is drawn to the fact that the
his thesis rests with its author.
of the thesis has been supplied
that anyone who consults it is
recognise that its copyright rests
or and that no quotation from
no information derived from it
shed without the author's prior
t.

EFFECTS OF SALINITY AND DIET ON THE NUTRITIONAL PHYSIOLOGY AND ALIMENTARY CANAL HISTOLOGY OF THE RAINBOW TROUT

by

MURDO GORDON MacLEOD

A thesis submitted for the degree of Doctor of Philosophy

UNIVERSITY OF STIRLING
Department of Biology

1975

ACKNOWLEDGEMENTS

I wish to thank Professor F.G.T. Holliday for suggesting this research project and for his continued interest in it as my supervisor. I am indebted to Dr. R.J. Roberts for access to the histological facilities and expertise of the Nuffield Aquatic Pathobiology Unit. The value of discussions with fellow research students and others at Stirling and at the SMBA Laboratory, Dunstaffnage, is gratefully acknowledged. Dr. P. Tytler is to be thanked for reading, and commenting on, the manuscript of this thesis. Finally, I record my sincere gratitude to my wife, Penny, for her encouragement and patience during the course of this study.

ABSTRACT

In rainbow trout (Salmo gairdneri Richardson) which had been acclimatized to the experimental salinity and temperature for at least 14 days, food intake was maximal in the intermediate salinities of 15.0 and 28.0 p.p.t. (parts per thousand), less in fresh water and 7.5 p.p.t. and minimal, by a statistically significant margin, in 32.5 p.p.t. sea water. There were marked day-to-day fluctuations in food intake.

Upon abrupt salinity increases of 7.5 or 13.0 p.p.t., there were decreases in growth rate which were related to decreases in food intake. Recovery of food intake and growth rate to pre-increase levels was complete within 14 days.

Absorption efficiency, in terms of total dry matter, total energy, and total nitrogen, was negatively related to salinity. Total nitrogen was absorbed considerably more efficiently than either energy or dry matter.

Conversion efficiency (K_1 and K_2) was estimated, also in terms of dry matter, energy and nitrogen, in trout of the 0+ and 1+ year-groups weighing from 50 to 150 g. Between 0.0 and 28.0 p.p.t., there was little effect of salinity on conversion efficiency. Between 28.0 and 32.5 p.p.t., however, there was a significant decline in food conversion efficiency. The salinity:conversion efficiency relationship was not significantly influenced by fish weight in the range studied. Dry matter and energy conversion were significantly lower than nitrogen conversion efficiency.

Neither food intake nor conversion efficiency was significantly affected by dietary sodium chloride supplements of up to 8.5% of the total ration.

Over the range of rations from 4% to 38% of dry weight per week, there was a rectilinear relationship between ration and weight increase (% increase in dry weight per week). From this relationship can be estimated the weight maintenance requirement, and the rate of weight loss during fasting. In the same experiment, conversion efficiency (K_1) increased from 0.00 at 4% dry weight per week (weight maintenance) to 0.19 at 38%, according to the equation $K_1 = 0.2115 - \frac{0.7699}{x}$.

Live weight loss between days 7 and 48 of fasting could be described by a straight line. Trout maintained in 32.5 p.p.t. sea water showed a significantly greater weight loss than those in salinities of up to 15.0 p.p.t.

In the liver and white epaxial muscle, n-hexane-extracted lipids fell significantly during fasting. In the white muscle, there was a corresponding slight increase in water content (except in 32.5 p.p.t. sea water). The volume of the gall bladder contents increased during fasting.

In the alimentary canal with its associated adipose deposits, there was a very highly significant correlation between extracted lipid and water content.

There was a negative relationship between salinity and the density of distribution of mucus cells in the intestinal and rectal epithelia of acclimatized trout. The luminal cross-sectional area of the intestine and rectum,

and the height of the intestinal villi tended to increase with salinity. The alimentary canal showed no evidence of histological degeneration after 48 days of fasting in various salinities.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS

ABSTRACT

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

1. INTRODUCTION 1

2. MATERIALS AND METHODS 12

2.1. Stock maintenance 12

2.2. Salinity: effects on food intake and conversion efficiency 14

2.2.1. Acclimatization 15

2.2.2. Feeding 15

2.2.3. Weighing and measuring 16

2.3. Salinity: effects on absorption efficiency 17

2.4. Sodium chloride dietary supplementation 18

2.5. Salinity: fasting 19

2.6. Keep-net: Airthrey Loch 21

2.7. Chemical Analyses 22

2.7.1. Freeze-drying 22

2.7.2. Homogenisation 22

2.7.3. Calorimetry 22

2.7.4. Lipid extraction 23

2.7.5. Nitrogen determination 23

2.8. Histology 25

3. RESULTS 29

3.1. Effects of salinity on food intake 29

3.1.1. Fluctuations in food intake 29

3.1.2. Acute effects of salinity 29

3.1.3. Chronic effects of salinity 30

	Page
3.2. Effects of salinity on food absorption	31
3.2.1. Dry matter	31
3.2.2. Energy	31
3.2.3. Total nitrogen	32
3.3. Effects of salinity on food conversion efficiency	33
3.3.1. Dry matter	34
3.3.2. Energy	36
3.3.3. Total nitrogen	37
3.4. Effects of ration on food conversion efficiency	39
3.5. Effects of dietary sodium chloride on food intake and conversion efficiency	42
3.6. Effects of salinity on fasting rainbow trout	43
3.6.1. Weight loss during fasting	43
3.6.2. Decrease in condition factor during fasting	46
3.6.3. Lipid and water content of certain organs and tissues before and during fasting	46
3.7. Keep-net: Airthrey Loch	49
3.8. Histology	51
3.8.1. Effects of salinity on the histology of the alimentary canal	51
3.8.2. Effects of fasting on the histology of the alimentary canal	54
4. DISCUSSION	56
4.1. Effects of salinity on food intake	56
4.1.1. Chronic effects of salinity	56
4.1.2. Acute effects of salinity	59
4.1.3. Fluctuations in food intake	59
4.2. Effects of salinity on food absorption efficiency	60
4.3. Effects of salinity on food conversion efficiency	63
4.4. Effects of food intake on conversion efficiency	80
4.5. Keep-net: Airthrey Loch	86
4.6. Effects of sodium chloride dietary supplementation	87

Page

4.7. Effects of salinity on fasting rainbow trout 88

4.7.1. Weight loss 88

4.7.2. Condition factor 90

4.7.3. Lipid and water content of muscle and liver 91

4.7.4. Lipid and water content of gut and peritoneal fat deposits 94

4.8. Effects of salinity on the histology of the alimentary canal 96

5. LITERATURE CITED 103

APPENDICES 117

LIST OF FIGURES

1. Day-to-day fluctuations in the food intake of rainbow trout maintained in salinities between 0.0 and 32.5 p.p.t.

2. Acute effects of salinity on growth rate.

3. The relationship between the acute effects of salinity on (a) weekly food intake and (b) growth rate.

4. Weekly food intake versus salinity in acclimatized trout.

5. (a) The effect of salinity on food absorption efficiency.
   (b) The relationship between faecal nitrogen content and salinity.

6. The relationship between conversion efficiency and salinity in rainbow trout in the weight range 50-95 g. (a) dry matter, (b) energy, (c) total nitrogen.

7. The relationship between conversion efficiency and salinity in rainbow trout in the weight range 80-155 g. (a) dry matter, (b) energy, (c) total nitrogen.

8. The relationship between growth rate and ration.

9. The relationship between conversion efficiency (K_1) and weekly ration.

10. Relative weight loss in rainbow trout of 100-155 g maintained without food in salinities between 0.0 and 32.5 p.p.t.

11. Relative weight loss in trout of 80-95 g maintained without food in salinities between 0.0 and 32.5 p.p.t.

12. The relationship between condition factor and duration of fasting, at various salinities, in rainbow trout of 80-95 g.

13. (a) n-hexane-extracted lipid as % of wet weight in white epaxial muscle during fasting in various salinities.
    (b) water content of epaxial white muscle during fasting in various salinities.

14. (a) n-hexane-extracted lipid as % of wet weight of liver during fasting in various salinities.
    (b) water content of the liver during fasting in various salinities.

15. The relationship between n-hexane extracted lipid and water content in (a) liver and (b) epaxial white muscle.

16. The relationship between extracted lipid and water content in the alimentary canal and associated adipose deposits.

17. Growth of rainbow trout maintained in Airthrey Loch, Stirling. (a) length, (b) weight.

18. The effect of salinity on the total cross-sectional area of the intestine and rectum in fed and unfed rainbow trout.

19. The effect of salinity on the height of the villi in the intestine of fed and unfed rainbow trout.

20. The effect of salinity on the distribution of mucus cells in transverse sections of the intestine and rectum.

21. Lipid/water relationships in (a) rainbow trout gut and adipose deposits, (b) brown trout gut and adipose deposits and (c) herring carcass.

