.001$)

Figures 24, 25 and 26 show the fate of ingested calories at different rates of food intake for experiments 1, 2 and 3 respectively. In all three figures it can be seen that percentages of ingested calories respired (Qr) are mirrored by the percentages of ingested calories retained as growth (Qg). As food intake increases the percentage of ingested calories respired first declines and then increases. Conversely, the percentage of ingested calories retained (Qg) first increases then declines. Also, in all three figures, as food intake increases, the percentage faecal calories (Qf) increases gradually. In Figure 24 it can be seen that the percentage of ingested calories respired is less at all feeding rates for fish in 10 ppt sea water than in fresh water. Conversely, the percentage of ingested calories retained is greater for fish in 10 ppt sea water than in fresh water. In Figure 25, no differences can be seen in the deployment of ingested calories at different ration levels between fish in fresh water and fish in 20 ppt sea water. In Figure 26 it can be seen that the higher percentage loss of ingested calories to faeces and respiration for fish in 28 ppt sea water combine to reduce the percentage ingested calories retained when compared with fish in fresh water.

4.5. Discussion and Conclusions

Metabolic Rates

In the present experiment, metabolic rates were markedly affected by feeding rate and salinity. With regard to the former factor, metabolic rates increased in direct proportion to feeding rate in all salinities. This confirmed previous findings by Paloheimo and Dickie (1966, a) and results of Edwards, Finlayson and Steele (1969) with plaice and dabs and Edwards et al. (1972) with cod. The most likely cause of this phenomenon is that the energetic costs of specific dynamic action increase disproportionately as the feeding rate is increased (Warren & Davis, 1969 ; Averett, 1969). Further examination of the effects of the specific dynamic action on metabolic rate will be made in Chapter 6. Paloheimo et al.(1966a) found after examination of the results of several workers that the levels of total metabolism of experimental fish could increase by a factor of 4 - 5X after an increase in ration level from the maintenance to the maximum level. The metabolic rates obtained in the present study were approximately twice as great at repletion ration than at the 1% ration level. It might be expected that the difference in total metabolic rates would be greater if the minimum and maximum rations were more extreme.

The actual metabolic rate values obtained in the present experiment may be compared with predicted values which can be obtained by utilising the estimates of Winberg (1956). He suggested that the following equation be used to estimate the respiratory rate of fish under natural conditions:-

$$2.\ T = a W^{b}$$

where T = Total Respiratory Rate in ml. O_2/hr

W = Weight of fish, g.

a and b are constants for the particular situation

Further explication of the equation, $T = aW^{b}$, is given in Appendix 1. Edwards, Steele and Trevallion (1970) and Solomon et al. (1972) both suggest that the above equation defines the respiratory rate of fish fed an unrestricted ration whereas fish fed a restricted ration have respiratory rates in between 'T' and '2T'. Comparisons of metabolic rates between fish used by different authors is difficult (Brett, 1962) as so often the experimental conditions are different. However, examination of Rao's data (from Fry, 1971) for a 100 g rainbow trout in fresh water at 15°C swimming at a low level of activity reveals an oxygen consumption of 12 ml/hr(16.24 mg/hr). This allows computation of an approximate value for 'a' to be made if 'b' is assumed to be 0.8 (c.f. Appendix 1). Thus a figure of 0.3014 for 'a' is obtained. If the values of 'a' and 'b' are then used to compute estimated metabolic rates for trout in fresh water on restricted and unrestricted rations the following results are seen:-

Restricted ration, T = 164 mg O_2/kg/hr

Unrestricted ration, 2.T = 328 mg O_2/kg/hr

Comparisons between these estimated metabolic rates and actual metabolic rates (Table 16) reveals some differences. Both estimated values underestimate the actual values. At the 1% ration this is probably caused by the fact that '1.T' is regarded as the respiratory rate at the maintenance ration. As fish grew actively at the 1% ration level their total metabolism would be higher than that at the maintenance level.

The condition of application of Winberg's equation, $2T = aW^b$ was that fish were in natural conditions. At the repletion ration level, fish were consuming more food than would fish in natural conditions which resulted in a higher determined metabolic rate compared with the estimated metabolic rate. Paloheimo et al. (1966a) state that when fish are fed to repletion the level of metabolism may be raised to that found in fish swimming at maximum speeds. Therefore, the difference between the estimated and the actual metabolic rate values can be very easily caused by differences in food consumption. This supports the proposition made by Solomon et al. (1972) that accurate estimates of total metabolic rate must take into account food consumption.

Comparisons of metabolic rates between different salinities are valid as all the experimental conditions were identical apart from salinity and the day of the experiment. Approximately the same size of fish was used in all tanks and they all expended equal quantities of energy on swimming activity as water flow rates in each tank were identical. Therefore any observed differences in the total metabolic rates between tanks on similar rations would have been caused by the environmental salinity. In agreement with Rao (1968, 1971) and Farmer and Beamish (1969) the metabolic rates of fish in 10 ppt sea water were lower than those of fish in fresh water on equivalent rations. This together with the fact that a lower percentage of ingested calories was devoted to metabolic needs in 10 ppt sea water tends to corroborate the hypothesis that the costs of osmotic and ionic regulation will be least at the isosmotic salinity. Trout held in 10 ppt sea water are in a medium very similar in osmotic pressure to that of the body fluids of the fish. At this salinity it is probable that the energetic costs of osmotic and ionic regulation will be small. This will be paralleled

by a fall in the total energy devoted to the maintenance of the fish with respect to the environment and the standard metabolic rate will fall. Rao (1968, 1971) has shown that standard metabolism is least at the isosmotic salinity for rainbow trout and this is matched by a fall in active metabolism at all levels of activity. The results obtained in the present work confirm his findings.

The similar total metabolic rates and percentages of ingested calories respired in fresh water and 20 ppt sea water again confirm the findings of Rao who found similar metabolic rates in fresh water and 20 ppt sea water at all levels of activity (interpreting Rao, 1971, Fig. 3. p. 207). This result indicates that the metabolic costs of ionic and osmotic regulation are similar in fresh water and 20 ppt sea water, both of these being approximately equidistant from the isosmotic point. These similar costs are reflected directly in the parity of total metabolic rates.

Care must be exercised when comparing the total metabolic rates of trout in fresh water and 28 ppt sea water as the food consumption rates were unequal at the repletion ration level. As food consumption has been shown to influence metabolic rate the figures for total metabolic rate in fresh water and 28 ppt sea water at repletion rations were adjusted and compared in units of mg O_2 consumed/kg fish/hr/g food consumed every hour. Average figures of 217 resulted for fresh water fish and 233 for 28 ppt sea water fish. Thus at all ration levels, weight adjusted and ration adjusted metabolic rates were higher in 28 ppt sea water. A number of causes may have been responsible. For example the energy expended in osmotic and ionic regulation will be higher in 28 ppt sea water than fresh water as the former is further from

the isosmotic point. Nordlie et al (1975) believe that fish in high salinity waters absorb large quantities of salts through both the integument and the intestine. This salt must be eliminated and energy is expended achieving this. Hence, there is a high expenditure of energy in high salinities. In the present experiment, the percentage of ingested calories respired is greater at all ration levels for fish in 28 ppt sea water than fresh water. These results again confirm those of Rao (1968, 1971) who found that the energetic costs of regulation were higher in 30 ppt sea water than freshwater for rainbow trout. This resulted in higher active and standard metabolic rates in 30 ppt sea water. In the present experiments the trout may have experienced some slight osmotic stress despite careful acclimation to the high salinity. This may have enhanced the metabolic rates of the fish to an undetermined extent.

Thus, in the present experiment, it was inferred that changes in the energetic costs of osmoregulation caused by environmental salinity were paralleled by observed changes in metabolic rate. These latter changes were also matched by changes in growth rate.

Food Consumption and Sea water death

In contrast to the previous experiment there were no significant differences in the food consumption rates of trout fed to repletion in fresh water, 10 or 20 ppt sea water. However, the results obtained for fish in 28 ppt sea water were in agreement with the previous experiment in that voluntary food consumption was significantly less in 28 ppt sea water compared with fresh water. The relationships between metabolic rate and food consumption have been fully discussed in the previous chapter and it seems that in the present experiment, although

large differences in metabolic rates were observed between different ration levels, relatively small differences caused by environmental salinity had little effect on voluntary food consumption. Wallace (1973) believed that metabolic rate and rate of food intake were affected to different degrees by the same environmental change and it appears that this applies to the present experiment. Even though the metabolic rates of fish were decreased in 10 ppt sea water below those in fresh water the food consumption was unchanged. Ultimately the former led to the improved growth rate in 10 ppt sea water. Although equal food consumption by fish in fresh water and 20 ppt sea water contrasts with results found in the previous experiment, this result was to be expected as total metabolic rates of fish in these two media were equal. The depressed food consumption rates of fish in 28 ppt sea water possibly resulted through an osmotic or ionic stress which was manifested in the mortalities during the fifth week of the third experiment.

The differences in the results of this and the previous experiment with regard to food consumption may be due to a seasonal effect as the demand-feeding experiment was carried out in the Spring during March/April 1974 and the present experiment carried out during the winter months of the same and the following year, 1974 - 5. Despite the fact that the photoperiod was similar in both experiments the fish may have been subjected to seasonal fluctuations in some aspect of their physiology. This concept of seasonal fluctuations in the appetite of trout will be discussed fully in the next chapter when further results can be called upon.

The specific cause of the death of three fish after the fourth week of the third experiment was not determined. However, it was assumed that the mortalities were the result of a failure to maintain the com-

position and ionic balance of the body fluids within acceptable limits. Conte and Wagner (1965) suggested that excessive changes in the composition of the body fluids of fish after transfer to sea water can upset other metabolic processes, for example the acid-base balance, which may result in an increase in the concentration of other solutes which cannot be excreted. Lutz (1972) found in the perch, Perca fluviatilis, that breakdown of ionic-level controlling mechanisms occurred in sea water. Sodium and chloride ions invaded muscle cells in large quantities and both the muscle tissue and the whole body lost significant amounts of water resulting in mortality. The fact that mortalities should occur with rainbow trout, a fully euryhaline fish, in the present experiment when held in 28 ppt sea water is at first surprising. Many authors (e.g. Landless, 1976b) have found that rainbow trout of a relatively large size (>80g) can tolerate salinities greater than 28 ppt. However, Landless (1976b) found that handling rainbow trout within three weeks of acclimation to sea water could result in mortalities. As fish were weighed after only two weeks acclimation to 28 ppt sea water in the present experiment, the fish may have been suffering from handling 'stress' which resulted in the reduced food consumption rates and later mortalities.

Faeces

The percentage of ingested calories voided as faeces increased significantly with increasing ration levels at all salinities. This was caused both by the output of faeces increasing with increasing rations and by the higher calorific value per g of faeces at the repletion ration levels of feeding. These results tend to confirm those of several workers (Dawes, 1930, 1931; Brown, 1946a ,b ; Kinne, 1960; Warren & Davis,

1967, Averett, 1969) who found decreasing digestibility of food with increase in food intake. The reasons for this have been attributed to the influence of the rate at which food passes through the alimentary canal on absorptive processes. Barrington (1957) states that frequent feeding of fish will lead to an increase in the rate of emptying the stomach resulting in defaecation of undigested material. This seems to be the case at the repletion ration level where the caloric density of the faeces was significantly higher than at other ration levels. However different results have been found by some authors, for instance Gerking (1955a) working on Lepomis macrochirus. He found that the efficiency of protein absorption remained constant over a range of rations. However, his study was on nitrogen content, not calories, which possibly accounts for the discrepancy. Davies (1964) found with the goldfish, Carassius auratus, that energy extraction efficiency (energy of ration - energy of faeces, urine, mucus etc. ÷ energy of ration) was least at low ration levels and increased directly with ration level. A possible explanation for this difference is that a smaller range of ration levels was investigated in the present experiment than in that of Davies who used maintenance to maximum rations. However, the results of the present experiment tend to confirm Dawes's (1930, 1931) assertion that more complete absorption of food occurs at the intermediate ration levels compared with high ration levels.

No observable difference was found in the absorption efficiences between fresh water, 10 ppt and 20 ppt sea water fish at any one ration level which would indicate that salinity at low and intermediate levels has no effect on the apparent digestibility of food. This was in agreement with the results of Smith (1973) who investigated the effects of

sea water on the digestibility of food in the rainbow trout. However, poorer absorption efficiencies were observed at all ration levels for trout held in 28 ppt sea water. This ultimately contributed to the slower growth of these fish compared with fresh water fish. The reason for this might have been that fish under mild osmotic stress are incapable of digesting and metabolising food as efficiently as 'normal' fish.

Actual values of the efficiency of absorption ranged from 83% in 20 ppt at the 1% feeding rate to 78.3% in 28 ppt at the repletion ration level. These values are lower, on the whole, than comparable values of other authors, for example Averett (1969) and Brocksen and Cole (1972) both of whom found absorption efficiencies of 85% except at high ration levels. No satisfactory explanation for this difference can be made. Two sources of error in the present experiments may have been present in the absolute measurement of absorption efficiencies but it was anticipated that the errors would be similar in both fresh water and sea water thus maintaining the validity of the experiments as comparative determinations. The first source of error was that some leaching of soluble faecal material may have taken place while faeces remained in the faecal traps. If this had occurred it would have led to an overestimate of the absorption efficiency. The second source of error was that no account of metabolic faecal material, i.e. that material in the faeces originating from the tissues of the alimentary canal as opposed to the food, was taken. This would tend to underestimate slightly the absorption efficiency. In a later experiment (Chapter 7) the metabolic faecal nitrogen of trout held in fresh water and sea water was measured and no appreciable differences were seen.

This supports the above assertion that the errors incurred by discounting metabolic faecal material from apparent absorption efficiency calculations were equal in fresh and sea water.

Carcass Analysis

The change in the relative proportions of carcass constituents with increase in feeding rate is in agreement with the results of other authors. In the present experiment as the feeding rate increased the carcass moisture content decreased (although not significantly), the true protein content increased and the oil content increased. The lack of significance in the difference between adjacent rations in any one salinity was attributable to variability in the observations. These results confirm those found by Gerking (1955, a) with the bluegill sunfish, Lepomis macrochirus. Brett, Shelbourn and Shoop (1969) also had similar results with sockeye salmon, O. nerka,.

A source of error inherent in this type of energy-balance study lies in the assumption that the calorific value of the fish tissues remains constant throughout the experiment. Usually this assumption is un-

avoidable as it is impossible to measure the calorific value of a fish before an experiment and use that fish later. The small discrepancies between the total calories ingoing and the total calories outgoing for each ration level in each salinity may have been caused by this error. It is possible that there may have been small changes in fish caloric density over the course of an experiment which would remain undetected. Fortunately such discrepancies were small and results were still valid for comparative purposes. According to Solomon et al. (1972) large changes in the calorific value of fish tissue occur only when fish are starving or recovering from a period of food shortage. Using condition factors as an approximate guide to the calorific value of fish before and after each experiment might give an indication of whether a large difference in calorific value was present between salinities. Although the condition factors of fish increased over the course of all the experiments, the rate of increase was approximately the same at all ration levels in all salinities. Therefore the experiments are still valid for comparative purposes.

The fact that the percentage balance figures are all less than 100% indicates that a loss of ingested calories is occurring which has not been accounted for. This loss is similar at all ration levels and all salinities being 1.5 - 7.5% of the ingested calories. It is possible that the estimate of the calories lost through excretion, Qe, was an underestimate. No account was made of urea excreted nor of mucus sloughed off fish continually throughout the experiment. In the case of the former, absolute quantities of urea excreted are likely to be small (Brett, 1962). However, mucus secretion may well have caused a loss in calories for which it was difficult to account. If Tables 21, 22 and

23 are examined, it can be seen that figures for percentage balance were similar between fresh water and sea water tanks at the same ration levels in different salinities. Thus, experiments are still valid for comparative purposes.

Growth and Energy Balance

An inverse relationship was found between the proportion of ingested calories respired and the proportion retained as growth. This supported the theory that metabolism and growth are complementary (Beamish & Dickie, 1967). It was found that where a change in total metabolic rate occurred, as in fish in 10 ppt sea water, an opposite change in growth rate was seen provided that the ingested ration was maintained constant.

Increases in food intake were divided between metabolism and growth. This is supported by the work of Edwards et al. (1969) with the cod and Solomon et al. (1972) with the perch. As seen in Tables 10, 11 and 12, the conversion ratios of food into weight gain initially were at an intermediate value at the 1% ration level, then decreased at the 1.5% level and then reached maximum values at the repletion level in all salinities. This is shown in Figures 24, 25 and 26 where as food intake increases the percentage of ingested calories retained rises and then falls. This is mirrored by the changes in the percentage of ingested calories respired. This would seem to confirm the results of Swingle (from Hastings, 1969) that there is an optimum ration level which results in the best food conversion ratios and either side of this optimum level, the conversion ratios become poorer (higher). The reason for this is that at low ration levels, a high proportion of the ration is metabolised for maintenance purposes while at high ration levels, poorer absorption efficiencies and increasing metabolic 'costs'

of specific dynamic action decrease the proportion of the ingested food available for growth.

Growth, measured as specific growth rate, was slightly faster for fish in 10 ppt sea water than in fresh water. Growth, measured as the percentage of ingested calories retained, was significantly greater for fish in 10 ppt sea water than in fresh water. This probably points to a greater accuracy in using calories gained rather than wet weight gain as the index of growth. Growth, measured both as calories retained and as specific growth rate was significantly slower for fish in 28 ppt sea water than for fish in fresh water. Growth in fresh water and 20 ppt sea water was similar. Therefore salinity, at certain levels, can influence growth probably indirectly through changing the total metabolic rate. This is in agreement with the results of several authors mentioned in the Introduction who have investigated the growth of salmonid fish in different salinities. These include Bullivant (1960), Saunders and Henderson (1969a, b, c), Otto,(1971) and Garrison (1971). One notable exception to the above results is a recent publication by Shaw, Saunders and Hall (1975) who found that different water salinities exerted no significant influence on the growth of Atlantic salmon, S. salar, at any feeding level. Possibly the explanation for this lies in the fact that their experiments were conducted during October and November, i.e. late autumn. Hoar (1965) has presented a hypothesis which accounts for seasonal changes in the physiology of salmonids whereby the fish are adapted to life in fresh water during autumn and winter and to life in the sea during spring and summer. Support for the acceptance of this hypothesis can be found in studies on temporal changes in various physiological-behavioural parameters: resistance to

sea water (Conte & Wagner, 1965; Conte et al, 1966); changes in salinity preference (Baggerman, 1960a; McInerney, 1964); and variations in thyroid activity (Baggerman, 1960a). If salmonids are adapted towards a fresh water existence during autumn and winter then perhaps growth will be better in fresh water at this time. In fact, examination of Figures 1 and 2 from Shaw et al. (1975) reveals slightly better growth and food conversion efficiencies in fresh water than in either of the two strengths of sea water at the higher ration levels. In addition the species of fish they used is normally a smolting fish and seasonal influences may not have such a marked effect on a non-smolting fish such as the rainbow trout. In the present series of experiments, no seasonal influence on growth rate was found.

The present experiment supports the observations of others that certain environmental salinities affect the growth of rainbow trout. Sea water of 10 ppt salinity exerted an effect in that growth was enhanced. This was caused by the lowered total metabolic rate of fish at this salinity which resulted from the reduced metabolic 'costs' of osmotic and ionic regulation. This latter fact was deduced following the results of Rao (1971) who found that the cost of osmotic and ionic regulation was proportional to metabolic rate. The fact that growth rates were similar for fish in fresh water and 20 ppt sea water indicated that the costs of ionic and osmotic regulation in these salinities were equal resulting in similar metabolic rates which led to similar growth rates. Poorer growth rates in 28 ppt sea water compared to fresh water resulted from a decrease in absorption efficiency in 28 ppt together with an enhanced metabolic rate. This may have been caused either by the increased energy expenditure necessary to maintain the

osmotic and ionic integrity of the fish or by the slight stress inflicted upon trout in this high salinity. Whatever reason, the number of ingested calories available for growth was reduced.

During the next experiment an attempt was made to confirm the fact that the maintenance requirement for energy was lower for fish in 10 ppt sea water than in fresh water.

In conclusion the metabolic rates, growth rates and faecal output of rainbow trout fed at three feeding rates and held in different salinities were measured and incorporated into energy-budget equations. The main observations of the experiment were that salinity and feeding rate influenced metabolic rate. Metabolic rate was lowest at low feeding rates and highest at high feeding rates. Metabolic rate was lowest in 10 ppt sea water, equal in fresh water and 20 ppt sea water, and highest in 28 ppt sea water. Apparent absorption efficiency of ingested food was inversely related to feeding rate and was unaffected by salinity except in 28 ppt sea water where significantly lower apparent absorption efficiencies were found. Growth rate was directly proportional to feeding rate and was also affected by salinity. Growth, as percentage of ingested calories retained, was fastest in 10 ppt sea water, equal in fresh water and 20 ppt sea water and slowest in 28 ppt sea water.

Chapter 5. Experiment 3. The Determination of Various Growth and Food Utilization Parameters of Trout in Different Salinities

5.1. Introduction

Environmental salinity has been shown in the previous experiment to affect the growth rate and metabolic rate of rainbow trout. Different salinities caused different changes in metabolic rate and from this it was inferred that the energetic costs of osmotic and ionic regulation were altered. The metabolic rate of trout was found to be least in 10 p.p.t. sea water, a salinity which is close to the isosmotic point and in which previous authors (e.g. Rao, 1971) have found the energetic costs of osmotic and ionic regulation to be at a minimal level. Metabolic rate was also similar for fish in fresh water and 20 p.p.t. sea water, these salinities being approximately equidistant from the isosmotic point. Thus, it was inferred that the energetic costs of osmotic and ionic regulation were similar in fresh water and 20 p.p.t. sea water. These deductions assumed that the metabolic cost of osmotic and ionic regulation was proportional to the metabolic rate. This was found by Rao (1971) also working with rainbow trout in sea water. It was proposed in the present experiment to test this deduction. An indirect method was adopted. This involved the determination of the maintenance, optimum and maximum energy requirements of trout in three salinities; fresh water, 10 p.p.t. and 20 p.p.t. sea water. No higher level of salinity was used in this experiment owing to the

difficulties experienced in holding trout for long periods in 28 p.p.t. sea water during the previous two experiments.

In theory, a decrease in the energy requirement of a maintenance system such as ion-osmoregulation will result in a decrease in the overall maintenance energy requirement and vice-versa with an increase. Changes in the maintenance requirements may conceivably alter the optimum energy requirement i.e. that ration which provides for the greatest growth with least intake, together with the maximum energy requirement i.e. that ration that just provides for the greatest growth rate. Therefore, an experiment was devised to determine the maintenance, optimum and maximum ration levels for fish in different environmental salinities and to attempt to correlate these with the results of metabolic rates found in the previous experiment. In addition, a further study of the voluntary food intake of fish in different salinities was incorporated into the experiments in an attempt to reconcile the apparently conflicting results on appetite found in the previous two chapters.

In pursuit of the above aims the experiment was carried out in two phases; fresh water with 10 p.p.t. sea water and fresh water with 20 p.p.t. sea water. As in previous experiments, fresh water held in one system acted as the control to compare with a saline water in the other system. A feeding trial was undertaken where six different ration levels were fed to the fish in both experimental systems. From the resultant growth rates of the fish several growth and food conversion parameters were determined for each salinity. These included specific growth rates, maintenance, optimum and maximum energy requirements, gross growth efficiencies and finally, net growth efficiencies. For the purposes of this experiment these parameters and their derivation will

now be reviewed.

5.2. Basic Considerations

Indices of Growth

The more frequently observed parameters of growth are gain in wet body weight or gain in length. These determinations are usually valid to use if the experimental duration is short. However, Phillips and coworkers (1963) have shown that if rearing of fish takes place over a period of months, significant changes in body composition may occur leading to altered calorific values of fish tissue. Gerking (1967) directs attention to the inadequacy of weight increase as a description of the growth process and substitutes energy or protein increase for use in laboratory experiments. In the present experiment, calories were used as comparisons were to be made with the previous chapter.

For the purposes of determining the maintenance, optimum and maximum energy requirements of fish in different salinities, gain in live body weight was used in determining the multiplicative or specific growth rates of fish fed different ration levels. The equation for this has been given by Brown (1957) as:-

$$\text{Specific Growth Rate (S.G.R)} = \frac{{}^{1}_{n}W_2 - {}^{1}_{n}W_1}{T_2 - T_1} \times 100 \quad \ldots\ldots\ldots 1$$

Where W_2 is the total weight of fish per tank at time T_2 and W_1 is the total weight of fish per tank at time T_1, T_2 being later than T_1. T_1 and T_2 are measured in days.

If growth rate at different ration levels is plotted against rate of feeding then, in theory, a curve of 'geometric shape' is produced. Such a curve is shown in Figure 27 and is adapted from Brett et al. (1969).

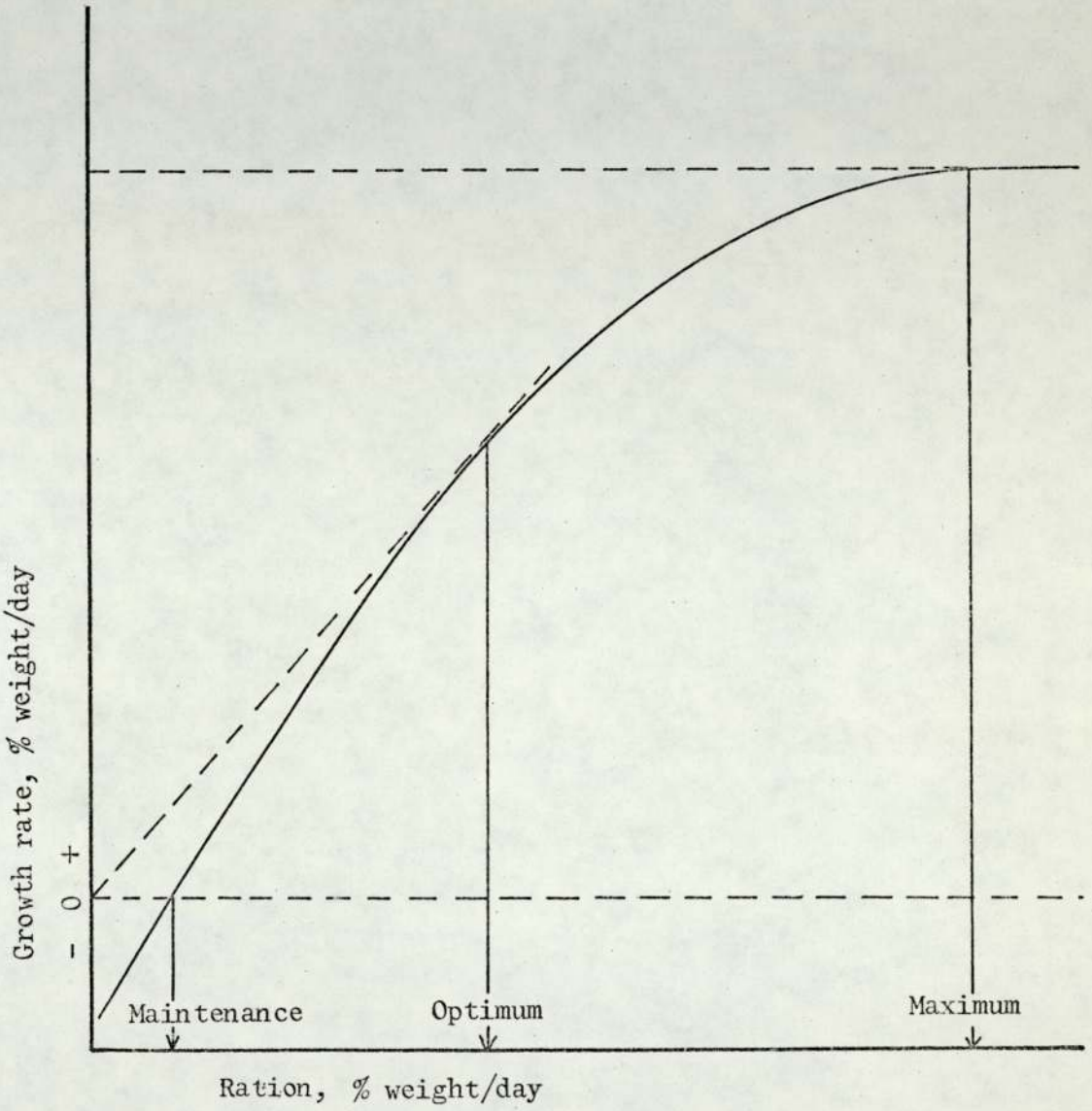


Fig.27. Geometric derivation of growth parameters with accompanying ration. After Thompson (from Brett et al. 1969)

Three parameters may be derived from this curve (Thompson from Brett et al, 1969). These are; the maintenance ration defined as that ration which just maintains the fish without any weight change; the optimum ration defined as that ration which provides for the greatest growth for least intake; the maximum ration defined as that ration which just provides for the maximum growth rate. Maintenance ration is determined from the intercept of the curve with the horizontal line originating from the point of zero growth. Optimum ration is determined by drawing a line from the origin to meet the curve tangentially. Dropping a vertical line from the meeting point to the abscissa determines the optimum ration level. Finally, the maximum ration is derived from the exact asymptote of the curve and dropping a vertical line to the abscissa.

Feeding Levels

Choosing ration levels for fish in experimental conditions must be undertaken carefully. The commonest method of reporting feeding rates involves rations consumed being related to the weight of the fish in the tank. Before choosing the feeding rates and the duration of the experiment it must be borne in mind that at high feeding rates over long periods the appetite of the fish will eventually become less than the quantity of food offered (Hastings & Dickie, 1972; Brett & Shelbourn, 1975). Also, care must be exercised when measuring the metabolic rates of fish fed on constant percentage rates over long periods. Paloheimo and Dickie (1966a) suspect that feeding a constant proportion of the body weight in food leads to a progressive rise in the level of meta-

bolism (c.f. Appendix 1) with growth until the active level of metabolism was reached. For these reasons the duration of each experiment was six weeks over which it was anticipated that differences in growth rates would become apparent but the time would be short enough to preclude the above problems.

A number of ration levels was chosen which would span a range of feed intakes from just above an estimated maintenance ration to repletion feeding. From previous experience the repletion level of feeding was anticipated as being approximately 2.0% live body weight/day. Accordingly the six ration levels were 0.5%, 0.75%, 1.0%, 1.5%, 1.75% and repletion feeding. As in other experiments food was fed three times per day except for the two lowest feeding rates which were fed once per day in order to give equal opportunity of feeding to all fish in the tanks. Those fish fed to repletion were not strictly on an ad libitum feeding regime as this implies unlimited access to food at all times. While the repletion method of feeding, outlined in the previous chapter, is akin to ad libitum feeding the difference is that unlimited food is available for only a limited time. Restricted feeding periods lead to food consumption rates below the maximum possible (Rozin & Mayer, 1964) and so repletion feeding will result in slightly lower food consumption rates than ad libitum feeding.

Indices of Food Utilization

Indices of food utilisation for growth are derived as simple ratios. Two such ratios are in common use. The first is defined as food required to produce one unit of weight increase and is known as the conversion factor or conversion ratio. This ratio was employed in the previous experiment.

$$\text{Conversion Ratio} = \frac{\text{Food fed, g. 'dry' feed}}{\text{Gain in live weight, g.}} \quad \ldots\ldots\ldots\ldots \quad 2$$

In hatchery and laboratory practice values can range between 1.2 and 3.0 depending upon conditions.

The second ratio is the inverse of the first and is defined as the growth produced by one unit of food fed. This is termed Gross Growth Efficiency and is most usefully determined in terms of calories or protein.

$$\text{Gross Growth Efficiency } (K_1) = \frac{\text{Weight gain, cal.}}{\text{Food fed, cal.}} \quad \ldots\ldots\ldots\ldots \quad 3$$

Values for this can range up to 0.5 as usually about 50% of food calories are respired.

A third, more infrequently used ratio is termed Net Growth Efficiency and involves the subtraction from the divisor of equation 3 an amount of calories which is equivalent to the maintenance requirement for the animal. This has been described in detail by Pentelow (1939), Brown (1957) and Swingle (from Hastings, 1969).

Thus:-

$$\text{Net Growth Efficiency} = \frac{\text{Weight gain, cal.}}{\text{Food intake - Maintenance Requirement, cal.}} \quad \ldots\ldots\ldots\ldots \quad 4$$

A further modification of equation 3 involves correcting the divisor by a factor of 'p', the assimilation coefficient (Winberg, 1956). Thus, a measure is obtained of the growth produced by one unit of assimilated food, or

$$K_2 = \frac{\text{Weight gain, cal}}{\text{p. Caloric Intake}} \quad \ldots\ldots\ldots\ldots \quad 5$$

The K-line

Apart from the investigation of the effects of salinity on the growth of fish it was considered that an investigation into the K-line phenomenon would be most valuable. Paloheimo and Dickie (1965) first proposed the existence of an empirical relationship between rations and growth efficiency, termed the 'K-line'. They (Paloheimo & Dickie, 1966b) re-examined the results of previous authors working on laboratory studies of food and growth in fishes. They found a regular variation of gross growth efficiency with changes in ration level. Gross growth efficiency has already been described in equation 3. Often, the regular variation in growth efficiency could be satisfied by a straight line, the K-line, of zero or negative slope if growth efficiency was plotted on a logarithmic ordinate. This relationship is given by the equation,

$$\log K_1 = \log. \frac{\text{Weight Gain}}{\text{Food Intake}} = -a - bR \quad \ldots\ldots\ldots\ldots 6$$

Where a and b are constants and R = Food Intake.
Food intake and weight gain are measured in the same units.
Interestingly, the actual growth efficiency seemed to be independent of body weight (Paloheimo & Dickie, 1965). Thus, the same absolute ration appears to lead to the same absolute growth independently of the fish size. This only holds true for ration levels between maintenance and maximum.

Paloheimo et al (1966a, b) demonstrated that a highly complex relationship existed between food, temperature, body size, metabolism and growth. Interactions of these were reflected in alterations of K-line gradients. Support for their findings has come from Brett et al (1969) with O. nerka and Le Brasseur (1969) with O. keta, both of whose data originally appeared conflicting but which were later appraised and re-

solved by Kerr (1971). Kerr considered that except for an initial positive phase at low ration levels, the K-line as originally described by Paloheimo and Dickie was probably the general form to be encountered. However, Kerr was of the opinion that varying levels of spontaneous activity induced by different ration levels were responsible for the laboratory K-line. He substituted this opinion in place of the more generally held theory that K shows a progressive decrease as the assimilation coefficient, 'p', decreases with greater rations. The available evidence indicates that 'p' may decrease significantly at high ration levels. Kerr's theory that it is varying levels of activity which account for the form of the K-line is not substantiated by Brett et al. (1969) whose fish were swimming at a constant low level of activity and who found a K-line relationship between growth efficiency and rations.

In the present experiments it was hoped that a relationship between gross growth efficiency and rations would be demonstrated. As the fish would be swimming at a constant low level of activity, so considerably reducing spontaneous activity, the theory of Kerr (1971) could be tested. In addition, it was considered of great interest to examine the effects of salinity on the position and gradients of such K-lines as could be produced.

5.3. Materials, Methods and Experimental Procedure

The design of the systems used in this experiment was essentially the same as the previous experiment. Two systems were employed, one holding fresh water and the other holding sea water. Each system included six tanks for holding fish which were used exclusively for growth studies. All fish were fed on 'Beta' floating trout food grade number four. Six different levels of feeding were assigned among the tanks of one system by using a table of random numbers. Similarly, the same levels were assigned among the tanks of the other system. The temperature of the water in both systems was maintained at 12± 0.5°C. The sea water in one system was made artificially from the constituents shown in Table 2.

The programme of study was conducted in two phases of six weeks duration each preceded by a two week acclimation period. Firstly, fresh water and 10 ppt sea water were used in the systems and secondly fresh water and 20 ppt. The first phase was conducted during the months of April - May and the second May - June. The methodology was identical for both experiments. The procedure adopted for the first phase of the experiment will now be described.

Rainbow trout of approximately the same size and weight, 17.5 cm and 60 g respectively were introduced into both experimental systems one containing fresh water, the other 10 ppt sea water. Twenty fish were allocated to each tank. The fish were then weighed on a 'batch basis' as described in Chapter 2 and several fish of different sizes were interchanged between tanks so that the average initial weight of fish per tank was approximately the same.

Six dietary ration levels had already been allocated between the

twelve tanks and each ration level was represented in both fresh and sea water. These ration levels were determined on a percentage body weight per day basis and were 0.5%, 0.75%, 1.0%, 1.5%, 1.75% and repletion feeding. The fish were then fed for two weeks on their assigned rations to acclimate them to general conditions. The method of feeding to repletion has been described in the previous chapter. After the two week acclimation period, fish in every tank were weighed individually using MS 222 as anaesthetic, as described in Chapter 2. Analysis of variance and Duncan's Multiple Range Test were then carried out on the individual fish weights and no significant differences were seen between any of the tank-weights of fish with the exception of the fresh water tank assigned a 1.5% ration which had a significantly lower average tank weight ($p < 0.05$) than the rest. This was borne in mind when examining the final data.

After the individual weighing the absolute food fed was adjusted upwards according to the feeding rate assigned to each tank in order to take account of the growth of the fish and the experiment started. The experiment lasted six weeks and fish were weighed every fortnight on a 'batch basis' except for the final weigh when individual weighing was carried out. After the final weigh, three fish from each tank were killed by a blow on the head, sealed in polythene bags and deep-frozen for future carcass analysis. After each weighing rations fed were re-adjusted according to feeding rate in order to take account of the growth of the fish. All tanks of fish were fed three times per day at 9.30 a.m., 2.00 p.m. and 5.30 p.m. except those fed at the 0.5% and 0.75% feeding rates. These were fed once per day at 9.30 a.m. to allow all fish an equal opportunity of feeding (cf. Brett et al., 1969). If this practice had

not been undertaken it was anticipated that the larger fish within the tanks fed at a low rate would eat the major portion of the food so resulting in unequal size distribution.

After the final weighing, all the fish were removed and replaced with new fish again 20 per tank. After three days the salinity of the sea water system was increased over two days to 20 ppt. Fish were then weighed on a 'batch basis' and the weights of fish per tank adjusted by interchanging fish between tanks. Fish were then fed at their assigned ration levels for two weeks to acclimate them to the general conditions. After the two weeks they were then weighed individually under anaesthetic and their weights per tank compared for significance using Analysis of variance and Duncan's Multiple Range Test. No significant differences were seen between the initial average tank-weights of fish. After the weighing the second experiment proceeded, in the same manner as before. This procedure has been summarised in Table 26.

In both phases of the experiment food consumed by fish in the two repletion-feeding tanks was measured daily. The total food consumed every fortnight was summed and converted into a percentage of the total initial fish weight fed per day. The following formula was used to determine the fortnightly weight-adjusted feeding rate per tank:-

$$\text{Weight-adjusted feeding rate per tank, g.food consumed/day/100 g fish} = \frac{\text{Total Food consumed per fortnight, g.}}{14 \times \text{Initial Total Weight of Fish/Tank, g.}} \times 100$$

Thus, three values were obtained for each repletion tank in both phases of the experiment. The means of the three fortnightly values were compared for significance using Students' 't' test.

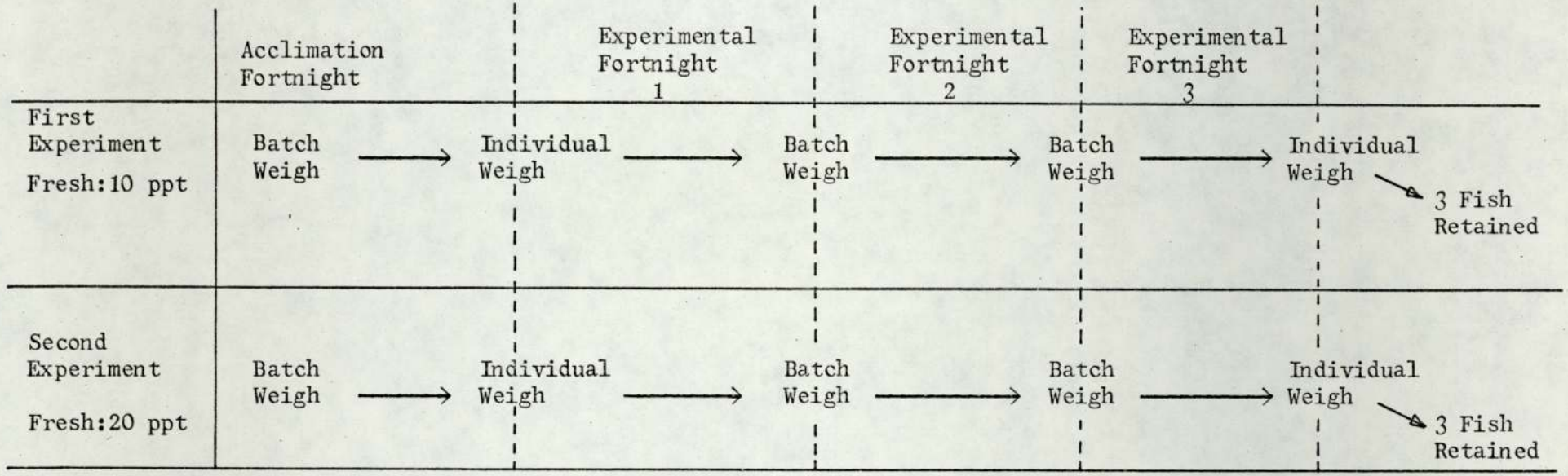


Table 26. The experimental procedure adopted for each tank of fish during the freshwater:10 ppt experiment and the freshwater:20 ppt experiment.

Fish retained and frozen after the first experiment (fresh water: 10 ppt sea water) were all analysed for proximate body composition. Of the fish retained after the second experiment (fresh water: 20 ppt sea water) only those fish from the 20 ppt sea water tanks were analysed. This was due to the fact that the mechanical failure of a deep freeze unit had caused the decomposition of some of the fresh water specimens. Thus, three groups of fish were obtained, each representing a different salinity. Each group was subdivided into six lots, these representing the different feeding rates and three fish were obtained for each lot. The following constituents were determined for each lot of fish:- dry matter, protein content and oil content. Three replicate determinations were used in each lot for each constituent and each carcass constituent was examined in turn by analysis of variance and Duncan's Multiple Range Test (Duncan, 1955) to investigate the between-salinity differences and the between-feeding rate differences.

In computing results the following formulae were used:-

1. Maintenance Energy Requirement, Kcal/Tank/Experiment $= \dfrac{M \times T \times C \times \text{Mean Total Fish weight, g}}{100}$

where M = Maintenance Ration as % Body Weight/day

T = Time, days

C = Caloric value of food, Kcal/g

The mean total fish weight is calculated from the formula given in Chapter 3.

2. Gross Growth Efficiency, % $= \dfrac{\text{Calories Retained} \times 100}{\text{Caloric Intake}}$

3. Net Growth Efficiency, % $= \dfrac{W \times 100}{I - M}$

where W is the calories retained over the duration of the experiment, Kcal

I is the caloric intake, Kcal

M is the maintenance energy requirement, Kcal.

5.4. Results

In this section, the results are presented in the following order:- food consumption by fish in the repletion tanks, proximate carcass analysis, growth and food conversion.

Food consumption

The mean weight-adjusted food consumption rates of fish fed to repletion are shown in Table 27. In both the first and second experiments, weight-adjusted food consumption was significantly greater ($p < 0.05$) by fish in sea water than by fish in fresh water. For example, the mean weight-adjusted food consumption rate by fish in 10 ppt sea water was 2.12% per day compared with 2.02% per day in fresh water. Similarly, figures of 2.08% per day for fish in 20 ppt sea water and 2.01% per day for fish in fresh water were obtained for the second experiment.

Proximate Carcass Analysis

Tables 28 and 29 show the values obtained for percentage dry matter, percentage protein content and percentage fat content of fish fed at different rates in three salinities. Each value is the mean of three replicate determinations. No significant differences were found in the mean values of any component between different salinities taking each ration level in turn. However, in all salinities, feeding rate was shown to have a marked effect on the levels of dry matter, protein and fat in carcasses as is shown in Tables 28 and 29. Percentage dry matter increased directly with feeding rate being 22.0 - 22.2% at the 0.5% feeding rate and 25.3 - 25.7% at the repletion feeding rate. Protein content decreased with increase in feeding rate being 69.3 - 69.6% at the 0.5% feeding rate and 66.8 - 67.0% at the repletion feeding rate. Results ob-

Experiment	Salinity	Weight adjusted[a.] Feeding Rate, % of body weight/day		Standard Error of Mean	Probability
1	Fresh Water	2.02	±	0.021	< 0.05
	10 ppt Sea Water	2.12	±	0.024	
2	Fresh Water	2.01	±	0.018	< 0.05
	20 ppt Sea Water	2.08	±	0.018	

Table 27. Mean Weight-adjusted feeding rates of fish fed to repletion in the first and second experiments

a. Means of 3 fortnightly values

Table 28. Proximate carcass analysis of fish fed at six different rates in three salinities. The percentage dry matter and protein content.

Salinity	Feeding Rate %	Dry Matter[a] % of Wet Weight ±	Standard Error of Mean	Probability[b]	Protein Content[a] % of Dry Matter ±	Standard Error of Mean	Probability[b]
Fresh Water	0.5	22.2	± 0.100		69.3	± 0.208	
	0.75	22.4	± 0.100		69.0	± 0.100	
	1.0	22.8	± 0.100		68.9	± 0.173	
	1.5	24.2	± 0.208		67.4	± 0.100	
	1.75	24.6	± 0.153		67.3	± 0.101	
	R	25.7	± 0.057		67.0	± 0.153	
10 ppt Sea Water	0.5	22.0	± 0.264		69.6	± 0.153	
	0.75	22.2	± 0.100		68.9	± 0.115	
	1.0	23.2	± 0.153		68.5	± 0.115	
	1.5	24.1	± 0.057		67.2	± 0.057	
	1.75	24.5	± 0.153		66.9	± 0.264	
	R	25.3	± 0.155		66.9	± 0.153	
20 ppt Sea Water	0.5	22.1	± 0.153		69.5	± 0.154	
	0.75	22.0	± 0.153		68.9	± 0.155	
	1.0	22.6	± 0.154		68.4	± 0.208	
	1.5	24.0	± 0.264		67.3	± 0.153	
	1.75	24.7	± 0.115		67.0	± 0.153	
	R	25.4	± 0.200		66.8	± 0.231	

R = Repletion Feeding

a = Mean values of three replicates

b = Means joined by the same line are not significantly different at the 5% level of probability

Table 29. Proximate carcass analysis of fish fed at six different rates in three salinities. The percentage oil content and calorific values.

Salinity	Feeding[c] Rate %	Fat content[a] % of dry matter	Standard Error of ± Mean	Probability[b]	Calorific Value Kcal/g dry matter
Fresh Water	0.75	22.3	± 0.200		6.006
	1.0	22.4	± 0.208		6.010
	0.5	22.5	± 0.153		6.042
	1.5	23.9	± 0.251		6.067
	1.75	24.2	± 0.100		6.069
	R	24.8	± 0.100		6.129
10 ppt Sea Water	1.0	22.0	± 0.264		5.949
	0.5	22.6	± 0.153		6.068
	0.75	22.6	± 0.173		6.028
	1.5	23.7	± 0.153		6.036
	1.75	24.2	± 0.115		6.067
	R	24.6	± 0.208		6.104
20 ppt Sea Water	1.0	22.2	± 0.153		5.962
	0.75	22.5	± 0.251		6.019
	0.5	22.7	± 0.208		6.072
	1.5	23.6	± 0.208		6.033
	1.75	24.3	± 0.115		6.082
	R	24.7	± 0.100		6.108

a = Mean value of 3 replicates

b = Means joined by straight lines are not significant at the 5% level of probability

c = Note. Feeding rates are ranked in order of increasing oil content

tained for percentage fat content were less easily interpreted. No significant differences were observed between mean percentage fat contents at the 0.5, 0.75 and 1.0% feeding rates. There appeared to be no observable trend in the fat contents within these feeding rates as the rates increased. However, at the 1.5, 1.75% and repletion feeding rates percentage fat content increased directly with feeding rate.

Estimated calorific values of the fish tissue are given in Table 29 for each rate. These were derived using values obtained from Brody (1945) of 5.65 Kcal/g protein and 9.45 Kcal/g fat. Calorific values were used later on in computing gross growth efficiencies and net growth efficiencies.

Growth

Mean specific growth rates of fish fed at different feeding rates in different salinities are given in Tables 30 and 31. In all salinities specific growth rate and food consumption were directly related. Minimum values at the 0.5% feeding rate ranged from 0.010 - 0.078%/day while maximum values found at the repletion feeding rate ranged from 1.122 - 1.148%/day. The specific growth rates of fish in 10 ppt sea water were higher at each feeding rate than those of fish in the equivalent fresh water tanks. The means of the three fortnightly specific growth rates at each feeding rate were compared either by Student's 't' test or the Fisher-Behrens test depending on the significance of the variance ratios. The specific growth rates of fish at the 0.5%, the 0.75%, the 1% and the 1.75% feeding rates were significantly higher ($p < 0.02$, $p < 0.05$, $p < 0.05$ and $p < 0.01$ respectively) for fish in 10 ppt sea water than fish in fresh water. The lack of significance in the differences at the other ration levels was attributed to higher variability

Feeding Rate	Salinity	Initial Mean Fish weight g/tank	Final Mean Fish weight g/tank	Mean Specific Growth rate[a] %/day/tank	± Standard Error of Mean	Probability	Maintenance Energy requirement For Experiment, Kcal	Total Food Consumed, g/tank
0.5%	Fresh	69.4	69.7	0.010	± 0.0006	<0.02	1045.7	291.9
	10 ppt	68.0	70.3	0.078	± 0.0085		955.3	289.0
0.75%	Fresh	66.4	76.9	0.348	± 0.0021	<0.05	1077.0	439.6
	10 ppt	67.6	79.3	0.381	± 0.0047		1013.9	449.6
1.0%	Fresh	69.0	88.3	0.585	± 0.0049	<0.05	1182.8	631.0
	10 ppt	69.9	90.7	0.618	± 0.0036		1109.2	642.1
1.5%	Fresh	58.8	89.7	1.006	± 0.0021	Not signif.	1116.5	858.7
	10 ppt	68.3	105.8	1.044	± 0.0170		1202.6	1003.5
1.75%	Fresh	64.2	102.4	1.110	± 0.0087	<0.01	1252.5	1112.0
	10 ppt	64.8	105.8	1.165	± 0.0060		1177.8	1131.7
R	Fresh	71.5	114.6	1.122	± 0.0101	Not signif.	1399.6	1432.1
	10 ppt	69.3	112.3	1.140	± 0.0036		1254.0	1460.5

Table 30. Weight change, specific growth rates and maintenance energy requirements of fish fed at different rates in fresh water and 10 ppt sea water.

a = Means of 3 fortnightly values

R = Repletion feeding

Feeding Rate	Salinity	Initial Mean Fish weight g/tank	Final Mean Fish weight g/tank	Mean a. Specific Growth rate %/Day/tank	± Standard Error of Mean	Probability	Maintenance Energy requirement For Experiment, Kcal	Total Food Consumed, g/tank
0.5%	Fresh	69.4	69.9	0.015	± 0.0039	Not signif.	1047.6	291.3
	20 ppt	68.2	68.5	0.010	± 0.0082		1028.4	287.1
0.75%	Fresh	70.1	81.9	0.372	± 0.0091	Not signif.	1143.0	465.6
	20 ppt	70.6	82.8	0.380	± 0.0075		1153.1	469.5
1.0%	Fresh	68.1	87.6	0.599	± 0.0061	Not signif.	1171.2	624.0
	20 ppt	70.9	91.1	0.595	± 0.0060		1218.1	649.0
1.5%	Fresh	68.5	105.1	1.018	± 0.0086	Not signif.	1305.6	1002.9
	20 ppt	69.6	107.7	1.039	± 0.0093		1332.8	1021.5
1.75%	Fresh	68.7	110.2	1.123	± 0.0076	Not signif.	1345.0	1192.3
	20 ppt	69.3	110.4	1.109	± 0.0112		1351.0	1199.3
R	Fresh	68.9	111.4	1.144	± 0.0106	Not signif.	1355.3	1377.0
	20 ppt	68.8	111.5	1.148	± 0.0072		1355.8	1424.9

Table 31. Weight change, specific growth rates and maintenance energy requirements of fish fed at different rates in fresh water and 20 ppt sea water.

a = Mean of 3 fortnightly values

R = Repletion feeding

in observed values at the 1.5% feeding rate and to the different rates of food consumption at the repletion feeding levels. Relevant significance values are shown in Table 30. Mean values for specific growth rate are shown plotted against feeding rate in Figure 28.

The specific growth rates of fish in fresh water and 20 ppt sea water are shown in Table 31. The means of the three fortnightly specific growth rates were found at each feeding rate and comparison between salinities made by Student's 't' test. These mean values are shown plotted against ration level in Figure 29. No significant differences were found between the specific growth rates of fish in fresh water and 20 ppt sea water at each feeding rate.

Figures 28 and 29 comprise curves which originate near the abscissa and rise with steadily decreasing gradients to an asymptote or possibly a point of flexion. In the absence of any suitable transformation, smooth curves were drawn in by eye passing through the mean values. These mean values are given in Tables 30 and 31, each being an average of three fortnightly determinations. Each curve was extrapolated downwards a short distance to meet the abscissa of the graph. In all cases the portion of the curves which ranged between the minimum and the maximum values of specific growth rate were of quasi-parabolic nature which nearly satisfied the following quadratic equations:-

Figure 28 - 10 ppt sea water - $y = -0.54x^2 + 2.07x - 0.82$

Fresh water - $y = -0.54x^2 + 2.08x - 0.89$

Figure 29 - 20 ppt sea water and fresh water - $y = -0.56x^2 + 2.16x - 0.92$

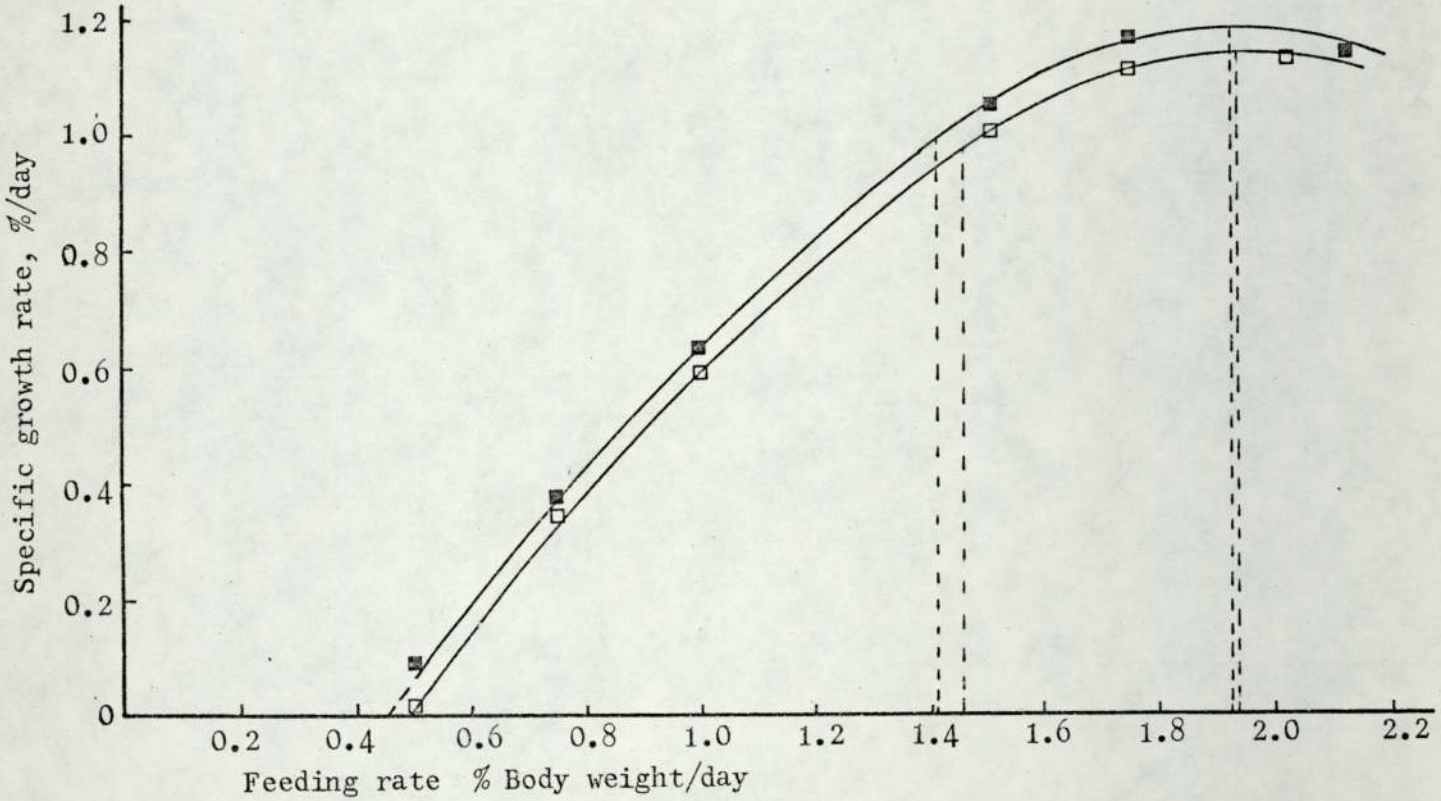


Fig.28. The variation of specific growth rate with increasing feeding rate in fresh water □ , and 10 ppt sea water ■ .
Each point is the mean of three values

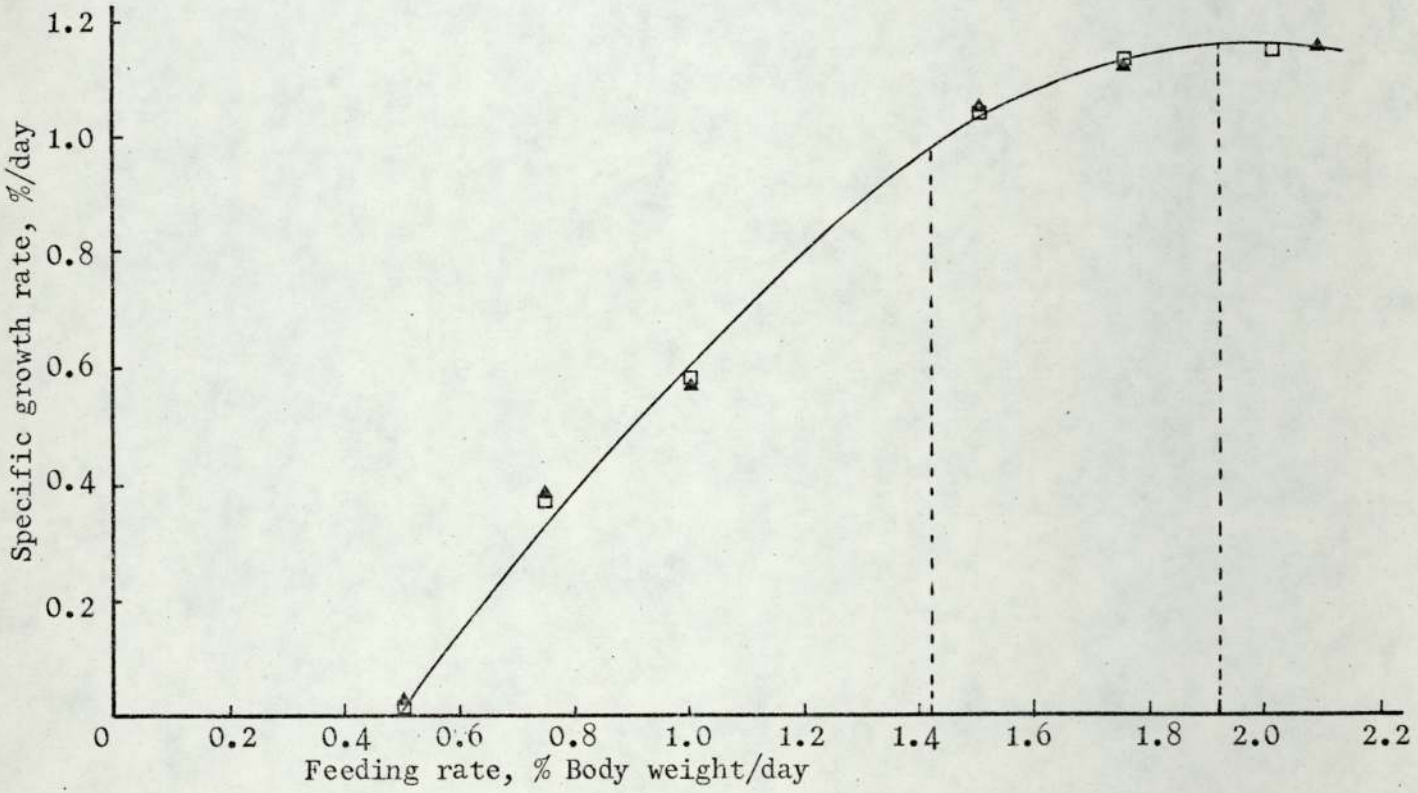


Fig.29. The variation of specific growth rate with increasing feeding rate in fresh water □ , and 20 ppt sea water ▲ .
Each point is a mean of three values

Comparison of the two curves in Figure 28 reveals that the specific growth rate curve for 10 ppt sea water is displaced upwards and slightly to the left from the fresh water curve. Derivation of the three parameters detailed earlier was attempted, these being the maintenance, optimum and maximum ration levels. A distinct difference in the maintenance ration values was observed these being 0.45% of the body weight per day for fish in 10 ppt sea water and 0.49% for fish in fresh water. The optimum and maximum ration levels were also lower for 10 ppt fish than fresh water fish but as the differences were small, caution must be practised in attributing significance to the differences. The optimum ration level was 1.41% of the body weight per day for fish in 10 ppt sea water compared with 1.43% for fish in fresh water. Finally, the maximum ration level was 1.93% for fish in 10 ppt sea water compared with 1.94% for fish in fresh water.

Comparison of the values plotted in Figure 29 revealed no observable difference in curves drawn for fresh water and 20 ppt sea water. Hence, only one curve is shown in the figure. The maintenance, optimum, and maximum rations were 0.49%, 1.42% and 1.91% respectively for both fresh water and 20 ppt sea water fish.

Maintenance ration levels were used to calculate the maintenance energy requirements for each tank of fish over the course of both experiments (freshwater : 10 ppt and fresh water : 20 ppt). These are shown in Tables 30 and 31. In absolute terms, maintenance energy requirements were 1.64 - 1.79 Kcal/100 g fish/day depending upon the salinity.

Indices of Food Conversion

Mean conversion ratio values for fish fed at different rates in different salinities are given in Tables 32 and 33. In all salinities, conversion ratio was markedly influenced by feeding rate. At low feeding rates, conversion ratios were high. As the ration was increased conversion ratios decreased to reach a minimum at the 1.5% feeding rate after which they increased once more. At the optimum point conversion ratios were just under 1.4 (Figures 30 and 31). The shape of the curves in all cases was near-parabolic with the parabola opening upwards. In Figure 31 (fresh water: 20 ppt sea water) only one curve has been drawn as separation between the two was impossible.

The means of the three fortnightly conversion ratio values at each feeding rate were compared either by Student's 't' test or the Fisher-Behrens test depending upon the significance of the variance ratios. Conversion ratios for fish at the 0.5%, the 0.75%, the 1% and the 1.75% feeding rates were significantly lower ($p < 0.001$, $p < 0.01$, $p < 0.02$, $p < 0.02$ respectively) for fish in 10 ppt sea water than for fish in fresh water. The lack of significance in the differences at the other ration levels was attributed to higher variability in observed values at the 1.5% feeding rate and to the different rates of food consumption at the repletion feeding levels. Relevant significance values are shown in Table 32.

No significant differences in conversion ratios were observed between fish in fresh water and in 20 ppt sea water at each feeding rate (Table 33). Mean conversion ratios for the fresh water:20 ppt experiment are shown plotted against feeding rate in Figure 31.

Feeding Rate	Salinity	Mean Conversion Ratio[a]	± Standard error of Mean	Probability	Total Food consumed Kcal	Total Energy Retained as Growth Kcal	Gross Growth Efficiency %	Net Growth Efficiency %
0.5%	Fresh	44.26	± 1.235	p <0.001	1066.3	8.05	0.75	39.08
	10 ppt	6.10	± 0.505		1055.7	60.47	5.73	60.13
0.75%	Fresh	2.05	± 0.014	p <0.01	1605.8	281.51	17.53	53.23
	10 ppt	1.93	± 0.021		1642.3	312.02	19.00	49.65
1.0%	Fresh	1.65	± 0.022	p <0.02	2304.9	527.31	22.88	46.99
	10 ppt	1.55	± 0.015		2345.5	575.74	24.55	46.57
1.5%	Fresh	1.38	± 0.050	N.S.	3136.7	907.96	28.95	44.94
	10 ppt	1.34	± 0.055		3665.6	1090.96	29.76	44.29
1.75%	Fresh	1.47	± 0.020	p <0.02	4061.9	1141.77	28.11	40.64
	10 ppt	1.37	± 0.015		4133.9	1218.67	29.48	41.22
R	Fresh	1.67	± 0.025	N.S.	5231.2	1357.18	25.94	35.42
	10 ppt	1.70	± 0.036		5334.9	1325.99	24.85	32.49

Table 32. The conversion ratios, gross growth efficiencies and net growth efficiencies of fish fed at different rates in fresh water and 10 ppt sea water.

a = Mean of 3 fortnightly values

R = Repletion feeding

Feeding Rate	Salinity	Mean Conversion[a] Ratio	±	Standard error of Mean	Probability	Total Food consumed Kcal	Total Energy Retained as Growth Kcal	Gross Growth Efficiency, %	Net Growth Efficiency %
0.5%	Fresh	33.26	±	5.058	N.S.	1064.1	12.07	1.13	73.15
	20 ppt	45.73	±	3.597		1048.7	8.05	0.77	39.65
0.75%	Fresh	1.97	±	0.034	N.S.	1700.7	318.63	18.73	57.13
	20 ppt	1.92	±	0.050		1715.0	322.92	18.83	57.47
1.0%	Fresh	1.61	±	0.042	N.S.	2279.4	534.30	23.44	48.21
	20 ppt	1.61	±	0.061		2371.4	542.44	22.87	47.03
1.5%	Fresh	1.37	±	0.031	N.S.	3663.4	1073.84	29.31	45.54
	20 ppt	1.36	±	0.025		3731.4	1103.23	29.57	45.99
1.75%	Fresh	1.43	±	0.021	N.S.	4355.3	1240.79	28.49	41.22
	20 ppt	1.45	±	0.047		4380.8	1235.09	28.19	40.76
R	Fresh	1.62	±	0.041	N.S.	5029.9	1338.28	26.61	36.42
	20 ppt	1.68	±	0.056		5204.9	1323.16	25.42	34.37

Table 33. Conversion ratios, gross growth efficiencies and net growth efficiencies of fish fed at different rates in fresh water and 20 ppt sea water

a = Means of 3 fortnightly values

R = Repletion feeding

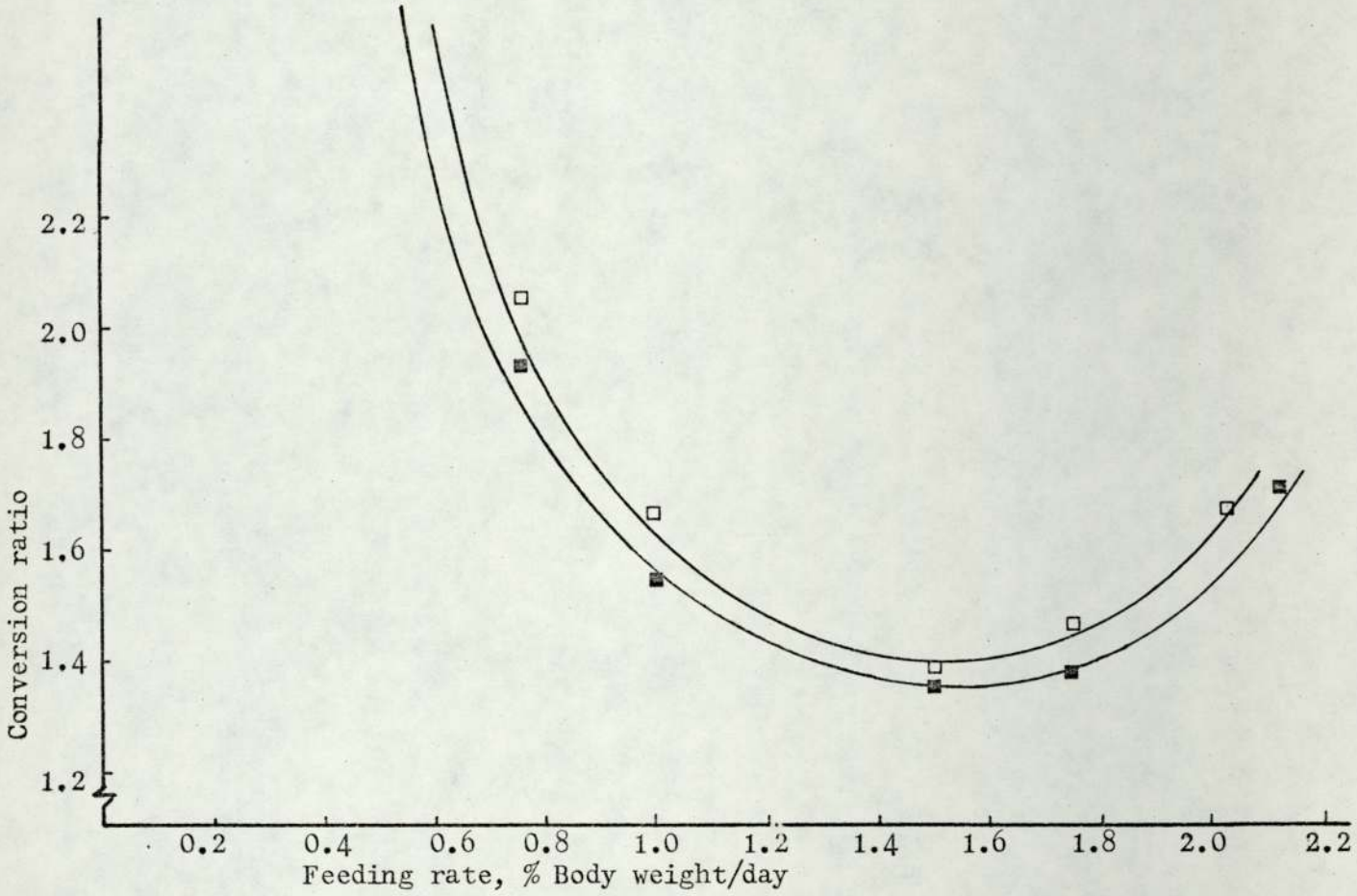


Fig.30. The variation of conversion ratio with increasing feeding rate in fresh water □, and 10 ppt sea water ■.
Each point is a mean of three values

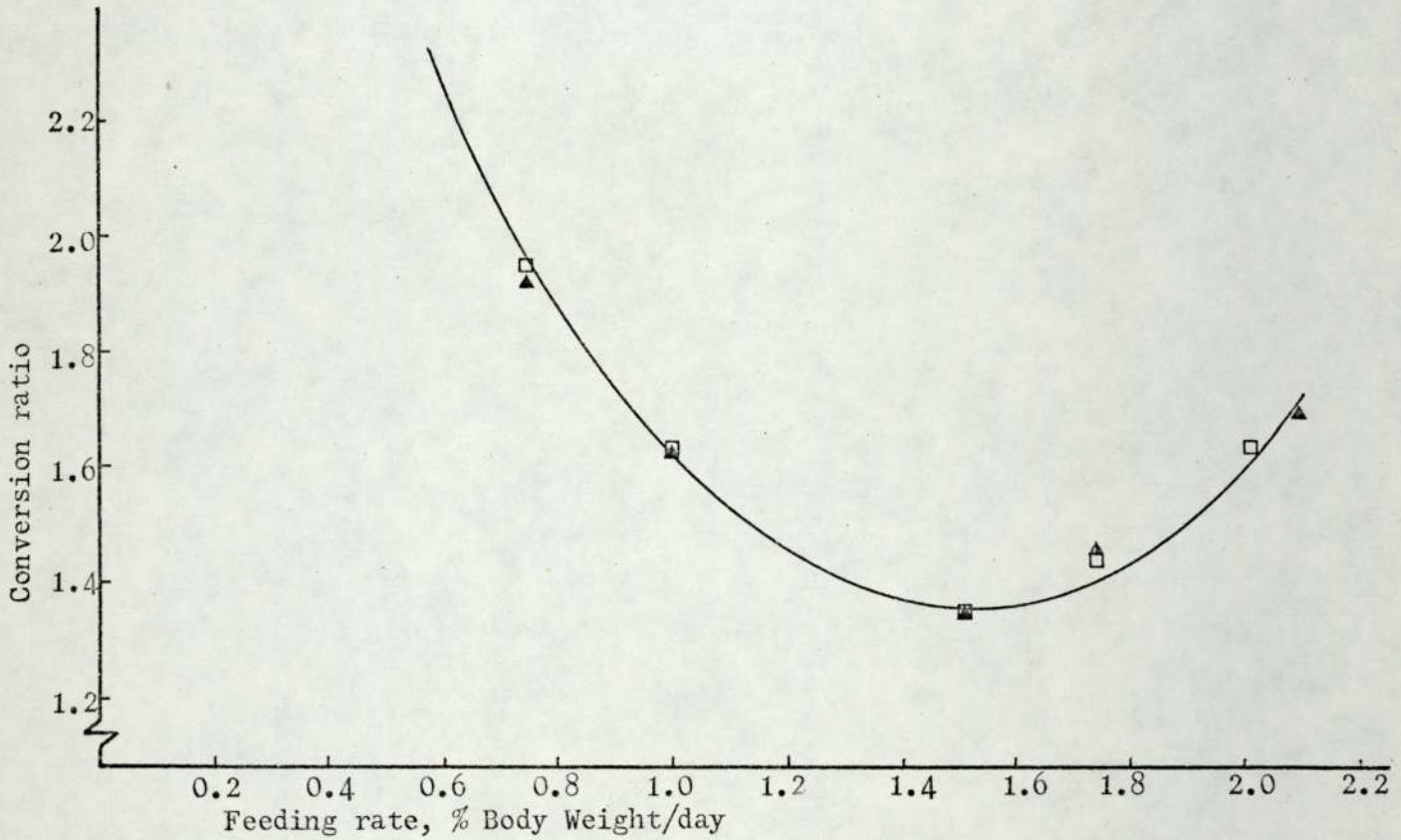


Fig.31. The variation of conversion ratio with increasing feeding rate in fresh water □ , and 20 ppt sea water ▲ .
Each point is a mean of three values

Live weight gains of each tank of fish over each six weeks' experimental phase were converted into energy units by multiplying by the appropriate factors obtained from Tables 28 and 29. Total food consumption per tank of fish was converted into energy units by multiplying by a factor of 4.0587 Kcal/g dry food. The derivation of this was described in the previous chapter. Gross growth efficiencies for fish at different feeding rates in different salinities were determined and are shown in Tables 32 and 33. At each ration level with the exception of the repletion feeding rate, gross growth efficiency was higher for fish in 10 ppt sea water than in fresh water. Figure 32 shows the gross growth efficiencies for the fresh water : 10 ppt sea water experiment plotted against feeding rate.

At each ration level, gross growth efficiencies were very similar for fish in 20 ppt sea water and in fresh water. Figure 33 shows the gross growth efficiencies for the fresh water : 20 ppt sea water experiment plotted against feeding rate. Both Figures 32 and 33 show curves which are close to the inverted forms of the conversion ratio curves described previously. In the absence of suitable transformations smooth curves were fitted by eye. Only one curve was drawn for Figure 33 as it was not possible to distinguish between separate curves for fresh water and 20 ppt sea water. All of the curves originated near the abscissa, rose with steadily decreasing gradient to a point of flexion and then descended with steadily increasing negative gradient. In the fresh water : 10 ppt sea water experiment (Figure 32) maximum gross growth efficiency was 28.75% at the 1.58% feeding rate for fresh water and similarly, maximum gross growth efficiency was 29.75% at the 1.56% feeding rate for 10 ppt sea water. In the fresh water : 20 ppt

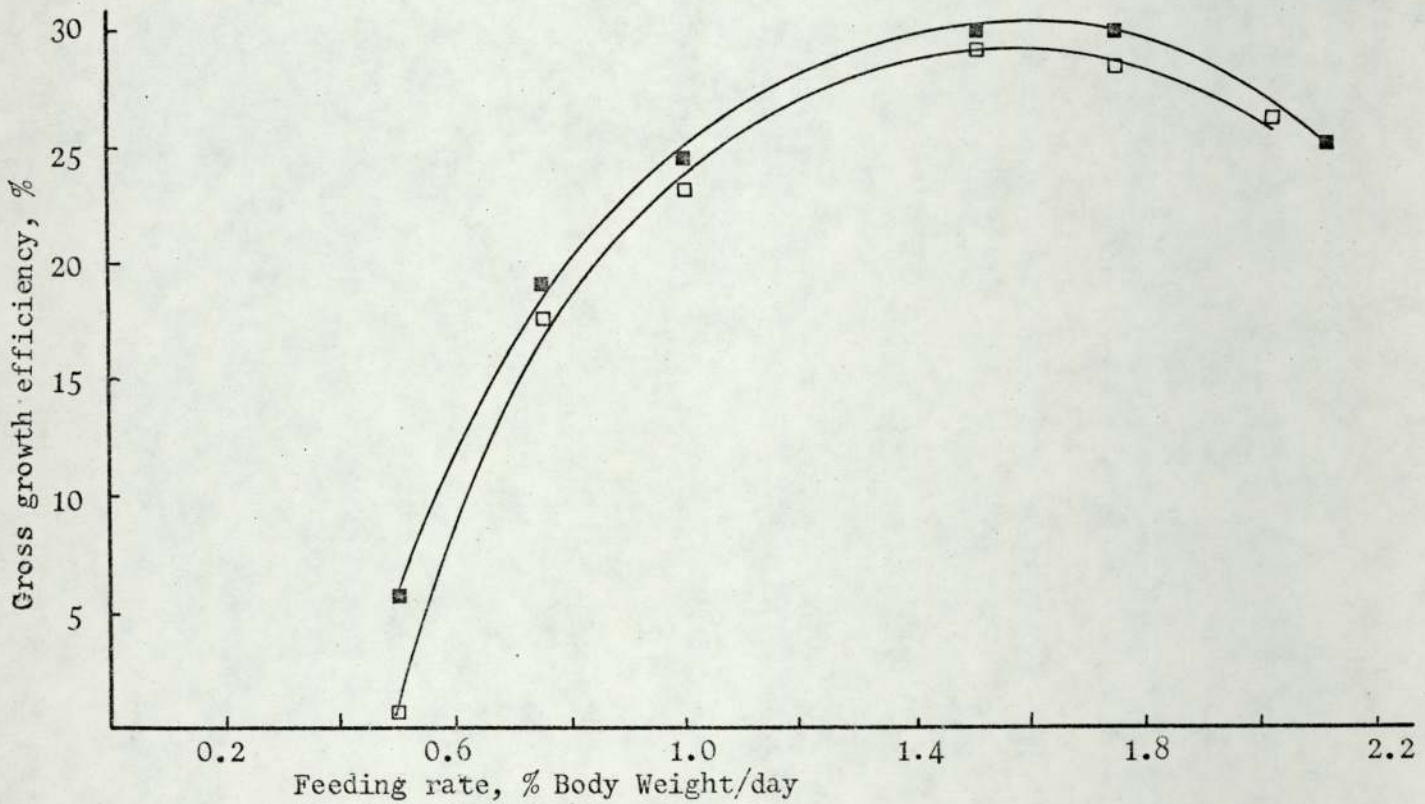


Fig.32. The variation in gross growth efficiency with increasing feeding rate in fresh water □ , and 10 ppt sea water ■ .

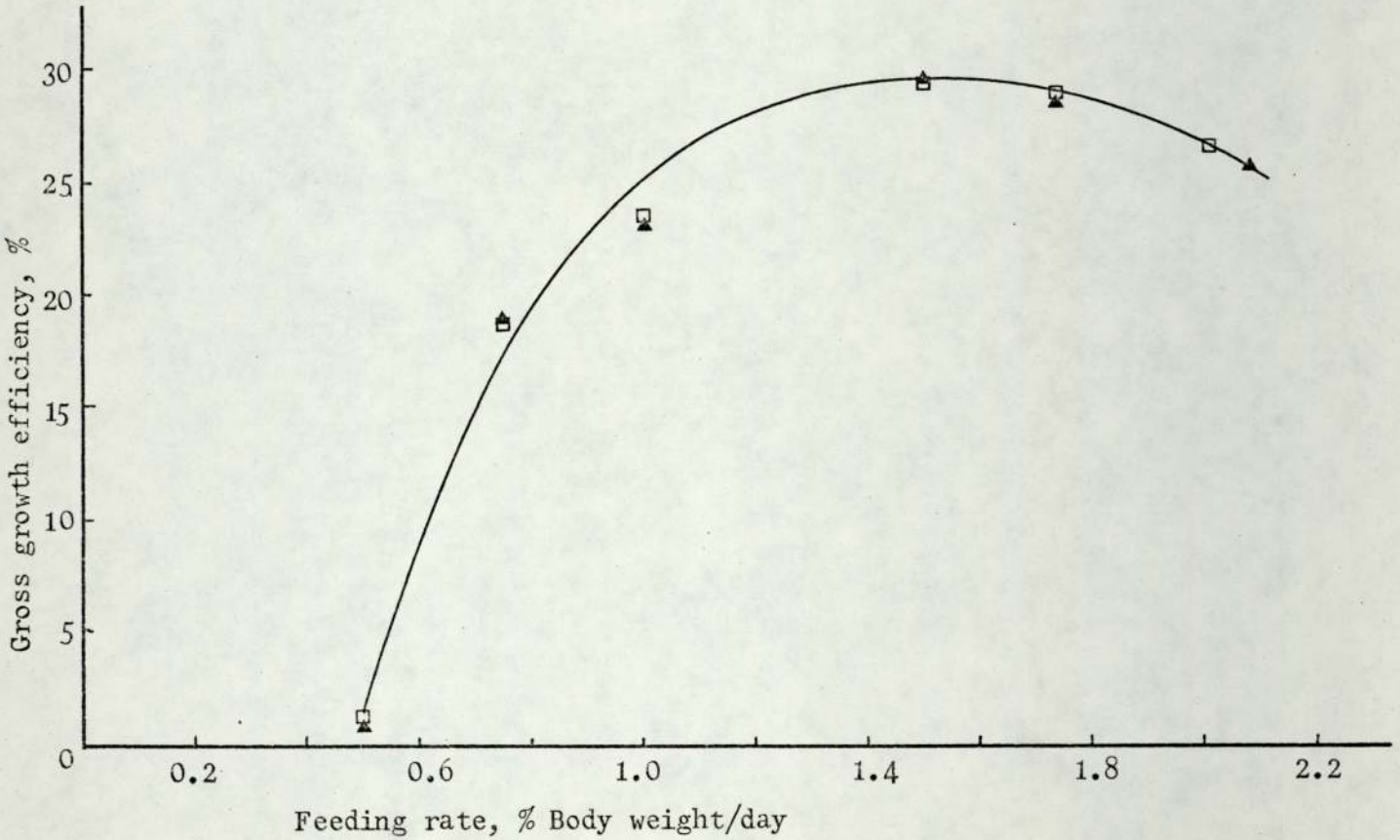


Fig.33. The variation in gross growth efficiency with increasing feeding rate in fresh water □ , and 20 ppt sea water ▲ .

sea water experiment (Figure 33) maximum gross growth efficiencies of 29.25% were obtained at the 1.53% feeding rate for both fresh water and 20 ppt sea water.

When the logarithms of the gross growth efficiencies were plotted against absolute food consumed per day curves were produced similar to those of Brett et al. (1969). Figure 34 shows two curves, both fitted by eye to the data, which correspond to the logarithms of the gross growth efficiencies of fish with increasing food consumption in fresh water and 10 ppt sea water. At a low food consumption level the logarithm of the gross growth efficiency was very low being almost zero for fresh water. The increase in log. gross growth efficiency is very rapid with increasing rations and at about 20 g food consumed/day the curves flexed and descended with a slight negative gradient. At all food consumption levels, with the exception of the repletion levels the logarithm of the gross growth efficiency was higher for fish in 10 ppt sea water than for fish in fresh water. Figure 35 shows a single curve fitted to the data by eye. The points on the graph represent the logarithm of the gross growth efficiencies of fish in fresh water and 20 ppt sea water at increasing levels of food intake. There was very little difference between the curves for fresh water and 20 ppt sea water and the logarithmic nature of the values made separation of the two curves impossible. The overall shape of the curve was similar to those shown in Figure 34.

Net Growth Efficiencies

The calculations of maintenance energy requirements of each tank of fish over the duration of each experiment have already been described and the values are shown in Tables 30 and 31. These values were used

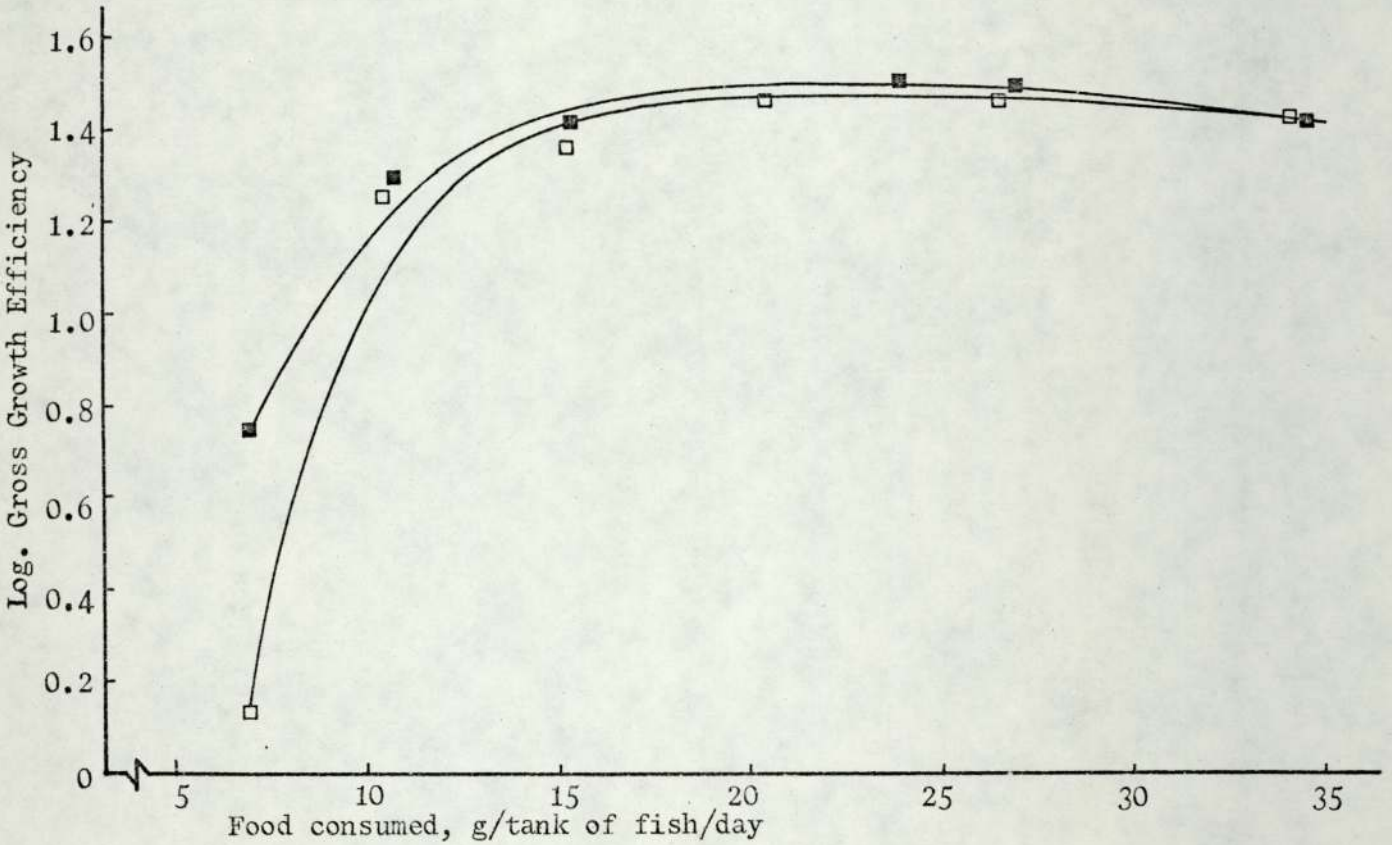


Fig.34. The variation in the logarithm of gross growth efficiency with increase in food consumption in fresh water □ , and 10 ppt sea water ■ .

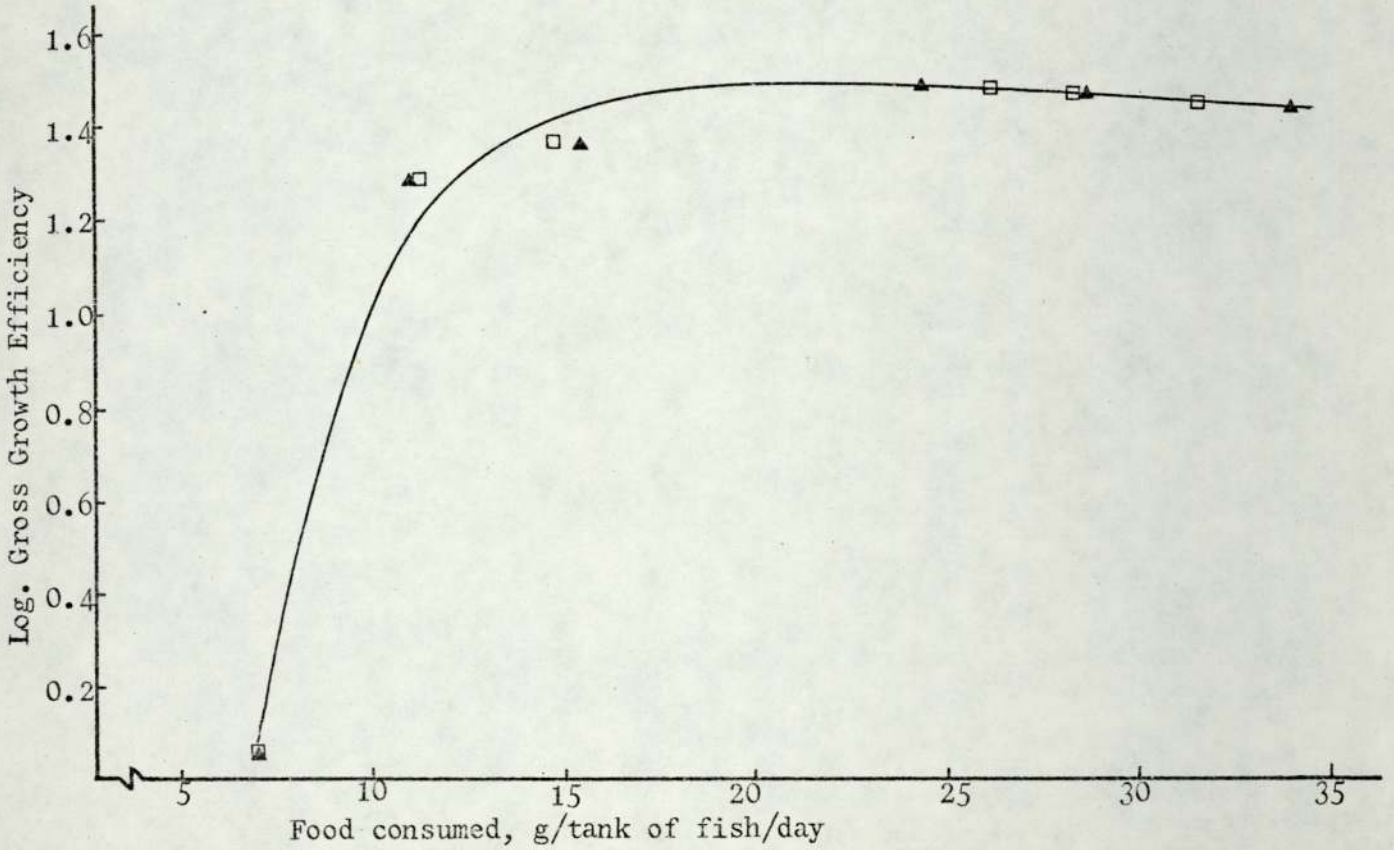


Fig.35. The variation in the logarithm of gross growth efficiency with increase in food consumption in fresh water □ , and 20 ppt sea water ▲ .

to calculate net growth efficiencies in the way detailed in the previous section. Net growth efficiencies were then plotted against feeding rate. Figure 36 shows results of experiment 1 (fresh water : 10 ppt sea water) and Figure 37 shows results of experiment 2 (fresh water : 20 ppt sea water). For the four sets of data involved, straight lines were fitted according to the method of Least Squares. In performing this operation, data obtained at the 0.5% feeding rate was omitted as values did not conform with the linearity of the relationship. In all cases, high correlation was found between net growth efficiency and feeding rate ($r = 0.95 - 0.97$) and these correlations were highly significant ($p < 0.01$). All four lines so produced had negative gradients. Using the method outlined in Wetherill (1972) for comparing gradients of lines together with Student's 't' test on paired data, it was found that no significant differences in either the gradients or the positions of the straight lines was seen taking each experiment in turn. From this it was concluded that net growth efficiency was unaffected by salinity but was inversely related to feeding rate. Actual values obtained for net growth efficiencies ranged from 5.75 - 49.6% at the 0.75% feeding rate to 32.5 - 36.4% at repletion feeding rates, (Tables 32 and 33).

To summarise the major results of this experiment, voluntary food intake was seen to be greater by fish in 10 ppt and 20 ppt sea water than fresh water. Salinity was found to exert no influence on body composition but feeding rate did have an effect. As feeding rate increased the percentage body dry matter and oil content increased as protein content decreased.

Specific growth rate was generally higher in 10 ppt sea water than

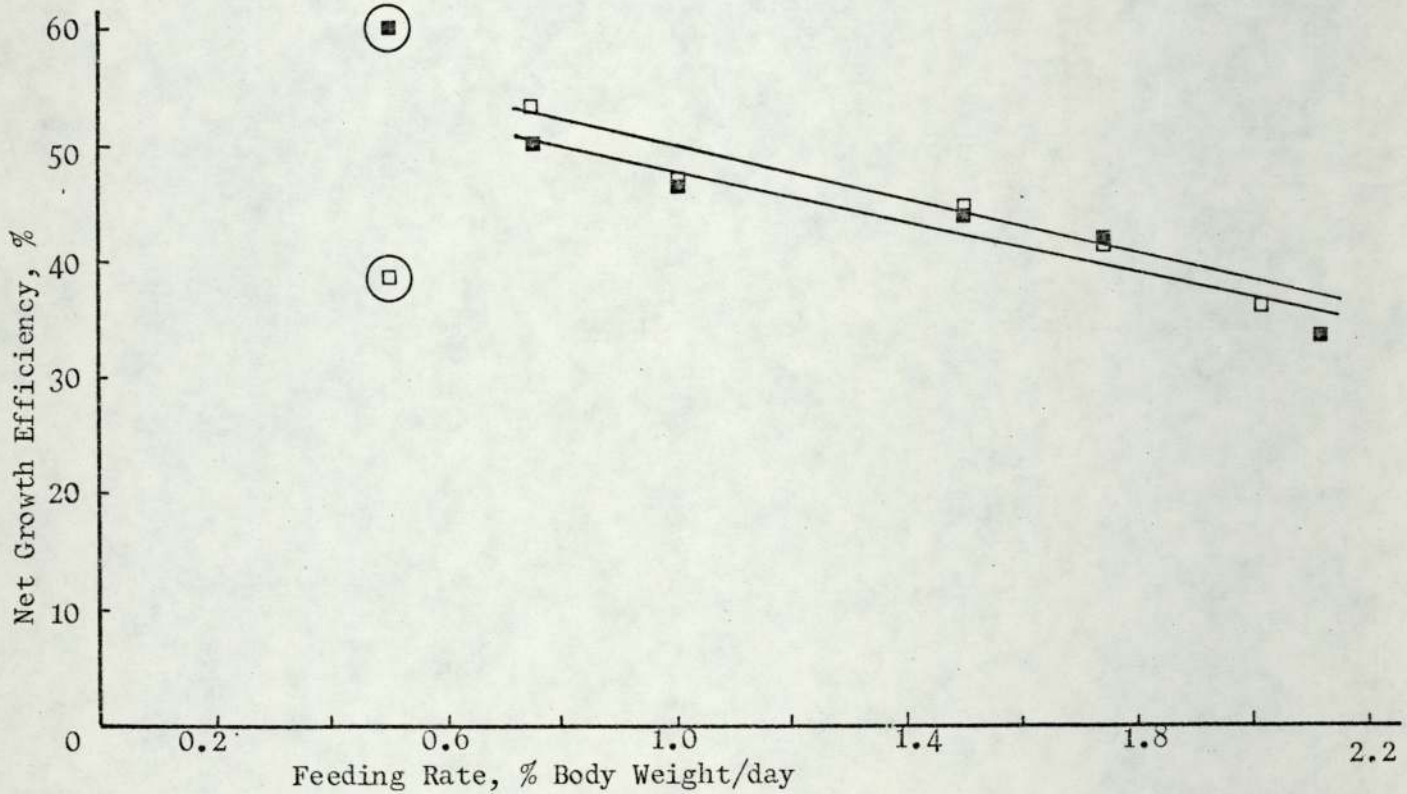


Fig.36. The variation in net growth efficiency with increasing feeding rate in freshwater □ , and 10 ppt sea water ■ .
Circled points were taken to be anomalous

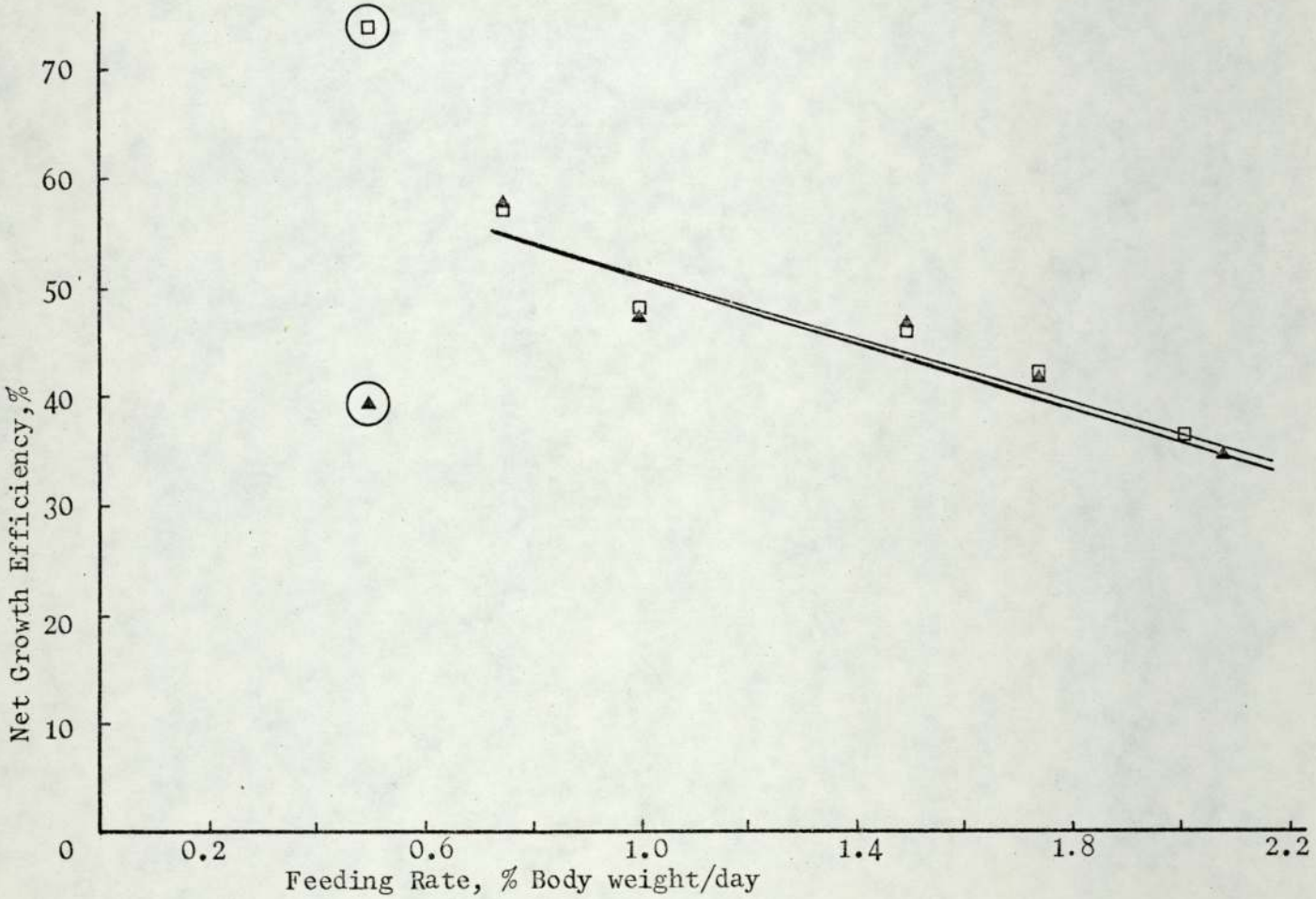


Fig.37. The variation in net growth efficiency with increasing feeding rate in fresh water □ , and 20 ppt sea water ▲ .

Circled points were taken to be anomalous

fresh water and was very similar in fresh water and 20 ppt sea water. The maintenance ration was lowest in 10 ppt sea water and was equal in fresh water and 20 ppt sea water.

Conversion ratio values were lower in 10 ppt sea water than fresh water and were very similar in 20 ppt sea water and fresh water. Gross growth efficiency values were higher in 10 ppt sea water than fresh water and equal in 20 ppt sea water and fresh water.

Net growth efficiencies were found to decrease as feeding rate increased and were unaffected by salinity.

5.5. Discussion

Growth

Faster growth rates in 10 ppt sea water and equal growth rates in 20 ppt sea water and fresh water were found. This was in good agreement with the results of the previous experiment. Again the conclusion is drawn that faster growth in 10 ppt sea water is a result of a reduction in the energy requirements for osmotic and ionic regulation which leads to an overall reduction in total metabolic rate. This resulted in a greater availability of ingested calories for growth. This conclusion is supported by the data obtained for the maintenance rations for trout in different salinities. The maintenance requirement is defined as that ration required to supply the needs of maintenance and activity with no growth and was found to be 0.45% for 10 ppt sea water and 0.49% of the total live weight per day for fresh water and 20 ppt sea water. A reduction in the energy requirements for osmotic and ionic regulation should result in a reduction in the maintenance energy requirement provided that the energy expended on activity is constant. In the present case, the maintenance requirement was reduced in 10 ppt sea water which supports the theory of reduced osmotic energy costs in this salinity. This reduction in maintenance energy required probably caused the improved growth rates and food conversion rates of fish in 10 ppt sea water. Fish in fresh water and 20 ppt sea water had identical maintenance energy requirements which indicates that the energy costs of osmotic and ionic regulation were similar in these two salinities. This conclusion was supported by metabolic rate determinations in the previous chapter.

A lack of significance in the differences between fresh water and

10 ppt sea water values for both conversion ratio and specific growth rate at two of the ration levels was apparent (Tables 30 and 32). At the repletion ration level the values were similar because of the difference in food consumption rates. At the 1.5% ration level, the lack of significance was caused by variability in individual observations. It was considered that the presence of two values out of the six not significantly different from the corresponding values did not detract from the conclusion that a real difference was apparent in specific growth rate and conversion ratio curves between fish in fresh water and 10 ppt sea water. However, it was considered that the differences observed in the optimum and maximum rations between the two salinities were so small as to be negligible.

Curves obtained for specific growth rate against feeding rate were similar to those of Brett et al (1969) who produced curves of a 'geometric' shape with sockeye salmon, O. nerka, at a similar temperature, 10° C. One difference in the curves between these two experiments is that in the present experiment, a point of inflexion may have been reached at high ration levels. All of Brett's curves reached a definite asymptote. However, the curves obtained in the present experiment closely resembled those obtained by Huisman (1974) with carp, Cyprinus carpio, who found definite inflexion points at high ration levels. It appears from Huisman's work that water temperature is important in determining the extent of the fall in the specific growth rate curve after the maximum ration point. At higher temperatures, the fall is large and at low temperatures, the fall is insignificant. This is probably because at high (relatively) temperatures and high feeding rates conversion efficiencies are poor owing to the high induced metabolic rates

with a concomitant reduction in energy available for growth purposes. In the present experiment the water temperature employed was at a mid-point in the range of temperature tolerance for trout. Thus, the decline in the specific growth rate curve after the maximum ration was small.

A comparison of the absolute maintenance energy requirements of fish found by different authors is shown in Table 34. Values obtained in the present experiment are of the same order as found by most of the authors cited in this table. Maintenance energy requirement is dependent upon many factors, for instance, fish size and water temperature. Brown (1946a) with S. trutta used environmental conditions similar to those of the present experiment. She found a maintenance energy requirement of approximately 1.10 Kcal/100 g fish/day. This value is lower than that found in the present experiment possibly because the level of activity of the fish in Brown's experiment was consistently lower than in the present experiment.

Results of food consumption by fish fed to repletion are in agreement with those of Experiment 1, the demand-feeding experiment and in contrast with the results of Experiment 2, the energy-balance experiment. This difference between Experiments may be explained by a seasonal variation in the appetite response to salinity. Seasonal changes in growth rate mediated by temperature or photoperiod are well noted (Gross, Roelofs & Fromm, 1965; Saunders & Henderson, 1969c; Smith, 1973) but similar fluctuations in voluntary food intake are less well documented. Seasonal growth cycles in fish have usually been attributed to seasonal changes in temperature because of the relationship between temperature and growth rate of poikilotherms.

Author	Species	Food	Average fish Weight g	Temperature °C	Calorific Value of Food Kcal/g dry	Maintenance Requirement			
						g dry weight/ 100 g fish/ day		Kcal/100g fish/day	
Davies (1964)*	Carassius auratus	Enchytraeus	35	21.5	5.8	0.3		1.74	
Brown(1946b)*	S. trutta	Minced Meat	50	20	5.7[a]	0.4		2.28	
Brown (1946a)	S. trutta	Minced meat	100	11.5	5.7[a]	0.193		1.10	
Pentelow(1939)*	S. trutta	Gammarus	17.5	19	4.23[b]	0.47		1.99	
Dawes (1930,* 1931)	Pleuronectes platessa	Mytilus	33.0	17	-	0.3		-	
Solomon and Brafield (1972)	Perca fluviatilis	Gammarus	10 - 19	14	4.23	0.31		1.30	
						10ppt sea water	Fresh water & 20ppt sea water	10ppt sea water	Fresh water & 20ppt sea water
Present Experiment	S. gairdneri	Pellets	70-110	12	4.059	0.405	0.441	1.64	1.79

a. The calorific value of minced meat was taken from Brody (1945) as 5.7 Kcal/g dry material

b. The calorific value of Gammarus was taken from Solomon and Brafield (1972)

* Data adapted from Davies (1964)

Table 34. Various maintenance energy requirements of fish found by different authors

In temperate regions annual cycles in temperature and photoperiod are nearly in phase and the individual effects of the two factors are difficult to distinguish in the natural environment. When these two conditions are kept constant, any fluctuations in growth, appetite or food conversion must be due to physiological changes within the fish brought on by an endogenous rhythm. Brown (1946a, b) postulated that endogenous rhythms were responsible for seasonal fluctuations in the growth rate of brown trout. Swift (1961), however, disagreed with this and maintained that temperature alone was responsible. Gross et al. (1965) working with the green sunfish, Lepomis cyanellus, concluded that seasonal growth cycles are initiated by the synergistic action of temperature and photoperiod.

In the present experiments an endogenous cycle in the effect of salinity on growth rate has not been demonstrated but a seasonal change in the appetite of fish in sea water is apparent. It was found that salinity had no effect on the appetite of fish during the winter months (Experiment 2) but had an enhancing effect on appetite during spring (Experiment 1) and summer (Experiment 3). Despite the fact that all experimental fish were maintained in constant temperature and photoperiod conditions during each experiment, before the experiments fish were held in out-door stocking tanks and were subject to natural fluctuations in temperature and photoperiod. Hence, the trout were 'in tune' to the season before the experiments and may have experienced a cycle in a physiological parameter related to season. Although it has been demonstrated that the time of year does not normally elicit changes in the digestive physiology of the rainbow trout (Windell & Norris, 1969) it is possible that both seasonal and salinity changes would produce an

alteration in some component of digestive physiology for example, rates of gastric evacuation and digestion.

Food Conversion

The changes in body constituents with increase in feeding rate are the same as those found in the previous experiment and by Gerking (1955a). As feeding rate increased, the oil content and caloric density increased. Conversely, moisture content decreased.

The near-parabolic shaped curves obtained when conversion ratio is plotted against rate of feeding (Figures 30 and 31) are similar to those obtained by Swingle (from Hastings, 1969). The explanation of this form of curve is that at low ration levels, most of the energy intake is metabolized for maintenance and activity requirements. As the rate of feeding increases a progressively larger proportion of ingested energy is surplus to maintenance needs and can be made available for growth purposes. Eventually an optimum feeding rate is reached where the conversion ratio is maximum. The explanation for the fall in conversion ratio with increasing rations beyond the optimum is not wholly resolved (Edwards, Finlayson & Steele, 1969). Apparently, beyond the optimum feeding rate, metabolism increases to the detriment of conversion ratio. The increase in metabolic rate includes an increase in the specific dynamic action of the food fed which can be high at high ration levels (Averett, 1969). This increase in metabolic rate is also enhanced by the increasing energy requirement for the gill pumps which consume more than twice the oxygen required by the heart at high feeding rates (Hughes, 1964).

The curves obtained when gross growth efficiencies were plotted against feeding rate (Figures 32 and 33) were the inverse of the conversion ratio curves. At low ration levels, where growth was minimal,

the gross growth efficiencies approached zero. Again, all the food ingested was deployed in maintenance and activity. Flexion points for these curves were found at slightly higher feeding rates + 0.14 - 0.15%/day, than the optimum feeding rates derived from specific growth rate curves. Again, the feeding rate at which maximum gross growth efficiency occurred was slightly lower for 10 ppt sea water than for fresh water but it would be unwise to attach too much importance to this small difference.

Higher gross growth efficiencies were obtained for fish in 10 ppt sea water than in fresh water and it is probable that this difference in the position of the curves was a real difference. Higher growth efficiencies are probably caused by the lower maintenance energy requirement needed by fish in 10 ppt sea water which enables proportionately more of the ingested energy to be available for growth. Actual values found for growth efficiencies (0.75 - 29.76%) depended on salinity and feeding rate. Edwards et al. (1969) working with Pleuronectes platessa and Limanda limanda found average gross growth efficiencies of 30%. Dawes (1930, 1931) and Pentelow (1939) found values of 20 - 40% with P. platessa and S. trutta respectively. The observation that maximum gross growth efficiencies were found at intermediate feeding rates is supported by Edwards, Finlayson and Steele (1972) with Gadus morhua who found a maximum gross growth efficiency of 24% at intermediate feeding rates. Also, Brett et al. (1969) with O. nerka found maximum gross efficiencies of 25% at 11.5^{o} C. Thus the absolute values obtained in the present experiment agree well with the results of other authors.

The K-line

Hastings and Dickie (1972) and Paloheimo and Dickie (1965) have discussed the derivation and use of the K-line in some detail. They maintain that the logarithm of the gross growth efficiency should be plotted against absolute food consumed rather than feeding rate as a percentage of the live body weight. This is because actual growth efficiency seems to be independent of body weight. A notable exception to this theory is provided in the work of Huisman (1974) who showed that the gross growth efficiency (K) was negatively influenced by increasing feeding rate. It was taken that a possible species difference existed as Huisman used carp (Cyprinus carpio) in his experiments whereas Paloheimo et al. (1965) analysed results of experiments on trout and plaice. Accordingly, Figures 34 and 35 show the logarithms of gross growth efficiencies plotted against absolute food consumption/day. The overall shape of the curves is similar to those obtained by Brett et al. (1969) with O. nerka at a similar temperature. At low ration levels, log. gross growth efficiency (G.G.E) is zero. With increasing rations log. G.G.E rises very steeply to a point of inflexion at intermediate ration levels and then falls gradually with a small negative gradient. This shape of curve in all salinities differs from that proposed by Paloheimo et al. (1966a, b) who stated that a linear relationship existed between log. GGE and food consumed. This was the K-line which had zero or negative gradient. However, the results of the present experiment comply with Kerr's (1971) analysis of the situation. He suggested that the K-line is a phenomenon observable only at high ration levels. He proposed that at low ration levels there was a curvilinear relationship between log. GGE and rations which had a very steep

positive gradient. At intermediate and high ration levels the relationship was linear with a negative gradient. This is more acceptable as by definition the gross growth efficiency must be zero at the maintenance ration. Huisman (1974) supports Kerr's conclusions and states that the linear relationship existing between log. GGE and food consumption is only apparent at levels above the geometrically determined optimum ration level. If log. GGE is plotted against feeding rate for the first experiment, to use this as an example, there are three points to the right of the optimum ration points on each curve (Figure 38). The relationship between these points may be curvilinear or linear with the curves having slight negative gradients. Thus it is probable that Kerr's and Huisman's assessment of the K-line phenomenon is justified in that true K-lines exist only beyond the optimum ration level.

Examination of equation 6 reveals the derivation of the K-line. This is:-

$$\log K_1 = \log \frac{\text{Weight Gain, cals.}}{\text{Food Intake, cals}} = -a - bR$$

Where R is the food intake in calories.

This is the linear form of the K-line where 'a' is the extrapolated intercept on the vertical axis and 'b' is the gradient of the line. Paloheimo and Dickie (1965) stated that "changes in the caloric content of the food, or the basic energy distribution within the animal imposed, for example, by environmental changes such as salinity, appear to affect the numerical value of 'a' but not of 'b'". Applying this to the present experimental results it would appear above the optimum ration level (Figure 38) that 'a', the level of the line, is higher for the 10 ppt sea water line than

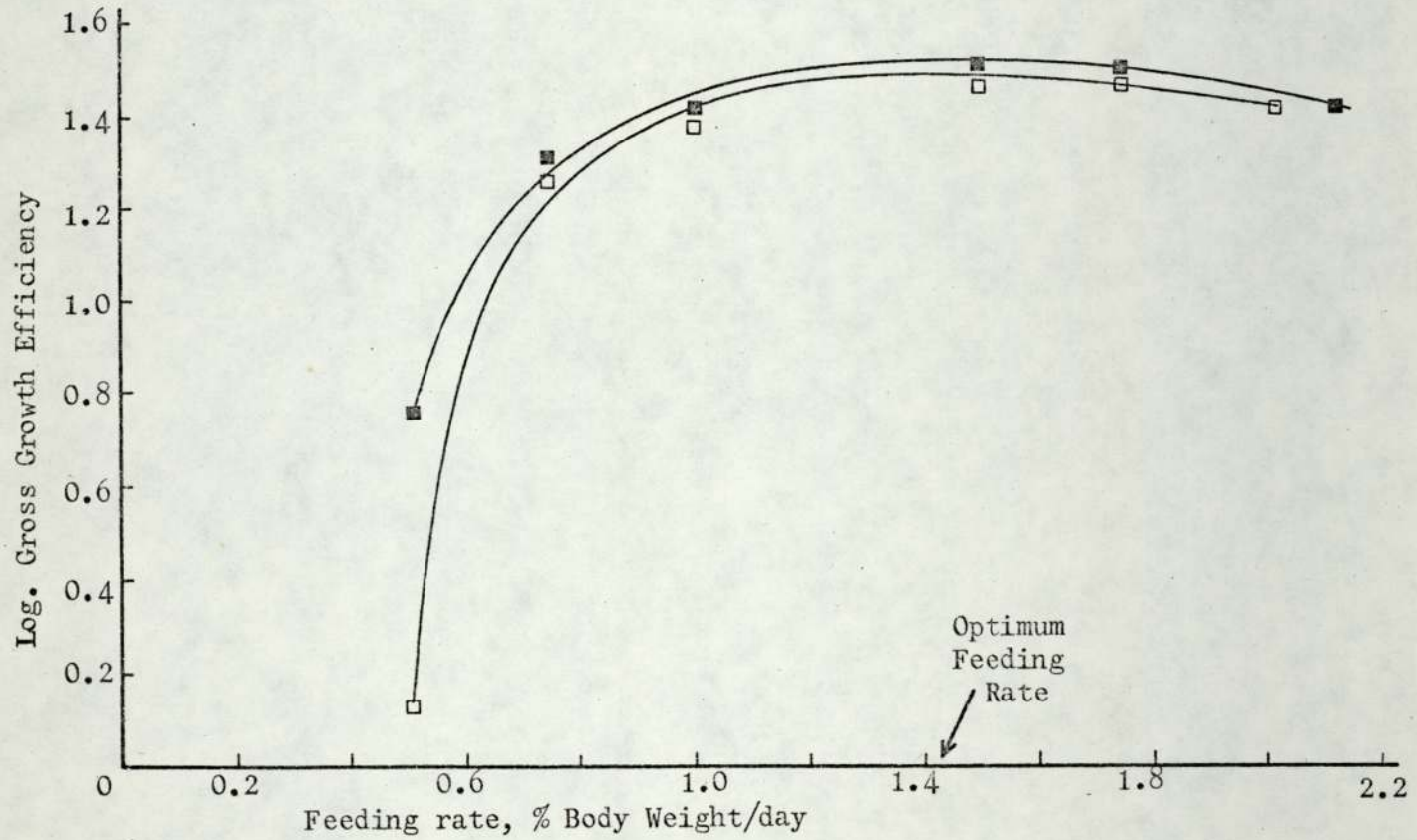


Fig.38. The variation in the logarithm of gross growth efficiency with increasing feeding rate in fresh water □ , and 10 ppt sea water ■ .

the fresh water line.

The gradients appear to be very similar and it is unlikely that a change in 'b' has occurred between the two curves. It is interesting that a displacement of the K-lines, as predicted by Paloheimo et al (1965) has occurred in the 10 ppt sea water/fresh water graph.

Kerr (1971) believed that the negative gradient of the K-line obtained at intermediate and high ration levels resulted from the increase in spontaneous activity of the fish with increasing ration levels. The results of the present experiment do not agree with this as all fish were swimming at identical, constant swimming speeds and negative gradients of the log. GGE curves were obtained above the optimum ration point. Hence, the decrease in log. GGE with increasing feeding rate must be related either to decreasing absorption efficiency or to increasing energy costs of specific dynamic action, or both.

Net Growth Efficiency

The concept of net growth efficiency was first proposed by Pentelow (1939). It provides a measure of the efficiency of conversion into growth of that proportion of the energy intake which is surplus to maintenance and activity requirements. If net growth efficiencies are plotted against feeding rate, usually a linear relationship is obtained, the line having either a negative gradient, as found by Averett (1969) with O. kisutch, or a zero gradient as found by Brett (1971, b) with O. nerka. In the present experiment, net growth efficiencies decline with increasing feeding rate at all salinities. This indicates that as food consumption increases either the absorption efficiency decreases or the energetic costs of specific dynamic action increase or both. As no difference was observed between the lines of best fit for net conversion efficiencies of

fish in either fresh water or salt water (Figures 35 and 36) it was concluded that the effects of salinity on absorption efficiency and energy costs of specific dynamic action were negligible. The fact that absorption efficiencies seemed to be unaffected by salinity was in agreement with the results obtained in the previous experiment for fish in fresh water, 10 ppt sea water and 20 ppt sea water. The inference that the energetic costs of specific dynamic action were unaffected by salinity was, at the time, surprising. In the previous experiment it was demonstrated that fish in 10 ppt sea water had lower total metabolic rates than fish in fresh water. It was anticipated that a change in the metabolic rate would result from a change in the proportions of metabolic substrates respired, these being protein, fat and carbohydrate. Any change in these proportions should lead, in theory, to a change in the specific dynamic action of the food fed (Ware, 1975). This was investigated in further detail in the next chapter.

Although the values found for net growth efficiency were higher than those found by Brett et al. (1969) with O. nerka the fact that increase in feeding rate resulted in decreased net conversion efficiencies was common to both.

The use of maintenance ration level in determining net growth efficiency has been in dispute. Brown (1957) reported that maintenance ration was difficult to determine as her fish kept adapting their growth rate to compensate for reduced rations. Pandian (1967) dismissed net growth efficiencies as being valueless. Also, Paloheimo et al. (1966b) considered that the difficulties inherent in obtaining satisfactory maintenance estimates precluded useful application of net growth efficiencies. In contrast, the present work and the results of Brett et al. (1969)

disagree with the above opinions. Brett believed that where stable growth rates are obtained, maintenance requirements can be determined accurately by extrapolation and valid measurements of net growth efficiency may be derived The reason that the above authors dispute the concept of maintenance requirement is possibly due to the fact that fish often change their level of activity under different feeding regimes. For example Beamish (1964b) has shown that fish can modify their levels of activity under different dietary conditions. In the present experiments, activity levels were the same between different tanks of fish thus making the energy expended on activity identical.

In conclusion, the overall patterns of growth and food conversion were the same as those found by Averett (1969) with O. kisutch . As feeding rates increased so gross growth efficiency increased from a low value, reached a point of flexion and decreased to an intermediate value. As feeding rates increased so net growth efficiencies decreased. The ration required to maintain trout at a constant activity level with nil growth was lower in 10 ppt sea water than in fresh water or 20 ppt sea water. This resulted in improved growth and higher gross growth efficiencies for fish in 10 ppt sea water. As maintenance costs were lower in 10 ppt sea water this supported the inference of the previous experiment that the energetic costs of osmotic and ionic regulation were least in the salinity closest to the isosmotic point. The similar gross growth efficiencies, growth rates and maintenance rations of fish in fresh water and 20 ppt sea water also supported the inference of the

previous experiment that the energetic costs of osmotic and ionic regulation were the same in these salinities.

Chapter 6. Experiment 4. The Determination of the Effect of Sea Water on the Apparent Specific Dynamic Action of Consumed Food.

6.1. Introduction

The results obtained in the previous experiment have shown that total metabolic rate and maintenance energy requirements of rainbow trout are lower in 10 ppt sea water than in either fresh water or 20 ppt sea water. As a result of this growth is faster in 10 ppt sea water. It was considered possible that an alteration in the specific dynamic action of consumed food could be elicited by environmental salinity on the grounds that the proportions of metabolic substrates, protein, fat and carbohydrate could be changed by an alteration in metabolic rate.

Specific dynamic action was first described by Rubner (1902) who termed it specific dynamic effect. Specific dynamic action (SDA) refers to the increase in metabolism following food consumption and is often defined as the metabolic cost of the utilisation of ingested food. The energy liberated as a result of SDA is generally assumed to be largely the result of the deamination of amino acids (Brody, 1945). Some heat production also follows the ingestion of carbohydrate and fat (Harper, 1971).

Energy requirements for absorption, digestion, transportation and deposition of food materials are regarded as being separate from SDA by some authors (Beamish, 1974). As the two components are difficult to separate experimentally usually both are determined concurrently and the term apparent SDA is used.

Some observations have been made on the increase in metabolic

rate subsequent to feeding (Saunders, 1963; Livingston, 1968; Kausch, 1969) but few attempts have been made to quantify this increase in terms of energy expenditure. However, it is likely that apparent SDA does account for a significant proportion of the ingested food energy as Pierce and Wissing (1974) state that the energy requirement for food utilisation was appreciably higher than that required for routine swimming activity in bluegills, Lepomis macrochirus. Apparent SDA has only been determined in fish by a few authors, e.g. Averett (1969) on coho salmon, O. kisutch, Muir and Niimi (1972) on aholehole, Kuhlia sandvicensis, Hamada and Ida (1973) on goldfish, Carassius auratus and Beamish (1974) on largemouth bass, Micropterus salmoides. Actual measurements of apparent SDA in fish have varied greatly in their magnitude and Warren and Davis (1967) estimate that between 5 and 40% of ingested food energy may be lost through SDA. The reason that these estimates of apparent SDA vary so much is that apparent SDA is dependent upon many factors the most important being the size of the ration consumed (Beamish, 1974), the nutritional content of the diet and the relative proportions of the metabolisable lipids, carbohydrates and proteins ultimately used as metabolic fuel (Ware, 1975). With regard to this latter point it is possible that salinity can change the proportions of the substrates respired by altering the total metabolic rate thus eliciting a change in apparent SDA.

The present experiment was designed to evaluate both the effect of feeding rate and salinity on apparent SDA in trout. Also the percentage of ingested calories lost through apparent SDA was determined under these conditions. The method used to find apparent SDA in this experiment has been used by other authors (e.g. Averett, 1969;

Beamish, 1974; Pierce et al, 1974). As in Chapter 4, indirect calorimetry was used in measuring total metabolic rate in terms of the quantity of oxygen consumed by a given weight of fish per unit time. The procedure was to determine the difference in oxygen consumption by a group of fish fully fed, and the same group in a post-absorptive condition. The period of time required for a fully fed trout to reach a postabsorptive condition was assessed from the relevant literature. Averett (1969) found that the oxygen consumption of coho salmon generally returned to pre-feeding levels within 24 hr. irrespective of meal size. Similarly, Beamish (1974) found apparent SDA was complete by 24 hr. irrespective of meal size with largemouth bass. However, Beamish (1964) found that the standard metabolic rate of brook trout Salvelinus fontinalis declined rapidly up to 48 hr after starvation after which there was relatively little change. Accordingly a period of 38.5 hrs (2 nights and 1 day) was chosen as being compatible with previous work.

6.2. Materials, Methods and Experimental Procedure

The experimental system used was essentially the same as in the previous experiment. However, only the six metabolism/growth tanks were used. Three of these tanks held fresh water throughout the experiment and were taken as the control while the other three held sea water. Three feeding rates were chosen: 1.0%, 1.5% and 1.75% body weight/day and these were represented in both the fresh water and the sea water tanks. The experiment was conducted in two phases, a. a comparison of fresh water and 10 ppt sea water; b. a comparison of fresh water and 20 ppt sea water.

The metabolism/growth tanks had been modified in the manner described in Chapter 2 so that nearly continuous measurements of total metabolic rates could be made. The use and operation of the metabolism/growth tank has been described in Chapter 2 in full.

Water temperature was maintained at $12 \pm 0.5^{\circ}C$ in both systems and photoperiod was 12 hr from 8.00 a.m. to 8.00 p.m. Daily rations were administered as three meals at 9.30 a.m., 1.30 p.m. and 5.30 p.m.

At the commencement of the first phase of the experiment (fresh water - 10 ppt sea water) 120 fish of approximately 70g each were weighed on a 'batch' basis and twenty fish were allocated to each of the six metabolism/growth tanks. Fish which were placed directly into 10 ppt sea water suffered no apparent ill effects. Fish were then fed at their pre-determined feeding rates for one week in order to acclimate them to general conditions. At the end of this period fish were starved for 12 hr and then reweighed on a 'batch' basis and the metabolism tests started. After this, fish were weighed at the end of every week

and their rations increased to maintain the initial feeding rates allocated to them and to take account of the growth of the fish.

When a metabolism 'run' was made on a metabolism/growth tank, oxygen consumption determinations were made every hour over a thirteen hour period during the daytime starting at 8.00 a.m. and finishing at 9.00 p.m.

During weeks 1, 2, 3, 6, 7 and 8 (Table 35) four days of metabolism runs were made every week. Week one will be now detailed as an illustration. On the first day, metabolism runs were undertaken on the three fresh water tanks, the fish being in a fed condition. At the end of the day feeding was discontinued in preparation for the unfed metabolism 'run'. On the second day, the same procedure was undertaken with the 10 ppt sea water tanks and when these runs had finished feeding was discontinued. On the third day, metabolism runs were undertaken on the three fresh water tanks, the fish now being in a postabsorptive condition. On the fourth day, the same procedure was undertaken with the 10 ppt sea water tanks. When these runs had finished on their respective days, feeding was continued. Exactly the same procedure was undertaken during weeks two and three. After week three, the salinity in the sea water system was increased over 3 days to 20 ppt and weeks 4 and 5 used to allow the sea water fish to acclimate to the new salinity. After this, the same procedure for metabolism tests was adopted for the second experiment (freshwater : 20 ppt sea water) during weeks 6, 7 and 8.

At the end of week three, a short experiment was undertaken to determine the total oxygen consumption of fish over a 24 hr. period. The salinities in the systems were held at fresh water and 10 ppt sea

Table 35. Experimental Procedure. Each cross denotes one metabolism test 'run'. Time runs vertically.

Week	Salinity	Feeding Rate		
		1.0%	1.5%	1.75%
1	Fresh	X	X	X
	10 ppt	X	X	X
			UNFED	
	Fresh	X	X	X
	10 ppt	X	X	X
2	REPEAT			
3	REPEAT			
4 & 5	Fresh 20 ppt	ACCLIMATION		
		1.0%	1.5%	1.75%
6	Fresh	X	X	X
	20 ppt	X	X	X
			UNFED	
	Fresh	X	X	X
	20 ppt	X	X	X
7	REPEAT			
8	REPEAT			

water and three days were devoted to the experiment. The first was to determine the oxygen consumption of fish fed at three feeding rates in fresh water and 10 ppt sea water every 2 hr over a period of 24 hours. At the end of this period, feeding was discontinued and the third day was used to determine the oxygen consumption of fish in a post-absorptive condition every 2 hr over a 24 hr period. From these results it was found that a slight fall in metabolic rate occurred during the hours of darkness (Figures 39, 40 and 41). From this a correction factor was derived which was applied to all mean daytime metabolic rate determinations in order to produce an estimate of the mean daily metabolic rate.

Mean metabolic rate values in terms of mg. O_2/kg/hr over both the 13 hr and the 24 hr periods were computed by calculating the area under the curves obtained when oxygen consumption values were plotted against time. Determining the oxygen consumption attributable to apparent SDA was found by determining the area between the two curves for the fed and unfed conditions.

All oxygen consumption values were converted to metabolic rates by dividing by the mean total weight of fish per tank for that week. As three replicate values were obtained for metabolic rates at each salinity and each feeding rate, comparison could be made by analysis of variance and subsequent Student's 't' test to investigate between-salinity and between feeding-rate differences. Relevant SDA values were compared by Student's 't' test.

6.3. Results

Metabolic rate values obtained for the 24 hr runs are shown in Figures 39,40 and 41. In order to clarify the figures only those values obtained for the fresh water tanks are shown. It was felt that showing 10 ppt sea water values was unnecessary as the end results were so similar. Hence, Figure 39 shows metabolic rate values of fish in fresh water fed at the 1% feeding rate and in the postabsorptive condition. Similarly, Figures 40 and 41 show fish fed at the 1.5% and 1.75% feeding rates respectively together with the corresponding post-absorptive metabolic rates. In all cases, a diurnal rhythmicity was observed in total metabolic rate. For both fed and unfed fish there was a slight fall in the metabolic rate during the hours of darkness. Differences between the mean daytime metabolic rates and the mean 24 hr rates were averaged for both the fed and the unfed fish. These were 6.15% and 3.62% respectively of the mean daytime metabolic rates in fresh water and 6.07% and 3.76% in 10 ppt sea water. It was considered that the differences between fresh water and 10 ppt values were so close as to make the differences spurious. Hence it was considered that the drop in total metabolic rate was similar for fresh water and 10 ppt sea water fish at all feeding rates.

In addition to the difference between daytime and nighttime metabolic rates a small fluctuation was observed in the daytime metabolic rates of fed fish at all feeding rates (Figures 39, 40 and 41). After each feed was given, there was a rise in the metabolic rates of the fish in that tank. This is most noticeable in Figure 39. The difference between feeding rates is quite marked. Feeding rate and metabolic rate are directly proportional. As the unfed metabolic rates are similar at all

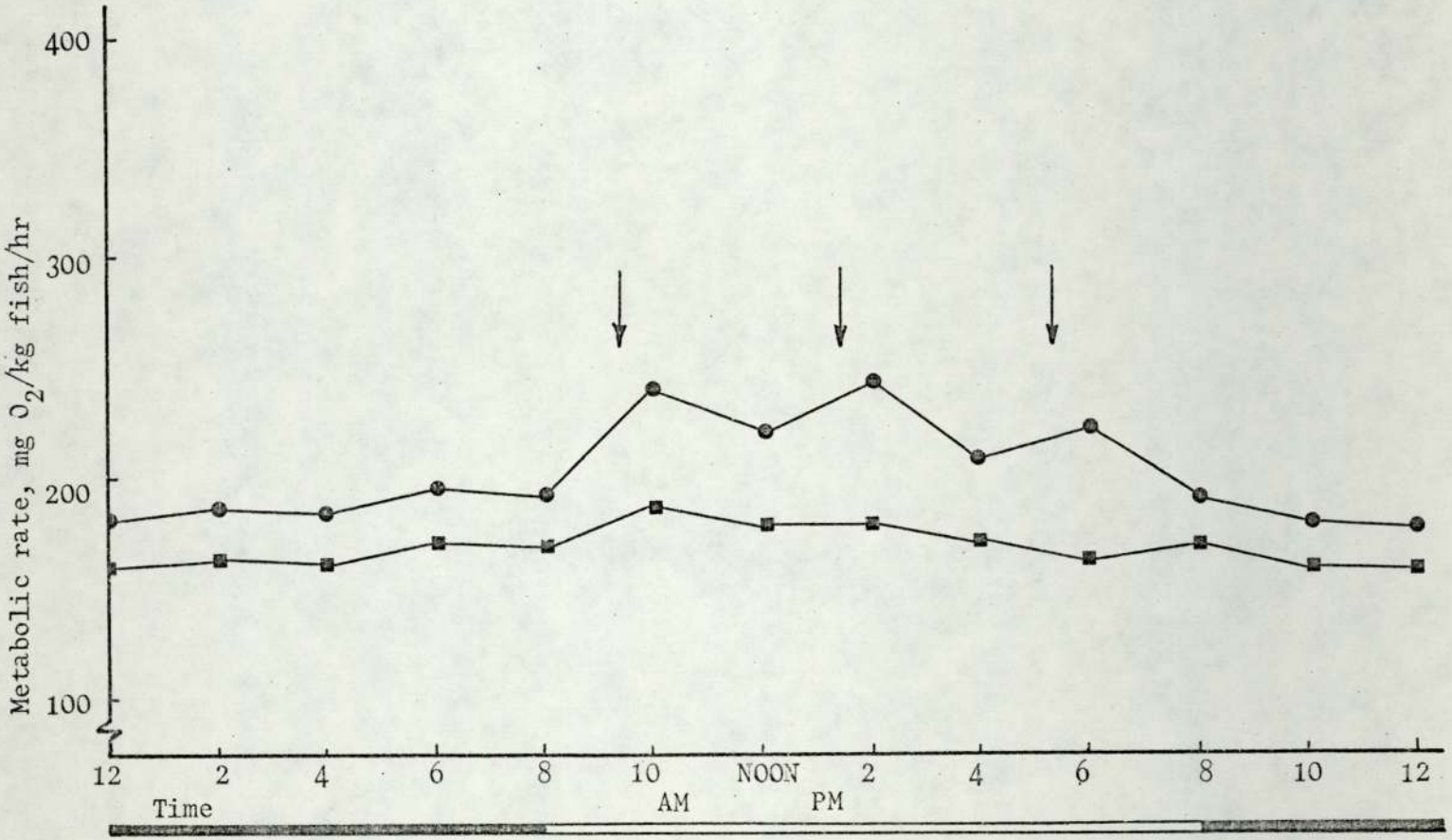


Fig.39. The variation in metabolic rate of one tank of fish fed at a 1% rate (upper line) and in a postabsorptive state (lower line) over two 24 hr. periods. Feeding times indicated by arrows. Fresh water tank

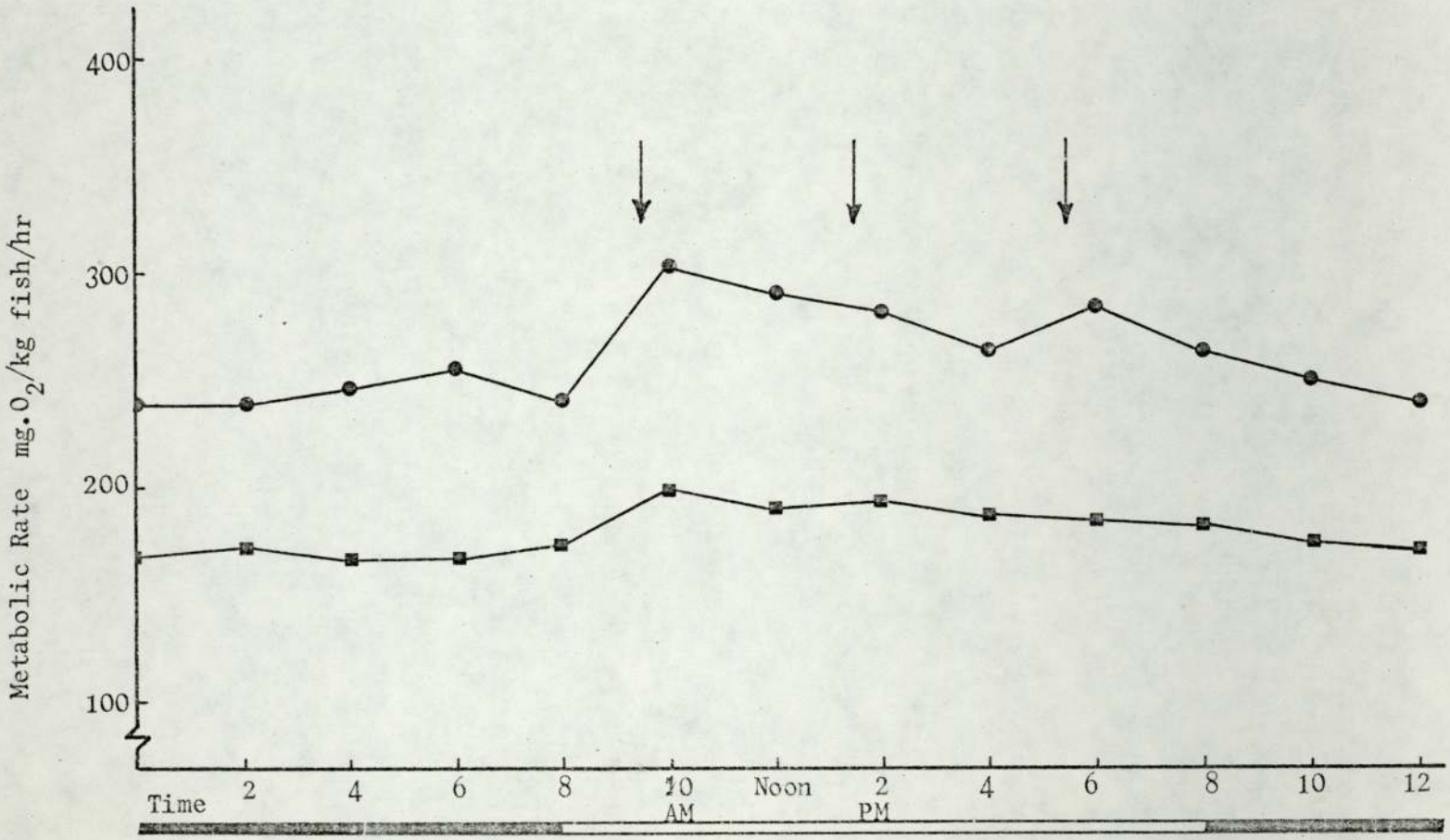


Fig.40 The variation in metabolic rate of one tank of fish fed at a 1.5% rate (upper line) and in a postabsorptive state (lower line) over two 24 hr periods. Feeding times indicated by arrows. Fresh water tank

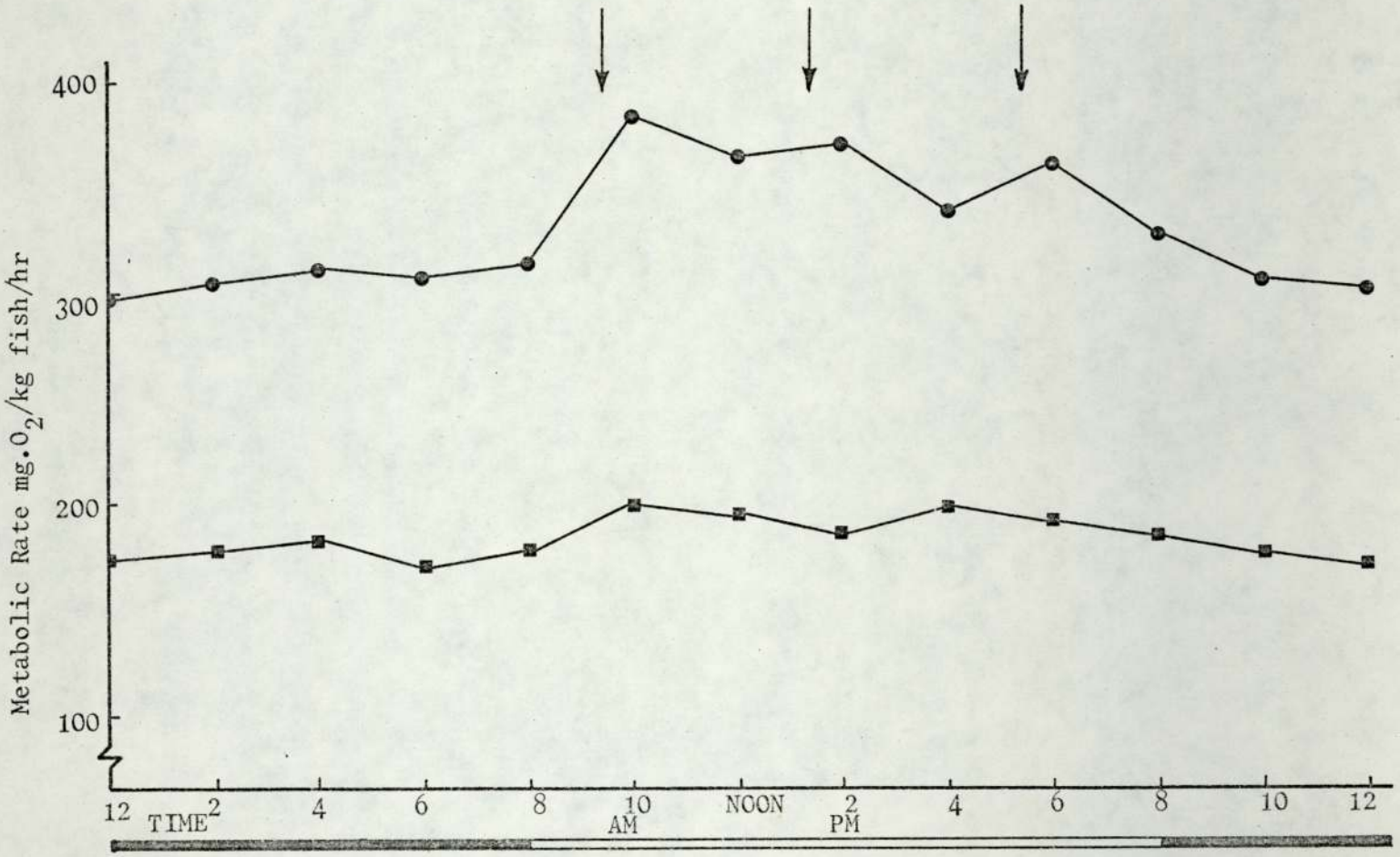


Fig.41. The variation in metabolic rate of one tank of fish fed at a 1.75% rate (upper line) and in a postabsorptive state (lower line) over two 24 hr. periods. Feeding times indicated by arrows. Fresh water tank.

feeding rates the oxygen consumption attributable to apparent SDA also increases directly with feeding rate.

Results of the 13 hr metabolic rate determinations are given in Tables 36 and 37. These show the mean metabolic rates of tanks of fish in different salinities in the fed and unfed condition at three different feeding rates. In the fresh water : 10 ppt sea water experiment in the fed condition metabolic rate increased significantly ($p < 0.001$) as feeding rate increased. At all feeding rates, metabolic rate was significantly lower for 10 ppt sea water than fresh water ($p < 0.05$ for 1.0% and 1.5% feeding rates and $p < 0.02$ for 1.75% feeding rates). In the unfed condition, metabolic rates were again significantly lower ($p < 0.001$) for 10 ppt sea water than fresh water. Apparent SDA values are expressed in the tables in both units of Kcal/kg fish/day and as a percentage of the ingested calories. Values for apparent SDA increased directly with feeding rate. In terms of the latter units, apparent SDA accounted for 5.46 - 5.66% of ingested calories at the 1% feeding rate and 17.45 - 17.33% at the 1.75% feeding rate. There was no significant difference in the values between salinities investigating each feeding rate in turn.

In the fresh water: 20 ppt sea water experiment in the fed condition metabolic rate increased significantly ($p < 0.001$) as feeding rate increased. At all feeding rates no significant differences were found between fresh water and 20 ppt sea water in both the fed and the unfed states. Values for apparent SDA again increased directly with feeding rate being 5.66 - 5.86% at the 1.0% feeding rate and 16.99 - 17.10% at the 1.75% feeding rate. The values obtained in fresh water and 20 ppt sea water were not significantly different at each feeding rate.

Feeding Rate	Salinity	Mean Metabolic Rate of Fed tank of Fish mg/kg/hr	Standard Error of ± Mean	Probability	Mean Metabolic Rate of Unfed tank of Fish mg/kg/hr	Standard Error of ± Mean	Probability	Mean Apparent SDA Kcal/ kg fish/ day	Mean Apparent SDA % of Fed Kcal/ day	Mean Apparent SDA Standard Error of ± Mean	Probability
1.0%	Fresh	202	± 3.30	p <0.05	175	± 2.45	<0.001	2.22	5.46	± 0.34	N.S
	10 ppt	180	± 0.94		152	± 1.63		2.30	5.66	± 0.19	
1.5%	Fresh	255	± 1.89	p <0.05	178	± 1.70	<0.001	6.32	10.38	± 0.44	N.S.
	10 ppt	231	± 3.30		152	± 1.89		6.48	10.65	± 0.29	
1.75%	Fresh	331	± 2.94	p <0.02	180	± 2.16	<0.001	12.39	17.45	± 0.52	N.S.
	10 ppt	304	± 4.65		154	± 1.70		12.31	17.33	± 0.36	

Each mean value shown is an average of 3 readings

N.S. = Not significantly different

Table 36. Mean metabolic rates of fed and unfed tanks of fish in fresh water and 10 ppt sea water together with values for apparent SDA

Feeding Rate	Salinity	Mean Metabolic Rate of Fed tank of Fish mg/kg/hr	Standard Error of ± Mean	Probability	Mean Metabolic Rate of Unfed tank of Fish mg/kg/hr	Standard Error of ± Mean	Probability	Mean Apparent SDA Kcal/kg fish/day	% of Fed Kcal/day	Standard Error of ± Mean	Probability
1.0%	Fresh	200	± 2.05	N.S.	171	± 1.70	N.S.	2.38	5.86	± 0.62	N.S.
	20 ppt	206	± 1.25		178	± 3.09		2.30	5.66	± 0.96	
1.5%	Fresh	261	± 1.70	N.S.	179	± 1.63	N.S.	6.73	11.05	± 0.44	N.S.
	20 ppt	259	± 2.05		180	± 2.16		6.48	10.65	± 0.23	
1.75%	Fresh	326	± 2.16	N.S.	179	± 0.82	N.S.	12.06	16.99	± 0.34	N.S.
	20 ppt	329	± 2.45		181	± 2.45		12.15	17.10	± 0.11	

Each mean value shown is an average of 3 readings

N.S. = Not significantly different

Table 37. Mean metabolic rates of fed and unfed tanks of fish in fresh water and 20 ppt sea water together with values for apparent SDA

6.4. Discussion and Conclusions

The fact that total oxygen consumption of fish undergoes a rhythmic diurnal fluctuation is in agreement with the results of several authors (e.g. Davis, 1964; Davies, 1966; Brett and Zala, 1975).

Sharp increases in oxygen consumption were observed immediately after feeding in the present experiment. This was undoubtedly due to the increase in activity of the trout during feeding. Trout are voracious feeders and exhibit much activity during feeding. Several minutes after feeding the trout resumed their normal activity within the tank, i.e. just maintaining their position against the flow of water. The post-prandial increase in respiration is in agreement with results found by Pierce and Wissing (1974) and Beamish (1974) the latter finding that oxygen consumption attributable to feeding procedure increased to a maximum in 1.0 - 3.5 hr after feeding.

In the present experiment the oxygen consumption was lower on average during the hours of darkness than during the hours of daylight. This again is likely to be caused by varying levels of activity as immediately after 'dawn' there was a rise in the oxygen consumption of fish even before the first meal fed at 9.30 a.m. (Figure 42). The reason for the nighttime low in activity which is increased as soon as 'dawn' occurs is that in the absence of any visual markers in the dark, fish were losing way against the flow of water in the tank. If the fish were slightly disorientated, it is possible that less energy would be devoted to swimming activity. As soon as the lights were turned on at dawn such visible landmarks as the water inlet and tank drain would enable the fish to resume their normal 'stationary' position by a small increase in swimming activity. The fact that the nighttime low in

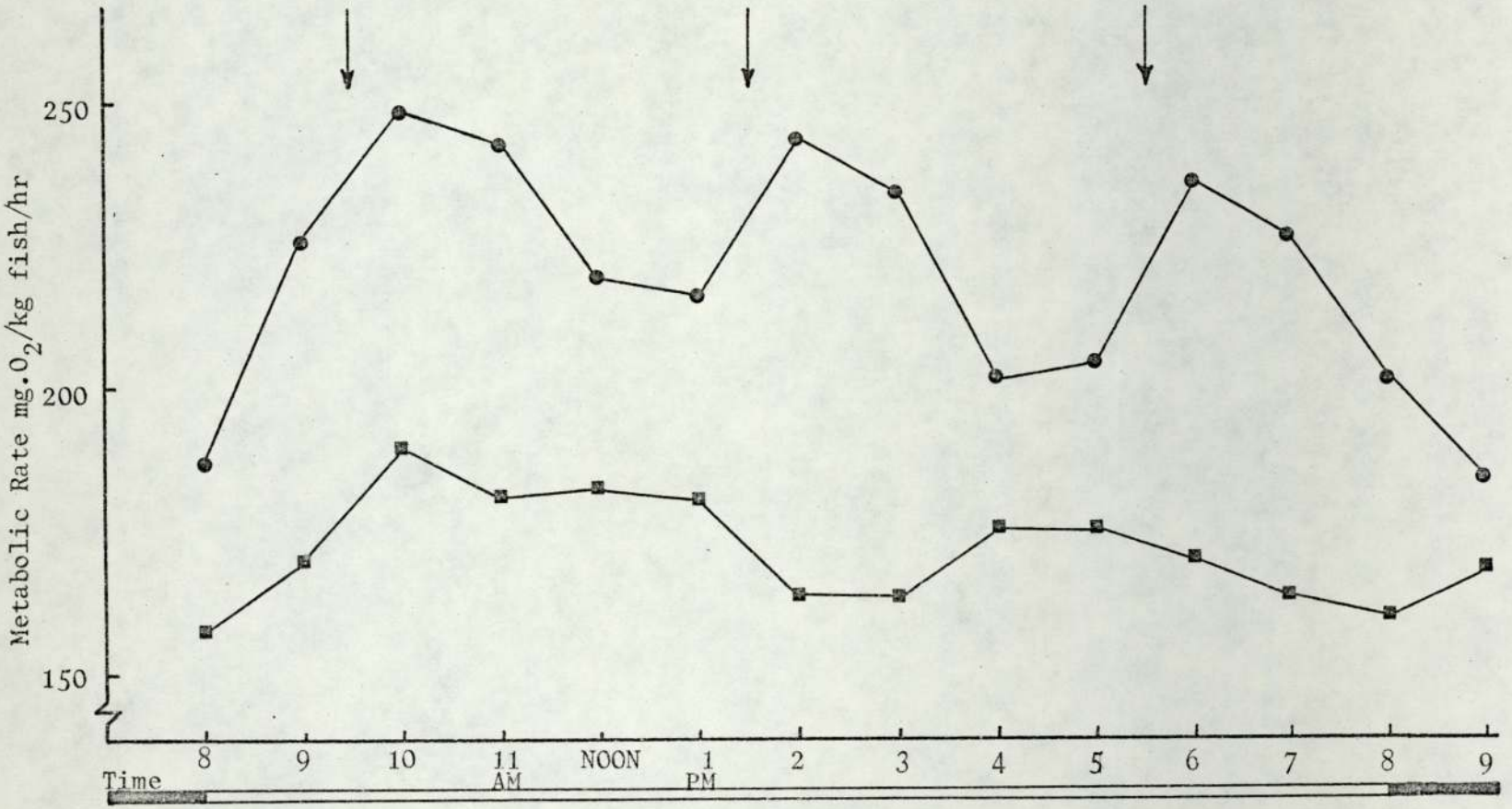


Fig.42. The variation in metabolic rate of one tank of fish fed at a 1.0% rate (upper line) and in a postabsorptive state (lower line) over two 13 hr. periods. Fresh water tank. Feeding times indicated by arrows.

activity occurred with starved fish confirms that it was variations in activity which were responsible. As the percentage drop in oxygen consumption during the hours of darkness was very similar in both freshwater and sea water tanks, comparison of metabolic rates between tanks was still considered valid. A similar result has been found by Davies (1966) working with goldfish, Carassius auratus. He found that the oxygen consumption was markedly reduced during darkness due to an inferred drop in the routine swimming activity of these fish. Contrasting results have been found by Pierce and Wissing (1974) who found that the nocturnal metabolic rates of bluegills, L. macrochirus, were 26% higher for both fed and unfed fish at all experimental temperatures tested. As the fish in both Davies' experiment and Pierce and Wissing's experiment were swimming at random it is likely that activity levels were the cause of the difference; there might also be species differences in diurnal swimming activity.

Interesting results on diurnal fluctuations in metabolic rate have been recently published by Brett and Zala (1975) who found that the metabolic rate of sockeye salmon was lowest at night and highest during the day, in agreement with the present experiment. In their experiment only one feed per day was administered from 8.30 to 9.30 a.m. They found a pre-dawn rise in the metabolic rate which increased to a peak just after the time of feeding. After this, metabolic rate gradually fell to reach a minimum the following night. Agreement is found between some of Brett and Zala's results and the results of the present experiment in that metabolic rate increased between 'dawn' and the first feed (Figure 42). This again is probably due to an increase in the activity of the fish in both experiments. The rhythm found in

the present experiment was not as dramatic as that found by Brett and Zala (1975, p. 2481, Figure 2) probably because only one feed was administered by the latter authors. In addition, the design of Brett and Zala's metabolism/growth tanks (Brett et al, 1971) probably afforded their fish greater opportunity for spontaneous activity than was possible by fish in the present experiment.

A phenomenon reported by Davis (1964) and not demonstrated in the present experiment was the presence of a pre-dawn increase in locomotor activity. Davis found with largemouth bass, Micropterus salmoides, and bluegill sunfish, Lepomis macrochirus, that the fish were able to anticipate their first feed of the day administered at 'dawn' by increasing their swimming activity. In the present experiment it is believed that the pre-feeding increases in oxygen consumption at 8.0 - 9.0 a.m. were caused by small increases in swimming activity as a response to the commencement of the day as described earlier.

Davies (1966) found that starvation as well as darkness reduced the oxygen consumption of goldfish again due to an inferred drop in the level of activity. Also Brown (1957) found with brown trout that spontaneous activity varied with feeding rate. This fact underlines the importance of maintaining swimming activity at a constant low level when measuring SDA rather than allowing fish to swim randomly.

The general increase in total metabolic rate with increased feeding rate is in agreement with the results of a previous experiment (Chapter 4) as are the actual values obtained. This phenomenon has been observed by many workers (Brown, 1957; Fry, 1957; Paloheimo et al., 1966a; Edwards et al., 1969; Edwards et al., 1972 and others) and is generally agreed to be caused by the disproportionate increase

in the energy dissipated by SDA as feeding rate increases (Warren et al., 1967; Averett, 1969). A result of this is that the level of total metabolism, α (cf. Appendix 1) is directly proportional to the feeding rate (Kerr, 1971).

The lower total metabolic rates of fish in 10 ppt sea water compared with fresh water together with similar metabolic rates in 20 ppt sea water and fresh water is in agreement with the results found in a previous section (Chapter 4). Thus there is confirmation that a salinity of 10 ppt being close to the isosmotic point causes a reduction in the total metabolic rate of rainbow trout either fed at any ration level or unfed. A strong inference can be drawn from these results with unfed fish. In the present situation unfed fish are expending energy for maintenance and activity only. As the energy expended on activity is equal in all tanks it follows that it is the maintenance energy requirement which has been reduced presumably through a reduction in the energy requirements for osmotic and ionic regulation. Similar metabolic rates by unfed fish in 20 ppt and fresh water confirm the hypothesis elaborated in Chapter 4.

Apparent Specific Dynamic Action

The values of the percentages of ingested calories dissipated through apparent SDA found in the present experiment fall within the wide range of 5 - 40% quoted by Warren and Davis (1967) and agree with figures found by Averett (1969) with coho salmon, O. kisutch. Averett found SDA figures of 10.7% to 17.3% of the assimilated food depending upon the ration size. If an assimilation coefficient of 0.8 is taken as standard, approximate figures of 8.6% to 13.8% of the ingested ration are found for the apparent SDA of Averett's salmon. Although the

figures found in the present experiment are more extreme than those found by Averett the order of magnitude is similar. Values for apparent SDA found by other workers include the following. Muir and Niimi (1972) with Kuhlia sandvicensis found apparent SDA accounted for 16 - 19% of ingested energy. Pierce and Wissing (1974) with L. macrochirus obtained figures of 12.7% and Beamish (1974) with Micropterus salmoides found values ($\pm$ 1 S.D) of 14.19 $\pm$4.19% of the ingested energy dissipated as apparent SDA. Thus there has been a wide range of values reported for apparent SDA. Absolute values are difficult to compare between publications as many factors may influence the magnitude of the SDA, e.g. the type of food, the quality of food, the caloric density and the frequency of feeding as well as environmental factors. A source of error apparent in the present experiment was that no account was taken of the energy expended in feeding. This would have led to a slight overestimate of the true apparent SDA. In practice this component is very difficult to measure and of the publications cited above only Beamish (1974) has included a measure of 'feeding activity' in his calculations. However, it was anticipated that such errors caused by omitting feeding activity from the present calculations would be negligible (cf. Pierce and Wissing, 1974).

The increase in the percentage of ingested rations dissipated as apparent SDA with increase in feeding rate is in agreement with results found by Averett (1964) but in contrast with results found by Beamish (1974). The latter author found that apparent SDA was independent of ration size. The results found in the present experiment, however, do corroborate results found in the previous section (Chapter 5) where net growth efficiencies were found to decline with increase in feeding rate. Increases in SDA occur when animals are unable to effectively

utilise food for growth either because of nutrient imbalances (Brody, 1945) or because of the metabolic state of the fish. For example, the inability of an animal to synthesise protein will result in deamination of the amino acids in the food leading to increased SDA.

The fact that no significant differences occurred in apparent SDA between different salinities at each feeding rate was surprising. Although no differences in net growth efficiencies occurred between salinities in the previous chapter it was anticipated that the change in energy turnover induced by salinity (10 ppt) might have affected the proportions of the basic metabolic substrates respired and hence might have affected apparent SDA. This hypothesis was not confirmed. Thus, it appears as if either there is no change in apparent SDA induced by low salinities or such a change is too small to be detected by the methods used in this experiment. However, it is interesting to note here that reductions in the total metabolic rates of fish in 10 ppt sea water are caused solely by reductions in their maintenance energy expenditure with no contributory reduction in SDA.

In conclusion, apparent specific dynamic action was successfully determined for fish held in three salinities and fed at three feeding rates. Feeding rate was found to affect apparent SDA in that as the feeding rate increased, the percentage of ingested calories lost through apparent SDA increased. Salinity had no effect on apparent SDA at any feeding rate. While this corroborated net growth efficiency evidence from the previous chapter the hypothesis that salinity induced a change in the proportions of metabolic substrates respired was not supported.

Chapter 7. Experiment 5. Determination of the Nitrogen-Balance of Trout held in Different Salinities

7.1. Introduction

In all previous experiments the effect of salinity on growth and conversion has been measured by changes in either live weight or calories. While it is desirable that conversion of food should be based on calorific values, because of the differences in the fat/protein ratio, calculations based on protein content are adequate when considering growth only (Brett et al., 1969). Indeed, Gerking (1962, 1967) has presented several reasons for considering increases in protein as the most valuable index of growth. Protein elaboration, the basis of cellular increase, is the most characteristic feature of growth. Proteins can be constructed only from amino acids rather than being derived from a variety of sources as is for example, fat. The main product of interest is often protein and the derivation of this product should be in the same terms.

Dietary protein is necessary for three main purposes (Cowey & Sargent, 1972):-

a. Maintenance

b. The repletion of depleted tissues

c. Growth and/or the formation of new additional protein.

The utilisation of protein is mainly affected by its amino acid pattern, by the caloric content of the diet, by the level of protein intake, and by the physiological state of the animal. It was the latter two factors which were of interest in the present experiment.

Tissue protein in animals may be regarded as being in a dynamic state with anabolic and catabolic processes occurring simultaneously

(Maynard & Loosli, 1969; Cowey & Sargent, 1972). The amino acids assimilated from the diet may enter either of these processes depending upon many factors. If they enter anabolic pathways, tissue protein is formed. If they enter catabolic pathways, the amino acids will be metabolized to simpler products which are subsequently eliminated. Accompanying this process is a release of energy, some of which may be harnessed for useful physiological work. A 'pool' of amino acids has also been envisaged (Allison, 1957) which acts as a receiver for protein catabolism and as a source for amino acid anabolism.

Generally, it may be said that a balance of nitrogen (protein) exists whereby dietary nitrogen as ingested protein is diverted into growth via anabolic processes or is excreted via catabolic processes or is eliminated as unabsorbed faeces. This may be expressed in the familiar nitrogen balance equation thus:-

Balance = I - (E + F)

Where I is nitrogen intake, E is nitrogen excretion and F is faecal nitrogen.

If the nitrogen balance is positive the animal is gaining new tissue by growth etc. and if negative, protein loss is occurring. If the nitrogen balance is zero, anabolism and catabolism are equal.

As growth, diet and metabolism are intimately associated it was considered of particular interest to investigate the nitrogen balance of fish in different salinities, this latter factor having previously been shown to elicit a change in the metabolism of trout. In addition, it was considered desirable to investigate the nitrogen balance of fish at several different levels of dietary protein intake in order to determine the maintenance and optimum protein requirements in different salinities.

In order to determine optimum and maintenance protein requirements of any animal it is desirable that protein should be fed to different groups of animals in a range of concentrations. This then enables the above parameters to be derived graphically if, for example, protein retention is plotted against protein intake. There are two methods of providing different groups of fish with a range of protein intakes. The first is to feed different groups of animals an identical diet but at different feeding rates. This method has the disadvantage that every dietary constituent including protein is being fed at a different rate to different groups. Such parallel changes in energy and mineral intake may cause changes in growth which might confuse the desired results of the effect of changes in protein intake on growth.

The preferable alternative to the above method is to formulate several different diets of varying protein concentrations and keep the concentrations of the other major ingredients, in particular energy, as similar as possible. This puts most of the emphasis on protein as the only variable. This method was used in the present experiment as the means of varying the dietary protein intake.

7.2. Methods of Protein Evaluation

Weight-Gain Methods

There are many methods of evaluating dietary protein some of which are better suited to a particular experimental situation than others (McLaughlan & Campbell, 1969). The rate of growth of an animal under defined dietary and environmental conditions is the simplest method of measuring the value of a particular protein level. The most widespread application of the weight-gain method of protein evaluation is found in the concept of protein efficiency ratio (PER) first introduced by Osborne, Mendel and Ferry (1919). PER is defined as the weight gain of an animal divided by the weight of protein consumed. This method has several drawbacks despite (or because of) its simplicity which include the following:-

a. the assumption that gain in body weight is constant in composition may not always be true

b. No allowance is made for maintenance requirements as it is assumed that all protein consumed is used for growth .

Thus the use of PER in the present experiment was considered to be impracticable as a greater degree of accuracy was required.

Nitrogen Balance Methods

Nitrogen balance may be determined from the equation outlined earlier:-

$$B = I - (U + F) \quad \ldots\ldots\ldots\ldots 1$$

If U and F are corrected for nitrogen of metabolic origin the biological value (BV) of that particular protein may be determined. Biological value is defined as the fraction of absorbed nitrogen utilised by the animal. The concept was first introduced by Thomas (1909, from

Mitchell, 1943) and was later refined by Mitchell (1924, from Bender, 1956) and others. The equation expressing biological value is as follows:-

$$BV = 100 \times \frac{I - (FN - MFN) - (UN - ENE)}{I - (FN - MFN)} \quad \ldots\ldots\ldots 2$$

Where I = Nitrogen intake

FN = Faecal nitrogen

MFN = Metabolic faecal nitrogen

UN = Urinary nitrogen

ENE = Endogenous nitrogen excretion

In this equation, the numerator represents the total nitrogen utilised for both growth and maintenance. Metabolic faecal nitrogen is that component of total faecal nitrogen which is not derived from the undigested, ingested food and comprises abraded cells from the gut wall and digestive juice residues (Maynard & Loosli, 1969). Endogenous nitrogen excretion is defined as the nitrogen excreted by animals fed a non-protein diet which is nutritively complete in all other aspects (Mitchell, 1943). Provided that all the variables which comprise the BV equation can be measured with accuracy, the use of BV is reliable. Eggum (1970) states that the use of BV provides accurate evaluations of a test protein.

Carcass-Nitrogen Methods

Bender and Miller (1953) described a method for estimating protein quality which they originally called net protein value. Later this was changed to net protein utilisation (NPU) (Miller & Bender, 1955). Net protein utilisation is defined as the fraction of ingested nitrogen utilised by the animal. Their equation for expressing this is:-

$$NPU = \frac{\text{Body N of test group - Body N of non protein group}}{\text{N consumed by test group}} \quad \ldots\ldots 3$$

Thus the percentage utilisation of nitrogen is measured by carcass analysis instead of the difference between nitrogen intake and output. This method is excellent for accurate NPU determinations but, being a carcass-analysis method, no figures can be obtained for the digestibility of the test protein, this latter often being a desirable parameter to determine. The control group of animals fed on a non-protein diet makes allowance for maintenance nitrogen requirements and permits the evaluation of poor proteins which do not promote growth.

In the present experiment it was decided that a nitrogen balance method of determining NPU for fish consuming different intakes in protein concentration would be employed. There were two main reasons for this decision. Firstly, it is often difficult to make fish accept a non-protein diet as would be required if the components of Equation 3 were determined. Feeding fish a non-protein diet has been achieved by only very few authors, for example Ogino, Kakino and Chen (1973) and Gerking (1955b) the latter author having to use force-feeding techniques. As it was anticipated that force-feeding would induce stress in trout this procedure was discarded.

Secondly it was estimated that a nitrogen-balance determination could be achieved in a shorter time than could a carcass-analysis estimation. An entire nitrogen-balance determination was anticipated as taking only five weeks to complete.

Thus, NPU values for fish fed at different levels of dietary protein in different salinities were determined using nitrogen balance techniques.

The equation used is similar to Equation 2 and the same components are determined. In fact, NPU is often defined as BV x Digestibility.

Thus, $$NPU = 100 \times \frac{I - (FN - MFN) - (UN - ENE)}{I} \quad \ldots\ldots\ldots 4$$

It can be seen that the difference between Equation 2 and 4 lies in the denominator where no correction for undigested food is made to I in Equation 4.

Nitrogen Balance with Fish

Several workers have undertaken nitrogen balance experiments with fish using either growth and carcass analysis methods or growth by difference (Equation 1) methods. The former type of experiment has been performed by Gerking (1955a, b, 1971) and Birkett (1969) who measured nitrogen retention during an experiment as the difference in total nitrogen content between a group of experimental fish at the end of a feeding experiment and a control group of fish sacrificed at the beginning of the experiment. Faecal and dietary nitrogen could be measured and the excreted nitrogen was calculated by difference.

The 'growth by difference' studies have been undertaken by Tunison and co-workers (1942) at the Cortland Hatchery, N.Y. and more recently by Atherton and Aitken (1970). Different researchers have used different methods to solve the many experimental difficulties inherent in this sort of work. Chief among these is the difficulty of separating the faecal and excreted nitrogen of an aquatic animal together with the breakup of faeces contributing towards soluble nitrogen in the water. The use of divided chambers (Post, Shanks & Smith, 1965; Smith, 1967) has contributed towards the separation of faecal nitrogen and excreted nitrogen (ammonia, urea etc.) but as yet, no entirely satisfactory method for accounting for soluble faecal nitrogen has been described.

The study by Tunison et al. (1942) was a modification of the Thomas-Mitchell biological value method with the difference that in order to measure metabolic faecal nitrogen and endogenous nitrogen excretion, fish were starved rather than fed a non-protein diet. This was due to the difficulty experienced in fish accepting a non-protein diet. As Tunison himself pointed out, faecal nitrogen voided during a fasting period will probably be less than the true MFN value as the absence of food in the gut would result in little gastric juice secretion or gut-wall abrasion.

The endogenous nitrogen excretion of animals is not an easily determined parameter. Several methods have been used by workers in the past for determining endogenous nitrogen excretion. These include starvation methods, feeding a non-protein diet, and extrapolation techniques as used in the present experiment. This latter method was chosen for several reasons. Starvation of fish followed by measurements of nitrogen excretion results in inaccurate estimates of endogenous nitrogen excretion because a starved fish has undergone changes in the proportions of basic metabolic substrates respired for energetic purposes. Savitz (1971a) showed that during starvation, fat is primarily oxidised for energy purposes. Hence there is probably a conservation of body nitrogen with a concurrent decrease in the nitrogen excretion rate. An improved method of determining endogenous nitrogen excretion (ENE) involves feeding a non-protein diet. This approaches the desired criteria for measuring ENE which theoretically is defined as the nitrogen excreted by an animal when consuming a non-protein diet which is otherwise nutritively complete (Mitchell, 1943). This technique has been used successfully by Gerking (1955b) and

Savitz (1969, 1971a, b) who provided bluegill sunfish, L. macrochirus, with daily oesophageal injections of a simple sugar to provide energy and to prevent undue tissue catabolism. One small criticism of this technique is that force feeding may cause large variability in rates of digestion (Windell, 1966).

Endogenous nitrogen excretion rates in the present experiment were determined by indirect techniques. That is, nitrogen excretion of fish fed different levels of protein in the diet was determined. When rates of nitrogen excretion were plotted graphically against protein consumption, extrapolation of the curve to the point of zero nitrogen intake gives the ENE rate. This technique dispenses with fish handling, anaesthetization, and oesophageal administration of food. As stated before, it was the objective in all experiments to maintain such handling at a minimum.

The principles embraced in the present nitrogen balance experiments will now be elaborated. In order to determine the nitrogen balance status of a tank of fish the fish were maintained in constant environmental conditions and fed at a constant rate for a period of time before the commencement of the experiment. This ensured that each tank of fish was in a relatively stable state of nitrogen balance with reasonably constant rates of nitrogen excretion and faecal output. The daily nitrogen excretion rate was determined by keeping fish in a static volume of water for a period of time and analysing aliquots of the water for the total soluble nitrogen content. This total nitrogen content included ammonia, urea, mucus-nitrogen and soluble faecal nitrogen. No attempt was made to distinguish between these variables with the exception of soluble faecal nitrogen which was corrected for in the calculations.

Faecal nitrogen was determined by collecting the faeces voided over the same period of time in a faecal trap. This resulted in an underestimate of the total faecal nitrogen due to the loss to the water of the soluble faecal nitrogen fraction. This was estimated in the following manner. Faeces were 'stripped' from fish regularly for digestibility measurements. Determining the apparent digestibility of the protein in a specific diet together with the absolute quantity of protein fed per day per tank of fish gave the theoretical nitrogen output in the faeces. The difference between this value and the determined value for faecal nitrogen collected in the faecal trap resulted in a value for the daily soluble faecal nitrogen loss.

Thus, $[N \times (100 - A.D.)] - T.F.N. = S.F.N.$

where N = Daily nitrogen intake, mg.

A.D. = Apparent nitrogen digestibility, %

T.F.N. = Total faecal nitrogen collected daily in a faecal trap, mg

S.F.N. = Soluble faecal nitrogen component, mg/day.

Thus, by subtracting from the total nitrogen consumed per day per tank of fish the total nitrogen excreted and the total nitrogen voided in the faeces, the nitrogen balance was determined. As this was positive in all cases it could be equated with total nitrogen retained, or growth.

7.3. Materials and Methods

Diet Formulation

Five diets were formulated which ranged in protein content from 60% of the dry weight to 20% in increments of 10%. All diets were made iso-caloric as well as having the vitamin and mineral contents the same. Dietary fibre was ignored on the basis that it is a difficult component to equalise in markedly different diets. It was anticipated that the period over which the diets were fed, 5 weeks, would be too short to allow unresolved differences to elicit a marked effect in any measured parameter.

The diets were of a semi-purified nature and it was hoped that this type of composition would bear a closer resemblance to the commercial diet used in earlier experiments than would diets of a purified nature. The composition of the diets used is shown in Table 38. The theory behind the formulation was to draw up a standard diet, Diet 1, and then to formulate all the other diets to have the same calorific value as Diet 1. Progressing from Diet 1 to Diet 5 it can be seen that the herring meal content (the major protein source) has gradually been reduced. The calories lost by such a procedure were replaced from various lipids and carbohydrate sources. Various constraints on the levels of the major ingredients had been decided before formulation; this will be elaborated later.

The calorific densities of the diets and of the various components of the diets were expressed on a metabolisable energy rather than a gross energy basis. The metabolisable energy is that proportion of the food which is available to the fish. The gross energy of a

Table 38. Composition (g/100g dry diet) of the experimental diets

	DIET				
Component	1	2	3	4	5
Herring Meal	83.93	69.64	55.36	41.07	26.79
Distillers Solubles	5.00	5.00	5.00	5.00	5.00
Cod Liver Oil	2.20	3.48	4.77	8.22	14.74
Corn Starch	2.87	3.35	3.84	4.33	4.81
Glucose	0	10.00	20.00	25.67	25.19
α-Cellulose	2.70	3.93	5.08	8.41	15.02
Mineral Mix	1.30	2.60	3.95	5.30	6.45
Vitamin Premix	1.00	1.00	1.00	1.00	1.00
Chromic Oxide	1.00	1.00	1.00	1.00	1.00

substance is the heat of combustion of that substance when exploded in a bomb calorimeter in an oxygen atmosphere. Not all of this energy is available to animals when food is ingested. Some is lost in the faeces, some in the urine, and a quantity is lost through SDA. When the energy lost through excretion and defaecation is subtracted from the gross energy intake, one arrives at that portion of the ingested energy actually capable of transformation in the body, the metabolisable energy (Maynard & Loosli, 1969). This may proceed another stage in that if the heat increment (SDA) is subtracted from the metabolisable energy, that portion of the ingested energy which appears as a product, e.g. growth or activity, is derived. This is known as the net energy of the food. However, derivation of net energy values is impracticable for this type of nutritional study as the SDA is very variable and is usually unknown. Thus, the evaluation of feeds based on metabolisable energy is a widely used practice in animal nutrition which takes into account energy losses through digestion and excretion and gives a more nearly correct evaluation of the production value of a feed. The only source of error which results from using metabolisable energy as opposed to gross energy of a food is derived from the presupposition of digestibility and excretion rates which may vary in practice from that anticipated. However, these errors were not envisaged as being significant as the fish in two corresponding tanks would be fed on the same diet thus keeping all results relative. Caloric values of major food groups were taken from Phillips (1972) in metabolisable energy terms as:-

Protein	=	4.0 Kcal/g food protein
Fat	=	8.0 Kcal/g food fat
Starch	=	1.6 Kcal/g food starch
Glucose	=	4.0 Kcal/g food glucose

(Phillips and co-workers, 1948)

Herring meal was the major source of protein in the diets and was taken to be 70% protein according to the supplier's analysis. A small amount of protein (1.25% of the dry weight of the diet) was derived from the distillers solubles (Scotoferm) which were included in every diet at a 5% level. Distillers solubles were included in the formulation solely as an aid in binding the dietary ingredients when pelleting. No other binder was used. Other ingredients used in all diets at the same level include the vitamin premix at 1% of the dry weight and a chromic oxide (Cr_2O_3) indicator, also at a 1% level. The composition of the vitamin premix is given in Table 39 where it is seen that vitamins A and D_3 have been omitted. This was deliberate as it was anticipated that the levels of herring meal in all diets would supply adequate quantities of these two vitamins.

The major source of lipid in most of the diets was cod liver oil. Lipid was also derived from the herring meal and a small contribution from the distillers solubles (0.25% of the dry weight of the diet) was also taken into consideration. Cod liver oil was used as the major source of lipid calories because it has been shown repeatedly (Phillips and co-workers, 1965) that the inclusion of fat in trout diets spares protein from catabolic pathways. Thus the spared protein can be utilised for growth (Phillips, 1969). Cod liver oil was chosen specifically for several reasons. It is highly digestible being a soft, unsaturated fat (Tunison & McCay, 1935; Sinnhuber, 1969). Also, being a fish oil it

Table 39. Composition of the vitamin premix used in experimental diets

Specification is Vitamin Premix No.4-C taken from Halver (1972), Appendix table III, p.655.

Note - Vitamins A and D_3 have been omitted.

Ingredient	Quantity, mg/lb. premix
D--Calcium pantothenate	600.0
Pyridoxine	500.0
Riboflavin	1,750.0
Niacin	6,250.0
Folic acid	100.0
Thiamine	250.0
Inositol	6,250.0
p-Amino benzoic acid	250.0
Biotin	5.0
Vitamin B12	0.25
Menadione sodium bisulphite	125.0
Vitamin E	2.178.0 I.U.
BHT antioxidant	800.0 U.S.P.
Ferrous carbonate	225.0
Copper sulphate	22.0
Choline chloride	40.000.0
Ascorbic acid	3.000.0

contains a high percentage of polyunsaturated fatty acids, most of which contain the omega-3 structural configuration (Ackman, 1967). Castell, Sinnhuber, Wales and Lee (1972) with rainbow trout showed a definite need for polyunsaturated fatty acids. Also linolenic acid (omega-3 fatty acid) was superior to linoleic acid in promoting growth and food conversion.

Fish are unable to biosynthesize fatty acids which are unsaturated in the omega-3 and omega-6 positions unless a suitable precursor is present in the diet (Sinnhuber, 1969). In experimental rations this is usually provided by corn, cottonseed, or soybean oil. However, corn oil has been shown to be low in omega-3 fatty acids and it is therefore unadvisable to use this as the sole fat source in diets. Accordingly, it was decided to use cod liver oil as the major lipid source as it is high in linolenic acids together with vitamins A and D. This lipid has been used as a partial lipid source with success by Wolfe (1951) and Halver (1957) and Phillips and Podoliak (1957) have considered that cod liver oil is safe to use as the sole lipid source during periods of rapid growth at medium-high water temperatures.

Fat levels ranged from 9.83% to 17.16% and it was considered that these levels were quite acceptable. Commercial diets range in fat content from 5.9 - 14.2% (Sinnhuber, 1969) and Higashi and coworkers (1964) found that rainbow trout grew well and showed no ill effects with 25% marine oil in the diet. In support of this, Kitamikado and co-workers (1964) found lipid levels up to 30% had little effect on protein digestion in rainbow trout.

The major source of available carbohydrate in most diets was pure glucose. Carbohydrate also derived from the corn starch, the herring meal (3.4% of weight of herring meal) and the distillers solubles (2.42% of the dry weight of the diet).

Trout can utilise only limited levels of carbohydrates according to Phillips and co-workers (1948). They found that levels of digestible carbohydrate higher than 12% of the wet weight of the diet (33% of the dry weight) caused accumulation of glycogen in the liver and eventual death. However, this figure has not been substantiated by other authors. Buhler and Halver (1961) showed that chinook salmon fingerlings tolerated and grew well on a diet containing 48% carbohydrate. However, there might have been a species difference in carbohydrate tolerance between trout and salmon. Nevertheless, a figure of 33% was adopted as the maximum level of carbohydrate in the diets of the present experiment. As soon as this level was reached, in diet No. 4 when the formulation was undertaken, cod liver oil was added to make up the calorific value to that equivalent to that of diet number 1. It was anticipated that the high levels of carbohydrate found in Diets 4 and 5 would not affect carbohydrate digestibility as Singh and Nose (1967) found that diets fed to rainbow trout with glucose levels of up to 60% did not affect the digestibility of that carbohydrate.

The quantities of minerals included in the experimental diets are shown in Table 40. The proportions of the ingredients were altered as the herring meal was deleted from successive diets in order to replace calcium and phosphorus. It was considered that minerals of a lesser importance would be adequately provided by the herring meal and distillers solubles present in every diet.

After formulation the experimental diets were mixed thoroughly in a rotary mixer and were then pelleted by a Christie Norris (Christie N. Norris, Chelmsford) pelleter into 3/8"-$\frac{1}{4}$" pellets. Before starting the experiment the major ingredients of the diets were analysed and the

Table 40. The composition of the mineral mixes used in experimental diets.

Diet	Di-calcium phosphate g/100g dry diet	Sodium chloride g/100g dry diet	Calcium iodate mg/100g dry diet
1	0.70	0.60	1.02
2	1.90	0.70	1.19
3	3.10	0.85	1.44
4	4.30	1.00	1.70
5	5.30	1.15	1.95

results are shown in Table 41. Moisture was determined by drying ground samples of diets at 50°C to constant weight. Protein was determined by measuring the total nitrogen content of triplicate samples of diets using the micro-Kjeldahl technique (A.O.A.C. Methods, 1970) and applying a factor of 6.25 to the results to convert total nitrogen to protein. Lipid in the diets was determined by subjecting triplicated samples to the Soxhlet method of lipid extraction for 8 hr using diethyl ether as the solvent. Ash determinations were made on dried, triplicated samples by heating diets in a muffle furnace (FR 610, A. Gellenkamp & Co. Ltd., Christopher Street, London) at 580°C for four hours. Chromic oxide was determined at a later date.

These analytical results together with the remaining estimated dietary components (Table 42) were used to calculate metabolisable energy contents of each diet. These were very similar ranging from 3.454 to 3.372 Kcal/g dry diet. As the difference between the highest and lowest values was only 2.43% of the lowest value it was considered that the difference was negligible for the purposes of the experiment.

Table 41. Analytical results of the composition of the experimental diets

Component	DIET 1	2	3	4	5
Moisture, % of food weight	7.08	8.66	8.84	8.14	8.31
Protein % of dry weight	62.42	53.70	42.80	30.12	22.57
Lipid % of dry weight	9.83	9.62	10.15	12.62	17.16
Ash % of dry weight	12.47	12.60	11.82	11.75	11.56
Chromic Oxide % of dry weight	1.00	0.93	1.04	1.02	1.02

Table 42. Estimated components in the experimental diets

Component	DIET 1	2	3	4	5
Carbohydrate (including nitrogen free extractives) % dry weight	8.14	18.14	28.14	33.33	33.33
Fibre, % dry weight	3.70	4.93	6.08	9.41	16.02
Metabolisable Energy Kcal/g dry diet	3.413	3.446	3.454	3.372	3.413

Experimental Procedure

The modification of ten of the 15 gal fish tanks together with their operation has already been outlined in Chapter 2 and Figure 10. The objective of the modifications was to allow these ten tanks to be used as nitrogen balance tanks whereby static volumes of water could be held in each tank and separation of faeces from the main body of water could be effected into a separate faecal trap.

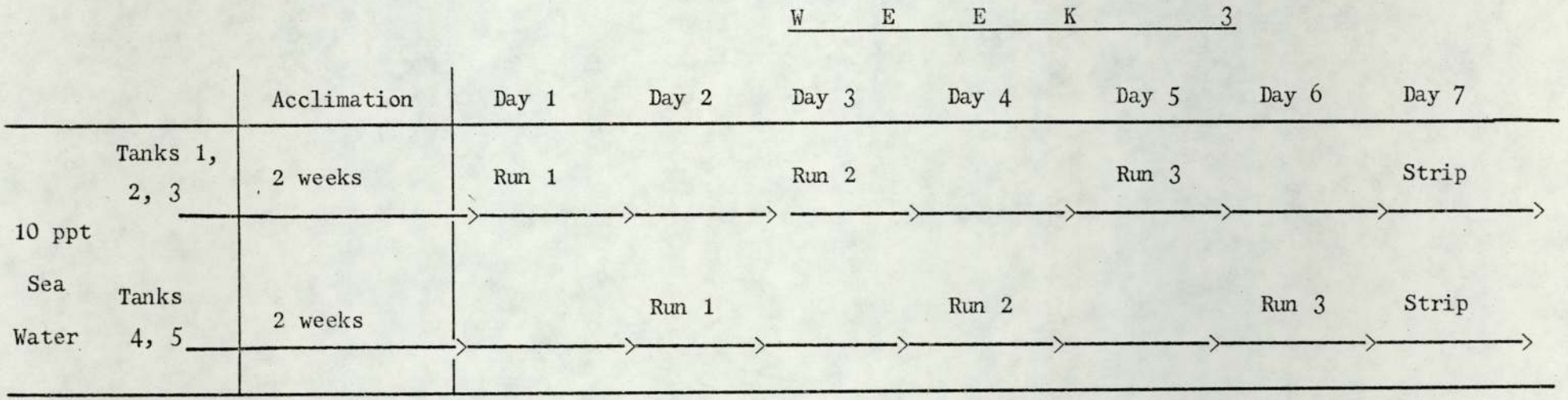
A standard procedure for determining nitrogen balance was adopted which measured the following parameters during each nitrogen balance test:- faecal nitrogen; soluble faecal nitrogen, this being the soluble nitrogen leached from the faeces during the test; and total non-faecal nitrogen which included ammonia, urea, mucus-nitrogen and the other minor nitrogenous excretory products. Accurate determination of these variables together with measurement of nitrogen intake enabled the components of the nitrogen balance equation , $B = I - (F + U)$, to be determined on fish in three salinities, fresh water, 10 ppt and 20 ppt sea water at five different levels of protein intake.

The two experimental systems were set up in the following manner. One system was holding fresh water, the other 10 ppt sea water. One hundred fish of approximately 110 g in weight each were weighed individually as detailed in Chapter 2 and 10 fish were allotted to each of ten tanks, five fresh water and five 10 ppt sea water. Analysis of variance and Duncan's Multiple Range Test were then carried out on the individual fish weights and no significant differences were seen between any of the tank-weights of fish. The five diets of different protein contents were then allocated among the ten tanks by using a table of random numbers so that each of the five diets was represented once in both the fresh water and the 10 ppt systems. All fish were then fed for one

fortnight on their allocated diet at a rate of 1% of the body weight per day. The ration was administered in three meals per day at 9.30 a.m. 2.00 p.m. and 5.30 p.m. During the experiment, all fish were weighed at weekly intervals by the 'batch' method and the rations increased in order to account for the growth of the fish and maintain feeding rate at the 1% level. Water temperature at all times was 12 $\pm 0.5^{\circ}C$ and photoperiod was 14 h commencing at 8.00 a.m.

The sequence of experimental determinations is given in Table 43 and is detailed below. During the first three weeks of the experiment one system held fresh water, the other 10 ppt sea water. The first two weeks were given to acclimating fish to both sets of experimental conditions. During the third week, nitrogen balance 'runs' were undertaken with the 10 ppt sea water tanks as detailed in Table 43. Altogether, three runs were performed on each of the five tanks. At the end of the third week, faecal samples were removed from the fish using a method outlined later on. During week four the salinity of the water in the sea water system was increased over two days to 20 ppt and the fish allowed to acclimate to this salinity for the rest of the week. Also, during week 4 nitrogen balance runs were undertaken on the fresh water fish using a sequence of operations identical to that used in the previous week. Faecal samples were again removed from fish. During the fifth week, nitrogen balance runs were undertaken on the fish in 20 ppt sea water in the same way. Faecal samples were taken from fish at the end of the week as before. At the end of week five, the fish were removed from the two systems and the experiment terminated.

The sequence of operations in undertaking a nitrogen balance run on one tank of fish is now detailed. Each run lasted 25 hr: the aim of the



Fresh water Nitrogen balances determined during week 4

20 ppt sea water balances determined during week 5

Table 43. Experimental procedure. Details of week three

experiment was to determine the total nitrogen output as faecal and non-faecal nitrogen over this period. Feeding was continued throughout the runs.

Total soluble waste nitrogen was determined by taking two 150 ml filtered water samples from the tank at the beginning of the run and two at the end of the run. The difference in total nitrogen was then considered to be the nitrogen eliminated by the fish in that period of time. The first samples were taken one hour after the start of a run (after the 'fresh' inflow of water had been shut off) to ensure thorough mixing of the tank and faecal trap water (cf. Fig. 10); the last samples were taken 24 hr later. These samples were stored in 150 ml glass bottles for later analysis and the ammonia in solution was 'fixed' by adding 0.1 ml concentrated sulphuric acid (Karzinkin & Krivobok, 1964) and refrigerating at $4^{o}C$. Total nitrogen in the samples was determined by macro-Kjeldahl technique (Golterman, 1969) on five 40 ml aliquots taken from each water sample and obtaining an average value.

Total faecal nitrogen was determined by dismantling the faecal trap and decanting off most of the clear water. Faeces were collected by filtering through a pre-weighed 14 cm Buchner filter paper. This was then air-dried for 24 hr and then oven-dried at $50^{o}C$ for 2 hr and when cool the dry filter paper and faeces were reweighed. The difference between this weight and the original weight of the filter paper gave the weight of dry faeces collected. The faeces were then removed from the filter paper, ground with a pestle and mortar and three aliquots were taken for analysis of total nitrogen. This was undertaken by micro-Kjeldahl estimation (A.O.A.C. Methods, 1970).

Estimation of the soluble faecal nitrogen component in the water

samples taken was undertaken in the following manner. The inclusion of a chromic oxide indicator at a 1% level in each of the five diets (refer to dietary formulation section) permitted an accurate measure of apparent digestibility of the nitrogen contained in the diets to be made. Faeces were stripped from each anaesthetised fish at the end of every week by running the fingers lightly along the underside of the fish from pelvic fins to cloaca. Faeces were collected in a beaker containing 20 ml distilled water to avoid contamination of the faeces by urine which was sometimes released during stripping. The water in the beaker, together with any released urine, was decanted off and the faeces washed on to a filter paper. This was then oven dried at 50°C for 3 hours and the dried faeces removed and ground with a pestle and mortar. Several aliquots were taken; half of these were used for determination of total nitrogen by micro-Kjeldahl technique and half used for the determination of total chromic oxide by the digestion and spectrophotometry technique described by Furukawa and Tsukahara (1966). The chromic oxide content of the five diets was also determined at this time. The optical density of digested faecal and dietary samples was determined by Unicam, SP500 spectrophotometer (Unicam Instruments Ltd., Cambridge, England). The apparent digestibility of the nitrogen in each diet was then determined from the following formula:

$$\text{Apparent Digestibility, \%} = 100 - \left(100 \times \frac{Cr_2O_3 \text{ in diet, \%}}{Cr_2O_3 \text{ in faeces, \%}} \times \frac{\text{N in faeces, \%}}{\text{N in diet, \%}}\right)$$

Values obtained in this manner were used to estimate the theoretical quantity of nitrogen voided in the faeces per day by multiplying the quantity of nitrogen consumed per day by the apparent digestibility of nitrogen for each diet. The difference between this value and the total weight of faecal nitrogen collected per day was taken to be the soluble

faecal nitrogen fraction. This value was then substracted from the total soluble nitrogen value (nitrogen excreted + soluble faecal nitrogen) and added to the faecal nitrogen figure to give corrected values for these quantities.

True digestibility of ingested nitrogen was calculated from the following equation:-

$$\text{True Digestibility, \%} = \frac{I - (FN - MFN)}{I} \times 100$$

where I = Rate of nitrogen intake, mg/100 g fish/day

FN = Rate of faecal nitrogen output, mg/100 g fish/day

MFN = Rate of metabolic faecal nitrogen output, mg/100g fish/day

Bacterial action on the waste products during accumulation has been shown to be negligible over a 24 hr period in both fresh water (Gerking, 1955b) and sea water (Wood, 1958). These authors have also shown that negligible quantities of nitrogen-containing materials are lost from aerated water during the same time period.

Three replicate determinations were made for each of the following parameters at each dietary level in each salinity: total nitrogen excreted, total faecal nitrogen and total nitrogen retained. Where valuable, statistical comparisons between two groups of triplicate determinations were undertaken using either Student's 't' test or the Fisher-Behrens test depending on the significance of the variance ratios.

7.4. Results

Digestibility and Metabolic Faecal Nitrogen

Apparent digestibilities of dietary nitrogen in diets containing different protein levels are shown in Table 44. The range in the apparent digestibilities was very small being 89.3 to 90.8. Apparent digestibility is shown in Figure 43 plotted against the weight of dietary nitrogen consumed per 100 g. of fish per day. Lines of best fit were drawn for the three sets of data using the Least Squares method. Lines for fresh water and 10 ppt sea water were superimposed. Linear relationships were evident for all three sets of data and the significance of correlation was high in each case ($p < 0.01$ for fresh water and 10 ppt; $p < 0.001$ for 20 ppt.). The gradients and positions of the three lines were compared for significance using the method outlined in Wetherill (1972) together with Student's 't' test on paired data. No significant difference between the gradients of any two lines could be demonstrated. No significant different in the position of the fresh and 10 ppt lines was found. However, the position of the 20 ppt line was significantly lower ($p < 0.05$) than that of fresh water and 10 ppt. Thus, it appears that a salinity of 20 ppt does slightly reduce the apparent digestibility of dietary nitrogen. In all salinities apparent digestibility increased slightly with increase in dietary nitrogen intake.

Apparent digestibility values were used to compute the mean soluble faecal nitrogen values as described in the previous section. Soluble faecal nitrogen loss over a 25 hr period was found to be 17.0% of the determined faecal nitrogen output in fresh water and 10 ppt sea water and 19.3% in 20 ppt sea water independent of protein intake. From these values together with the determined faecal nitrogen values, total

Table 44. Apparent and true dietary nitrogen digestibilities of diets of different protein contents

Salinity	Diet No.	Mean Total Faecal Nitrogen mg/100g fish/ day [a.]	± Standard Error	Apparent Digestibility, %	True Digestibility, %
Fresh Water	1	9.2	± 0.057	90.8	91.8
	2	7.9	± 0.100	90.8	91.9
	3	6.4	± 0.153	90.6	92.0
	4	4.9	± 0.100	89.7	91.8
	5	3.7	± 0.100	89.8	92.4
10 ppt Sea Water	1	9.2	± 0.115	90.8	91.3
	2	7.9	± 0.153	90.8	91.4
	3	6.4	± 0.115	90.6	91.3
	4	4.9	± 0.100	89.7	90.8
	5	3.7	± 0.155	89.8	91.0
20 ppt Sea Water	1	9.3	± 0.057	90.7	91.6
	2	8.3	± 0.378	90.3	91.4
	3	6.9	± 0.151	89.9	91.2
	4	5.1	± 0.113	89.3	91.3
	5	4.0	± 0.321	89.7	91.4

a. Means are of 3 replicate determinations

Diets are ranked in order of decreasing protein content; diet 1 is the highest containing 60% protein

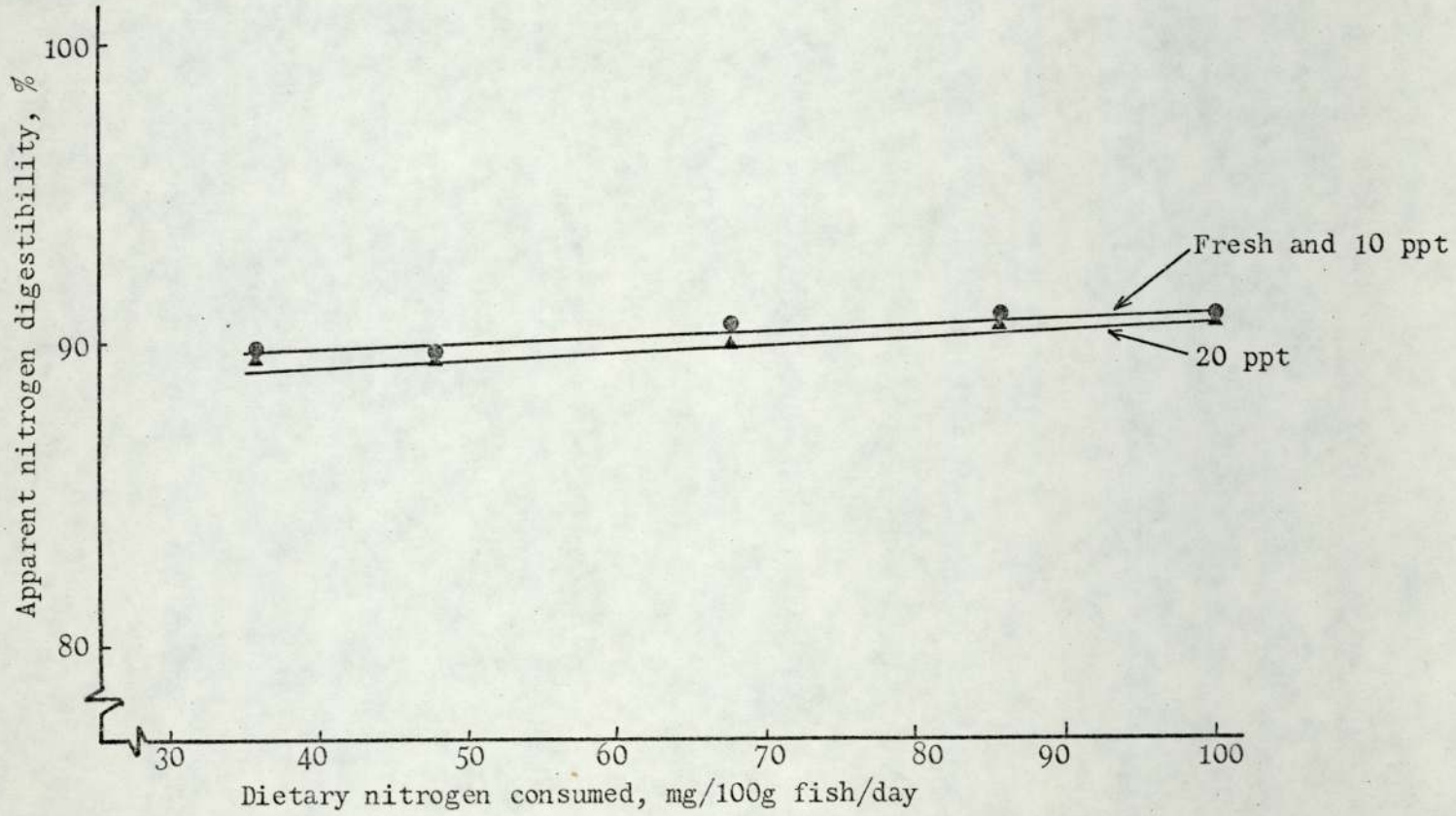


Fig.43. Variation in apparent digestibility of dietary nitrogen with increasing nitrogen intake rate in fresh water ● , 10 ppt seawater ● and 20 ppt seawater, ▲. Each point is a mean of 3 values

faecal nitrogen was estimated. These values are shown in Table 44. Plotting mean total faecal nitrogen output against rate of dietary nitrogen consumed was undertaken in the manner of Nose (1967) in order to determine metabolic faecal nitrogen by derivation methods (Figure 44). Straight lines were fitted to the three sets of data using the Least Squares method (significance of correlation - $p < 0.001$ for each set of data) and the lines extrapolated downwards to intercept the ordinate of the graph. These points were of interest as it was taken that metabolic faecal nitrogen is equal to the faecal nitrogen loss at zero nitrogen intake. Metabolic faecal nitrogen was found to be 97 mg/100g dry diet, 48 mg/100g dry diet and 91 mg/100g dry diet for fresh water, 10 ppt and 20 ppt sea water fish respectively. The gradients and positions of the three straight lines were compared for significance using the methods mentioned earlier and no significant differences were found between any two lines. Hence, the differences in metabolic faecal nitrogen values is probably spurious. Nevertheless, the above values were used in NPU computations.

Values obtained for metabolic faecal nitrogen at each salinity were used to determine values for true digestibility of ingested protein (Table 44) using the equation given in the previous section. These values were plotted graphically against dietary nitrogen consumption rate in Figure 45. Straight lines were fitted to the three sets of data using the Least Squares method. The gradient of all three lines was almost zero indicating that the true digestibility of ingested nitrogen did not vary significantly with the level of intake of nitrogen. This true digestibility value was approximately 91.5%.

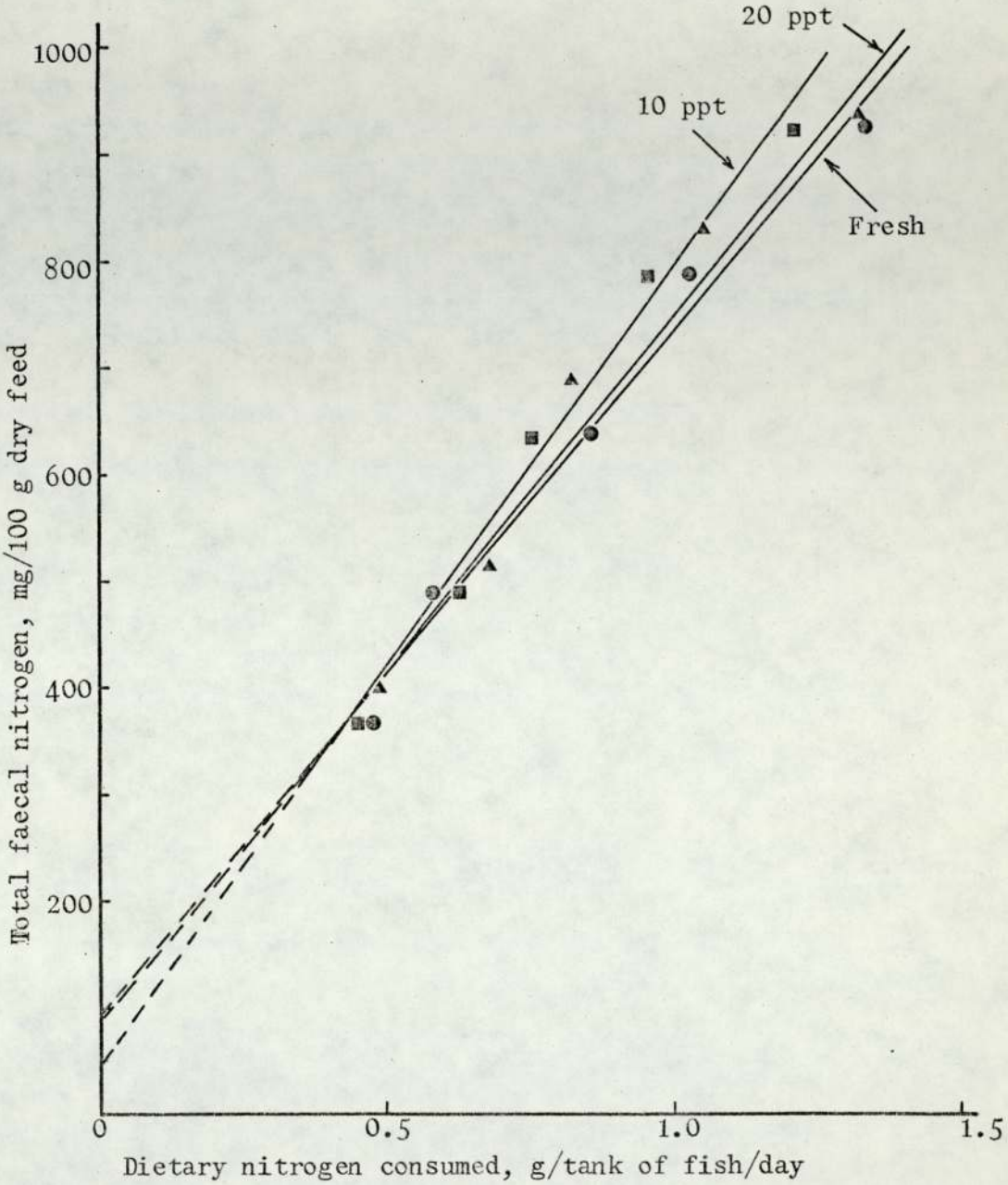


Fig.44. Variation in total faecal nitrogen voided with increasing dietary nitrogen intake in fresh water ●, 10 ppt sea water ■, and 20 ppt seawater, ▲. Each point is a mean of 3 determinations

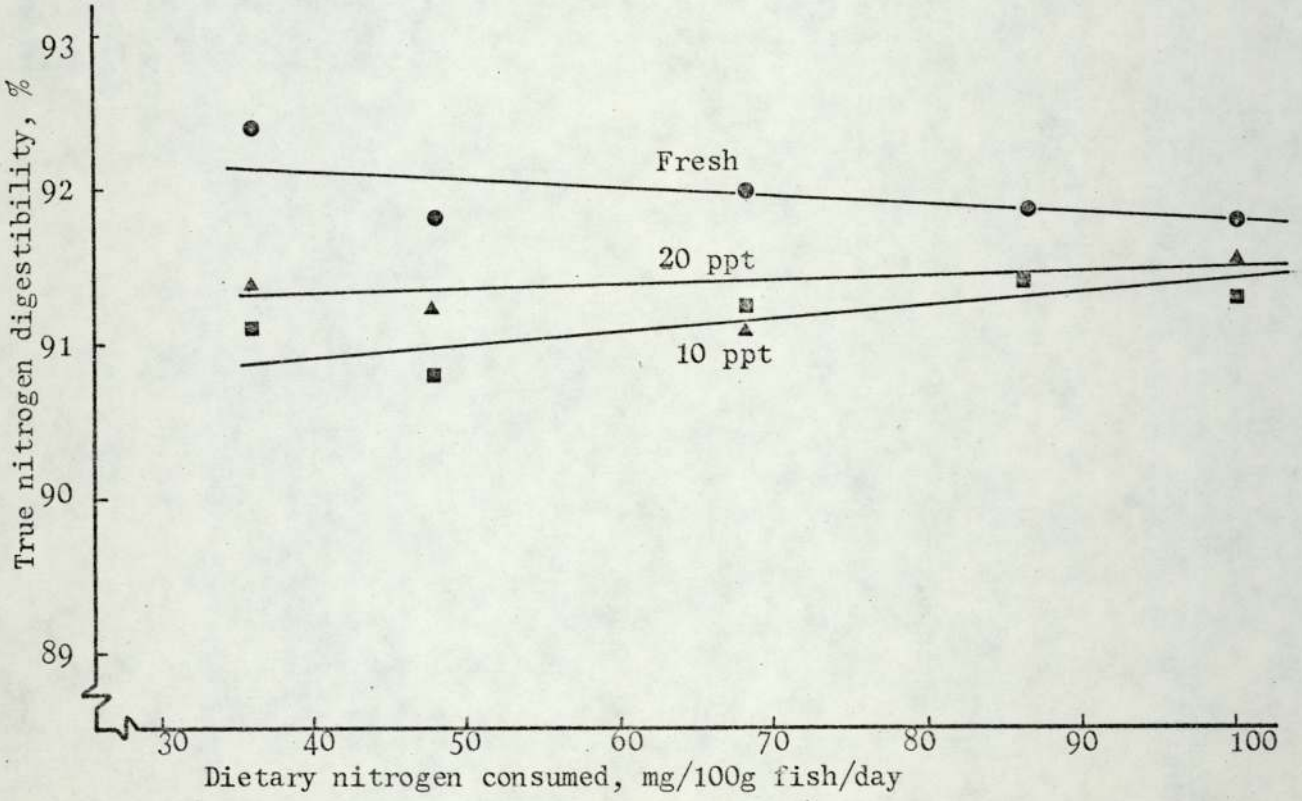


Fig.45. Variation in true digestibility of dietary nitrogen with increasing nitrogen intake rate in fresh water ● , 10 ppt sea water ■ , and 20 ppt sea water ▲ . Each point is a mean of 3 determinations

Using Student's 't' test on paired data it was demonstrated that the true digestibility of dietary protein was significantly higher ($p < 0.01$) for fresh water than for 10 and 20 ppt sea water. No significant difference was demonstrated between 10 and 20 ppt sea water lines.

Non-Faecal Nitrogen Excretion

Total nitrogen excreted as ammonia, urea, mucus-nitrogen and other nitrogenous compounds of minor importance is shown in Table 45. Each value is the mean of three separate test runs and each has been corrected for soluble faecal nitrogen. There was a direct relationship between protein level in the diet and nitrogen excretion at all salinities. Non-faecal nitrogen excretion was significantly lower in 10 ppt sea water than in 20 ppt or fresh water with the exception of the lowest protein containing diet, number 5. There was no significant difference in non-faecal nitrogen excretion between fresh and 20 ppt sea water. Relevant probability values are given in Table 45.

Total non-faecal nitrogen excreted per day by each tank of fish is plotted against dietary nitrogen consumed in Figure 46. All three curves produced were similar in overall shape but different in actual position on the graph. Each curve was taken as having two halves, a left-hand linear portion, and a right-hand curvi-linear portion. The linear portions were fitted to each group of three points by the Least Squares method. Correlation coefficients were high being 0.995, 0.988 and 0.995 for fresh water, 10 ppt and 20 ppt sea water respectively and correlation was highly significant ($p < 0.01$, $p < 0.02$, $p < 0.001$ respectively). The curvilinear portions of each curve were fitted to the data by eye. Each of the straight lines was extrapolated back to the ordinate and the intersection points noted. These points were

Table 45. Non-faecal nitrogen excretion of fish fed different levels of dietary protein

Diet	Salinity	Mean total nitrogen[a] Excreted per day mg/100 g fish	± Standard Error of Mean	Probability
1	Fresh	62.2	± 0.305	p <0.001
	10 ppt	59.0	± 0.153	p<0.01
	20 ppt	61.7	± 0.462	
2	Fresh	49.2	± 0.300	p <0.01
	10 ppt	47.1	± 0.265	p<0.01
	20 ppt	49.2	± 0.321	
3	Fresh	36.8	± 0.400	p <0.02
	10 ppt	34.9	± 0.252	p<0.01
	20 ppt	37.5	± 0.208	
4	Fresh	30.6	± 0.557	p <0.01
	10 ppt	26.5	± 0.200	p<0.01
	20 ppt	31.1	± 0.850	
5	Fresh	25.2	± 0.755	Not signif.
	10 ppt	23.9	± 1.365	Not signif.
	20 ppt	25.6	± 0.666	

a Means are of 3 replicate determinations

Note - Diets are ranked in order of decreasing protein content; diet 1 is the highest containing 60% protein

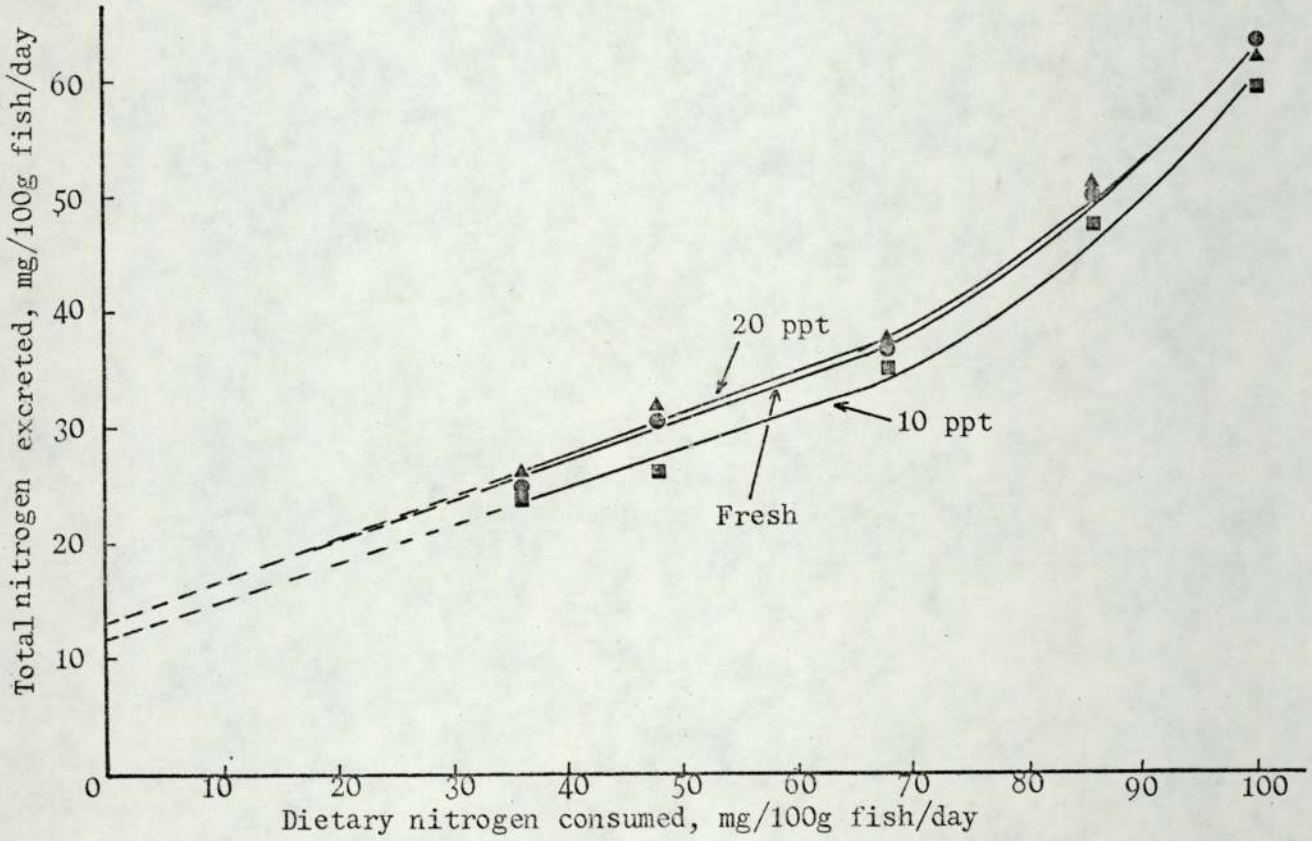


Fig.46. Variation in total nitrogen excretion with increasing nitrogen intake rate in freshwater ● , 10 ppt sea water ■ , 20 ppt sea water ▲ .
Each point is a mean of 3 determinations

taken as representative of the endogenous nitrogen excretion of fish at that particular salinity. Here, endogenous nitrogen excretion was taken as the nitrogen excretion rate of fish fed a nutritively complete diet which was absent in protein. Endogenous nitrogen excretion values were similar in fresh water and 20 ppt sea water being 12.9 and 12.8 mg/100g fish/day respectively. A lower value was found in 10 ppt sea water this being 10.6 mg/100g fish/day. These values were subsequently used in computing NPU's.

Differences between the lines were examined using the methods outlined earlier. No significant difference between the gradients of any two lines was found (examining only the left-hand curves). No significant difference was demonstrated between the position of the freshwater and 20 ppt lines (examining the entire curves). However, the 10 ppt sea water line was significantly lower ($p < 0.01$) than the fresh water and 20 ppt lines. Hence, the difference between the two endogenous nitrogen excretion values of 12.8 and 10.6 is probably a real difference.

Nitrogen Retained and Net Protein Utilisation

Following the basic theory behind nitrogen balance, the nitrogen retained (+ve balance) was computed at each protein level in each salinity by subtracting from the nitrogen intake per day the mean faecal nitrogen losses and mean non-faecal nitrogen losses. The results of these calculations are shown in Table 46. Each value is the mean of three estimates. There was a direct relationship between protein level in the diet and nitrogen retention in all salinities. Nitrogen retention was significantly higher in 10 ppt sea water than in 20 ppt or fresh water with the exception of the lowest protein containing diet, number 5.

Table 46. Estimated nitrogen retention and net protein utilisation at different protein levels in different salinities

Diet	Salinity	Nitrogen Retained[a] mg/100g fish/day	±	Standard Error	Probability	NPU,%
1	Fresh	28.6	±	0.252	p<0.001	42.5
	10 ppt	31.8	±	0.252	p<0.01	42.9
	20 ppt	29.0	±	0.258		42.7
2	Fresh	28.9	±	0.265	p<0.01	49.7
	10 ppt	31.0	±	0.361	p<0.01	48.9
	20 ppt	28.5	±	0.300		49.1
3	Fresh	24.8	±	0.551	p<0.05	57.6
	10 ppt	26.7	±	0.153	p<0.001	55.6
	20 ppt	23.6	±	0.153		54.9
4	Fresh	12.5	±	0.681	p<0.01	54.9
	10 ppt	16.6	±	0.208	p<0.02	57.7
	20 ppt	11.8	±	1.167		53.2
5	Fresh	7.1	±	1.457	Not Signif.	58.2
	10 ppt	8.4	±	0.737	Not Signif.	54.1
	20 ppt	6.4	±	0.528		55.9

a Means are of 3 replicate estimates

Note - Diets are ranked in order of decreasing protein content; diet 1 is the highest containing 60% protein

There was no significant difference in nitrogen retention between fresh and 20 ppt sea water. Relevant probability values are given in Table 46.

Nitrogen retained plotted against protein level in the diet was undertaken as described in the previous section and three dose-response curves were produced (Figure 47). The left-hand portions of each curve were fitted to the data by using the Least Squares method. Correlation coefficients were high being 0.999, 0.996 and 0.998 for fresh water, 10 ppt and 20 ppt sea water respectively and correlation was highly significant ($p < 0.01$) in all cases. These straight lines were extrapolated down to intersect the abscissa. These points of intersection were taken to be the maintenance protein requirement at that salinity. These values were 24.5 mgN/100g fish/day for fresh water, 20.4 for 10 ppt sea water and 24.9 for 20 ppt sea water. Differences between the lines were examined using the methods outlined earlier. No significant differences between the gradients of any two lines were found (examining only the left-hand curves). No significant difference was demonstrated between the positions of the fresh water and 20 ppt sea water lines (examining the entire curves). However, the 10 ppt sea water line was significantly higher ($p < 0.01$) than the fresh water and 20 ppt lines. Hence, the difference between the two maintenance nitrogen values of 24.9 and 20.4 is probably a real difference.

Maintenance nitrogen values were converted to a percentage protein in the diet basis by multiplying by a factor of 0.625. Thus maintenance protein values of 15.3%, 12.8% and 15.6% are obtained for fresh water, 10 ppt and 20 ppt sea water respectively.

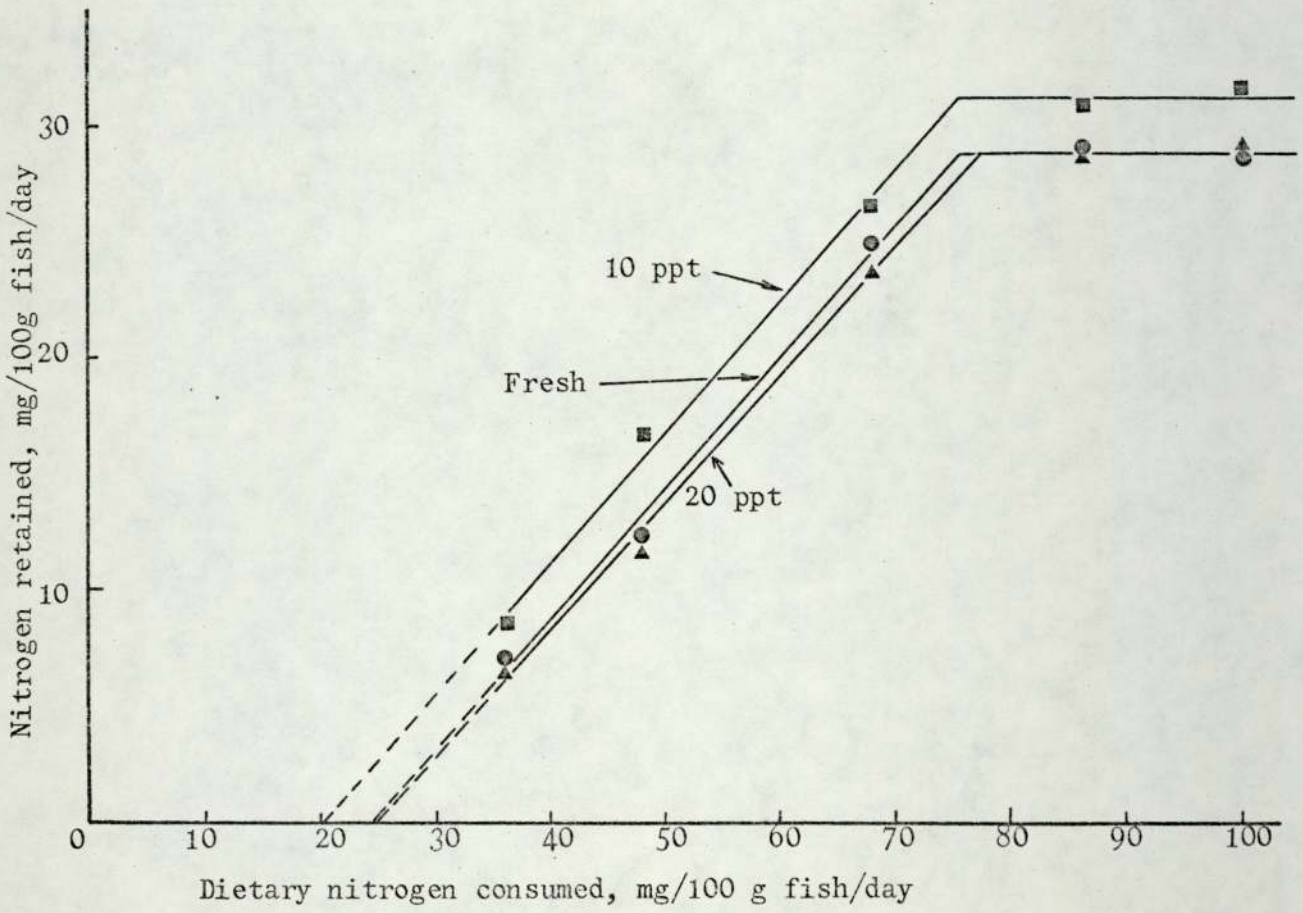


Fig.47. Dose-response curves of nitrogen retention against nitrogen intake rate in fresh water ● , 10 ppt sea water ■ , 20 ppt sea water, ▲ . Each point is a mean of 3 estimates.

Minimum nitrogen levels in the diet needed to promote maximum growth were taken from the intersection points of the ascending and horizontal portions of each curve. Figures were the same for fresh water and 10 ppt sea water being 75.5 mgN/100g fish/day. Figures were a little higher for 20 ppt sea water, 77.5 mgN/100g fish/day. It is not believed that these figures are significantly different. These figures were converted to a percentage protein in the diet basis by multiplying by a factor of 0.625. Thus optimum dietary protein levels of 47.2% and 48.4% were found for fresh water / 10 ppt and 20 ppt sea water respectively.

Net protein utilisation values were calculated for each dietary protein level in each salinity. Values are shown in Table 46. In all salinities NPU was inversely related to protein intake. Values were generally highest (54.1 - 58.2%) at the lowest level of protein intake and lowest (42.5% - 42.9%) at the highest level of protein intake. No obvious difference in NPU values was observed between salinities except at the lower levels of protein intake where NPU's were lower for 20 ppt than fresh water. Thus, although dietary protein level had a marked influence on NPU values, salinity did not exert such a pronounced effect. NPU's have been shown graphically in Figure 48.

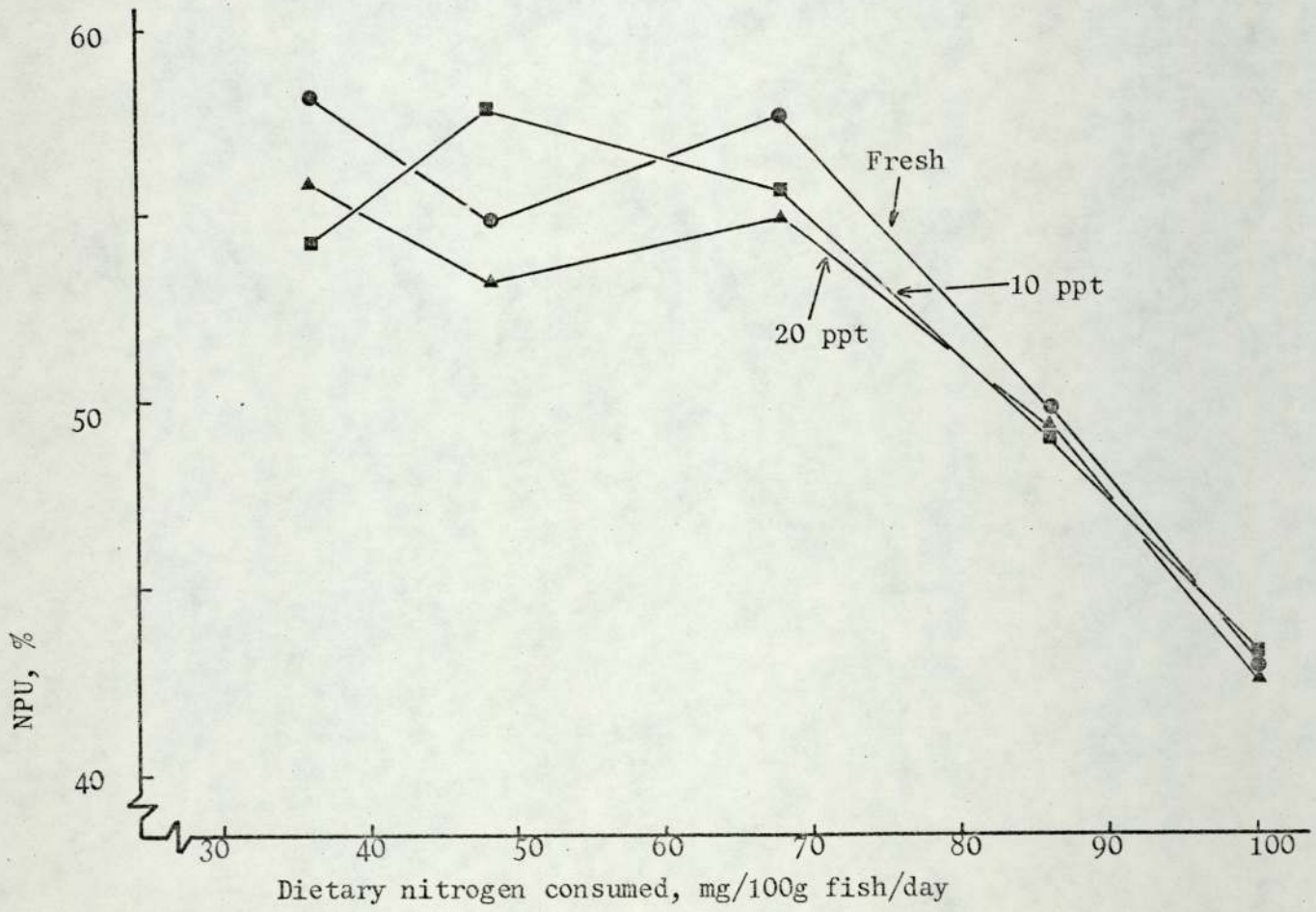


Fig.48. Variation in NPU with increasing nitrogen intake rate in fresh water ● , 10 ppt sea water ■ , and 20 ppt sea water ▲ .

7.5. Discussion and Conclusions

The general increase in the apparent digestibility of protein with the increase in protein level in the diet (Figure 43) is in agreement with results found by Nose (1967) with rainbow trout and Ogino and Chen (1973a) with carp although all these authors found more of a decrease in apparent digestibility at the lower protein levels. It has been suggested that this phenomenon is caused by the disregard of metabolic faecal nitrogen when calculating apparent digestibility. This theory is supported when true digestibility values are plotted against protein intake (Figure 45) and straight lines which are almost horizontal are produced. The true digestibility of dietary protein at all salinities (91.5%) is in good agreement with results found by previous workers (Nose, 1967, 91.9% for white fish meal; Ogino et al., 1973a, 92.7% for commercial trout pellets) and it was considered that the figure used in computing metabolisable energy values for protein, i.e. 90% digestibility was justified.

One of the values found for metabolic faecal nitrogen (48 mgN/100g food consumed for 10 ppt sea water) fell just outside the range quoted by Nose (1967) which was found for rainbow trout; this was 50 - 150 mgN /100 g food consumed. However, the two other values found for fresh water and 20 ppt sea water were well inside this range and it was considered that the differences found in metabolic faecal nitrogen values between salinities were spurious. This was because the three straight lines (Figure 44) were not significantly different from each other in either position or gradient. However, the positive conclusion can be drawn from these results that metabolic faecal nitrogen is constant regardless of dietary protein level as is evidenced by the straight lines of

Figure 44. This is in agreement with results obtained by Mitchell and Bert (1954) with rats and Nose (1967) and Ogino, Kakino and Chen (1973) with fish. Thus, it appears that even though dietary fibre increased from diets 1 to 5 this had no effect on the true digestibility of the protein in the diets or on the metabolic faecal nitrogen values. Also, the increase in dietary starch from diets one to five could not have been large enough to elicit a decrease in protein digestibility as found by Kitamikado, Morishita and Tachino (1964). Although there were some significant differences between salinities with regard to both apparent and true digestibility of dietary nitrogen, the overall differences were slight on all occasions. However, it is interesting to note that salinity can induce small changes in digestibility possibly through changes in the moisture and ion content of the gut brought about by sea water ingestion.

The increase in nitrogen excretion rate with the increase in nitrogen consumption is in agreement with the conclusions reached by Paloheimo and Dickie (1966b) and Kausch (1969) and the results of Birkett (1969) and Savitz (1971b). However, a slight difference was apparent in the shapes of the curves obtained in the latter two publications and the curves obtained in the present experiment. Birkett (1969) found a linear relationship between nitrogen excretion and nitrogen absorption. Savitz (1971b) found a curvilinear relationship between nitrogen excretion and nitrogen consumption with the rate decreasing as consumption increased. In the present experiment above the 40% protein level, the rate of nitrogen excretion gradually increased (Figure 46). This was undoubtedly due to the fish reaching their maximum rate of protein synthesis at about the 40% protein level (diet 3) and any protein ingested which was in excess of this capacity for synthesis was excreted. Thus

as the amount of protein ingested increased above the 40% level, increasingly more was deaminated and excreted as waste nitrogenous materials.

It was considered appropriate to derive endogenous nitrogen excretion values from the graph of nitrogen excretion against nitrogen intake. Some authors (Birkett, 1969; Gerking, 1971) have derived ENE values from graphs of nitrogen retention against nitrogen intake by maintaining the position of the 'y' intercept is equivalent to ENE rate. While this is undoubtedly justified it was considered that deriving ENE values from nitrogen excreted/nitrogen intake figures would only draw upon one set of experimental errors those being introduced in the measurements of total nitrogen excretion. Deriving ENE values from nitrogen retained/nitrogen intake figures would have drawn upon two sets of experimental errors inherent in the determination of nitrogen retained these being introduced in the measurements of both nitrogen excreted and faecal nitrogen.

Values obtained for the endogenous nitrogen excretion rates of various fish vary a lot depending upon environmental conditions (Table 47). Three factors will affect the value of the ENE rate; water temperature, fish weight and the method of determination. Savitz (1969) found that ENE rate and temperature were directly proportional. Gerking (1955b) found ENE rate was logarithmically related to body weight in fish as it is in higher animals. Finally, the ENE rate of a starved fish (cf. Tunison et al., 1942; Fromm, 1963) will probably be lower than that of a comparable fish fed a glucose diet. This has been demonsirated by Savitz (1971a) and is due to the reduction in total metabolic rate of the fish with starvation together with an increase in the proportion of body fat respired for energy. Thus, extreme caution must be used when comparing ENE rates between various

Table 47. Endogenous nitrogen excretion rates of fish

Author	Species	Fish Weight g	Temperature °C	Endogenous Nitrogen Excretion mg/100g fish/day
Gerking* (1955b)	L. macrochirus	100	25.9	12.60
Savitz (1969)	L. macrochirus	100	23.9	11.54
		100	15.6	4.85
		100	7.2	5.55
Ogino et al. (1973)	Carp	78 - 370	20	7.20
		133 - 215	27	8.60
Tunison et al. (1942)	S. fontinalis	?	10.5	5.4
Fromm (1963)	S. gairdneri	129	12 - 14	13.6
Birkett (1969)	Pleuronectes platessa	0.171-33.64	17	5.6 - 20.8
	Solea vulgaris	0.248-56.6	17 ?	
	Perca fluviatilis	100-152.4	17	
Present Experiment	S. gairdneri	120	12	10.6 - 12.9

* Interpreting Gerking (1955b; Fig.2, p.287)

publications. Nevertheless, the values found in the present experiment are of the right order of magnitude and fall within the range of values reported.

Lower nitrogen excretion rates in 10 ppt sea water compared with fresh water and 20 ppt sea water is in good agreement with previous results concerning metabolic rates. It appears that the reduction in total metabolic rate induced by 10 ppt sea water elicits a concomitant reduction in the rate of protein catabolism which is reflected in the reduction in total nitrogen excretion. The lower ENE values in 10 ppt sea water also indicate reduced nitrogen catabolism and reduced maintenance protein requirements.

No attention has been paid to the output of muco-proteins by fish in the present experiment. Gerking (1952) regards mucus as an excretory product and not a component of faecal nitrogen. No practicable methods could be undertaken in the present experiment for estimating mucus nitrogen. Thus, nitrogen excreted in mucus was included in the measurement of total nitrogen in water.

When fitting curves to the nitrogen retained/nitrogen consumed data (Figure 47) it was decided to use discontinuous response curves which consisted of two linear portions each, one with a zero gradient and the other with a positive gradient. This type of dose-response curve has been used widely in nutritional studies (Hegsted, 1948; Delong, Halver & Mertz, 1958; Hegsted & Chang, 1965; Almquist, 1970; Ogino & Saito, 1970; Zeitoun, Halver, Ullrey & Tack, 1973). However, the use of this type of curve has been criticised by Cowey, Pope, Adron and Blair (1972) who would prefer to see a continuous response curve fitted to nitrogen gain/nitrogen intake data. Nevertheless, in the

present experiment a discontinuous response curve consisting of two linear sections was used at each salinity so that the dose-levels of nitrogen in the diet which resulted in both zero and maximum rate of nitrogen retention could be determined. This method assumes that nitrogen retention and intake are directly proportional until the point where maximum protein synthesis occurs. Here, inflexion of the curve occurs.

Nitrogen consumption levels resulting in maximum growth were very similar at all three salinities. If these values are converted to a percentage protein in the diet basis, values of 47.2 - 48.4% emerge. It is not probable that the difference between the highest and lowest values obtained is significant using these methods. The actual values are 2 - 3% units higher than is usually quoted as the optimum protein level for trout. (Halver, Bates & Mertz, 1964). A part of this overestimate will be caused when converting nitrogen to protein values by using the factor of 6.25. This assumes that all the nitrogen is present as protein and so will result in an overestimate of true protein by including non-protein nitrogen, e.g. amino acids. Also, protein requirement is dependent on temperature (Delong, Halver & Mertz, 1968) with optimum requirements rising as temperature increases. Thus, it is probable that protein requirements will be higher at 12°C, the temperature of the water in the present experiment, than at 10°C, the water temperature in Halver's et al (1964) experiment.

An observable difference occurs in the maintenance protein requirement between different salinities. This value represents that amount of nitrogen which is required to balance protein used for the energy costs of activity, digesting food, and other aspects of metabolism.

In agreement with the results of Chapter 4 it was found that maintenance protein requirements were least in 10 ppt sea water (12.8%) and greatest in fresh water and 20 ppt sea water (15.3%). Again it appears that the energy required for the processes of osmotic and ionic regulation is least in 10 ppt sea water. As a proportion of this energy will be derived from dietary protein it is to be expected that maintenance protein requirement will be least in 10 ppt sea water. Equal maintenance protein requirements in fresh water and 20 ppt sea water confirm that protein is used in similar quantities for osmotic and ionic regulation in these two salinities.

While the values obtained for maintenance protein requirement agree well with those found by Zeitoun et al. (1973) who also investigated the protein requirements of rainbow trout in different salinities one difference is apparent in that their fish showed faster growth in 20 ppt than 10 ppt sea water above the 40% protein level in the diet. No explanation is offered to account for this difference.

At all salinities, maintenance nitrogen requirements were found to be approximately twice that value found for endogenous nitrogen excretion in different salinities. This is in agreement with several workers who have found ENE rates are conservative measures of maintenance nitrogen requirements (Birkett, 1969; Savitz, 1969, 1971b; Gerking, 1971). The reason for this is that the maintenance nitrogen is that quantity in the diet which is required to satisfy the entire requirements of protein catabolism with nil growth. This value will depend on the endogenous nitrogen excretion, that quantity of nitrogen excreted at a constant rate from the continual catabolism of tissue proteins and that quantity of dietary nitrogen which must be catabolised

in order to satisfy the endogenous requirement. This latter quantity is directly dependent on the biological value of the protein fed (Allison, 1957). This has been demonstrated in carp by Ogino and Chen (1973b) who showed that maintenance nitrogen requirements increased as the biological value of the dietary protein decreased. Thus, maintenance nitrogen requirement will always be greater than the ENE rate.

The utilisation of dietary protein is affected by a number of factors which include total energy intake, the protein to calorie ratio of the diet and the environmental conditions. In the present experiment, the latter two factors have varied in an attempt to determine any interaction of salinity with protein level in the diet (protein/calorie ratio). From the data in Table 46 and Figure 48 it can be seen that net protein utilisation by rainbow trout decreases, generally, with increasing dietary protein level. This is in agreement with results obtained by Miller and Payne (1961) working with growing rats, Ogino and Saito (1970) with carp and Cowey, Pope, Adron and Blair (1972) and Cowey, Adron, Brown and Shanks (1975) with plaice, Pleuronectes platessa. The reason for this decrease in NPU with increase in protein level is that progressively more of the protein ingested is being used as an energy source. This is reflected in the increase in nitrogen excretion rates with increase in protein level (Figure 46). It is not clear from the data whether the relationship between NPU and protein level is linear as proposed by the above authors.

Values obtained in the present experiment fall within the wide range determined for plaice by Cowey et al (1972). Comparable values include the following:- at the lowest level of protein intake, the

NPU value was 54.1-58.2% in the present experiment compared to 60.9% found by Cowey et al.(1972) at similar protein intake. At the highest level of protein intake the NPU value was 42.5%-42.9% in the present experiment and 39.8% in Cowey's experiment at similar protein intake. Thus, in the present experiment NPU values comparable with those of Cowey et al. (1972) were found.

Values for NPU in different salinities were very similar for fish fed on diets 1 and 2. No observable difference was apparent between salinities at the other levels of protein intake apart from lower values found for 20 ppt fish compared to fresh water fish at the 40%, 30% and 20% dietary protein levels. As no difference was observed in either nitrogen retention or excretion at these dietary protein levels the difference in NPU's must be due to the slightly lower apparent absorption efficiency comparing 20 ppt sea water to fresh water.

In conclusion, successful nitrogen balance studies were undertaken on trout held in fresh water, 10 ppt sea water and 20 ppt sea water and fed at five different rates of protein intake. The chief observation was that a salinity of 10 ppt sea water resulted in a lower endogenous nitrogen excretion rate and maintenance protein requirement than was seen in 20 ppt sea water and fresh water. This had the effect of increasing nitrogen retention in the former salinity at all levels of protein intake. Overall net protein utilisation was unaffected by salinity. Thus, salinity of low values elicits a lessening of the maintenance energy requirements by lowering the energy required to maintain osmotic and ionic equilibrium. This is reflected directly in many of those parameters determined in the present experiment, in particular growth and nitrogen retention which are enhanced.

Chapter 8. FINAL CONCLUSIONS

The main thesis with which this investigation was initiated was whether salinity *per se* was capable of eliciting a change in the growth rate of rainbow trout. It has been demonstrated that a salinity of 10 ppt causes a lowering of the total metabolic rate together with a decrease in the maintenance energy and protein requirements which together result in an increase in the growth rate and gross growth efficiency in this salinity. A salinity of 28 ppt caused an increase in the total metabolic rate of trout together with a decrease in the apparent absorption efficiency of energy which resulted in a decrease in growth rate. No differences in many parameters were observed between freshwater and 20 ppt fish. Thus, in the present experiment different salinities were capable of producing different physiological effects.

It was inferred that the reason for these changes in metabolic rates and maintenance energy requirements was that alterations in the energy devoted to osmotic and ionic regulation caused by different environmental salinities were reflected in the changes in metabolic rates. Whilst this view is possibly simplistic (Parry, 1964) it does account for the majority of the observations found in the present series of experiments. As well, the theory receives credence from other sources (Rao, 1968, 1971; Brett, 1974).

While the main conclusion has been that selected salinities can influence growth rate in the laboratory it is probable that such effects would not be observable in the majority of farming situations. This is because the 10 ppt seawater situation is rarely found in the industry where usually coastal salinity values range between 20 and 34 ppt.

In addition, the effects of fluctuating seawater temperatures and supplementary food occurring as plankton would help to mask any changes in growth rate effected by salinity.

The present experiments would suggest that the increases in growth caused by a salinity of 10 ppt are probably too small to warrant large-scale control of salinity in the fish farming situation. Strict control of water salinity would be expensive as land-based tanks with both freshwater and seawater inflows would be necessary. It is probable that the small increase in growth afforded by low salinities could not justify this sort of capital expenditure by itself. Thus it is considered that at the present time, achieving optimum salinities for growth should be secondary in importance to achieving optimum temperature and dietary conditions. With regard to the latter point, it is conceivable that a small reduction in protein content of the diet could be managed with no loss in growth performance if constant low salinity conditions were to be achieved.

Two exceptions to the above general arguments are immediately apparent. The first lies in the observation that selected salinities apparently affect the voluntary food consumption of trout to different degrees depending on the time of year. It would be of value to undertake a long-term study over a year to investigate the duration and extent of the enhanced appetite response. If this were to be significant then the increases in growth rate caused by increased food intake in 10 and 20 ppt may well be large enough to justify strict salinity control.

Secondly, a limited application of salinity control may rest with the rearing of small trout for a short period. Trout as small as

10 - 20 g can tolerate successfully intermediate salinities (Zeitoun et al., 1973) and at this stage are fast growers. The lessening of maintenance energy requirements caused by low salinities together with the low water and space requirements for individual fish may make environmental control economically feasible (Brett, 1974). Thus the application of salinity control may well be a useful tool in fish farming in the future.

APPENDIX 1. Body weight and metabolism

Generally, the logarithm of oxygen consumption, T, is linearly related to the logarithm of body weight, W (Beamish & Dickie, 1967) thus,

$$T = a\ W^{b}$$

or

$$\log T = \log a + b \log W$$

where 'a' and 'b' are constants, 'a' being the level of metabolism and 'b' being the weight exponent. Winberg (1956, 1961) in extensive reviews concluded that under standard conditions particular values for 'a' and 'b' characterise almost all fish species.

The level of metabolism, 'a', appears to be markedly affected by changes in a variety of conditions relating both to the fish and its environment. These conditions include swimming activity, temperature, food consumption, digestibility of food, species of fish and many more. Usually the values for 'a' range between 0.1 and 0.3.

The weight exponent, 'b' has been found to lie midway between that value which would result in surface proportionality (0.67) and that value which would result in weight proportionality (1.0). A value of 0.8 appears to give adequate description to the relationship between weight and metabolism among the majority of fishes under reasonably constant conditions (Paloheimo & Dickie, 1966a). However, it may vary slightly with temperature or with swimming activity (Brett, 1965).

Winberg (1956, 1961) postulated that the basic energy balance equation,

$$p\ R = T + \frac{\Delta W}{\Delta t}$$

where R = Energy of ration

p = Assimilation coefficient

T = Energy dissipated in metabolism

ΔW = Energy retained as growth in time, Δt

could be used to derive an index of 'routine' metabolism which was

$$T = pR - \frac{\Delta W}{\Delta t}$$

Estimates of routine metabolism derived from this equation agreed very well with actual determinations of oxygen consumption under similar experimental conditions. This agreement between the results of respiration experiments which are generally run for a few hours and the results derived from laboratory feeding experiments which may last several weeks gave assurance that both determinations were reflecting features of a long-term energy budget.

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