22. Electrical output of the Phillipson micro-bomb calorimeter *versus* weight of benzoic acid combusted.

23. Energy *versus* electrical output in the Phillipson micro-bomb calorimeter.

LIST OF TABLES

		Page
1.	A summary of the statistical significance of differences between food intakes in the five salinities investigated.	30
2.	Energy and ash contents as % dry wt. of faeces in salinities between 0.0 and 32.5 p.p.t.	32
3.	The effect of salinity on K_1 (dry matter) in rainbow trout of the 1+ year-group weighing from 115 to 155 g.	33
4.	The effect of dietary sodium chloride on food intake and conversion efficiency.	42
5.	The effect of salinity on some alimentary canal tissue dimensions in fed and fasting trout.	52
6.	A survey of absorption efficiency results from a range of fish species and diets.	61
7.	The lipid/water relationship in the gut of rainbow and brown trout and the carcass of herring.	95

LIST OF PLATES

1. Transverse section of the intesting showing general structure.

2. Transverse section of intestine showing the striated border of the columnar epithelial cells.

3. Transverse section of intestine illustrating a typical density of goblet cells in the mucosa of rainbow trout maintained in fresh water.

4. Transverse section of intestine illustrating a typical density of goblet cells in the mucosa of rainbow trout maintained in 32.5 p.p.t. sea water.

5. Transverse section of intestine showing the depressions developed in the mucosa of rainbow trout maintained in 32.5 p.p.t. sea water.

6. Transverse section of the rectum showing the greater prevalence and size of goblet cells on the outer edge of the spiral septum.

LIST OF APPENDICES

		Page
1.	Food intake	117
2.	Food absorption	118
3.	Food conversion efficiency	119
4.	Fasting	120
5.	Effect of ration on conversion efficiency	122
6.	Phillipson Micro-bomb calibration	123
7.	Histological techniques	124

1. INTRODUCTION

The rainbow trout, Salmo gairdneri Richardson, is indigenous to the waters of western North America. Since 1874, its range has been extended by man to all continents except Antarctica (MacCrimmon 1971). One of the earliest introductions - in 1885 - was to the hatchery at Howietoun, Stirling, from which the trout for the present study were obtained.

The farming of rainbow trout has now attained a certain economic importance in several parts of the world, especially in North America, Europe and Japan. According to MacCrimmon (op.cit.) the species 'offers an unrealized potential on all continents if warranted by a protein and market demand.' The potential of the species as a source of protein is, however, limited by its requirement, as a carnivore, for a diet containing a high level of first-class protein. In the more affluent countries, however, there is undoubtedly a potential, if not already an existing, market demand for rainbow trout both as a dietary luxury and for the stocking of game-fishing waters.

Salmo gairdneri has both migratory (anadromous) and non-migratory races, the former being known as the steelhead trout. In Europe, at least, the non-migratory type is more commonly produced by fish farms. Both forms are euryhaline, i.e. both are able to tolerate a certain range of environmental salinities, which tends to become broader as the fish increases in size (Busnel and Drilhon, 1944; Parry, 1958,

1960; Jensen, 1966; Conte and Wagner, 1965). The euryhalinity of the species has been exploited in a number of mariculture units, notably in Norway and Denmark but also in Scotland (Jensen, 1966; Sedgwick, 1970).

Jensen (1966) cites temperature as the main motivation behind the seawater culture of rainbow trout in Norway. Even in southern Norway, river temperatures are so low that the effective growing season for rainbow trout is restricted to the period between May and mid-October. In western and northern Norway, he stated, the mean sea temperature is higher than the mean river temperature throughout the year. The optimum temperature for rainbow trout growth is 12°C (Atherton and Aitken, 1970) and the preferred temperature (determined by behavioural experiments) is 13.0-13.6°C (Garside and Tait, 1956). Below about 5°C, feeding and growth rates approach zero. Further reasons for the unsuitability of Norwegian rivers for fish culture - e.g. steep gradients and rapid fluctuations in level - are mentioned by Sedgwick (1970), who also stated that some diseases of rainbow trout are less prevalent in sea water and that 'the fish are said to make a better conversion or utilization of given quantities of food in salt water than in fresh water.'

The topographical and climatic aspects of the problem of rearing rainbow trout in river water in Norway, and the advantages of rearing them in sea water, have been recognized as having features in common with the situation in certain parts of Scotland. It was noted that the

commonly-held belief given expression by Sedgwick (1970) that rainbow trout 'grow better' in sea water had no sound empirical basis and it was therefore decided to determine experimentally (1) whether growth in Salmo gairdneri is affected by salinity and (2), if growth is affected, does the effect occur at the level of (a) food intake or (b) food conversion efficiency. Further physiological and histological studies were designed and carried out to contribute to an understanding of any observed influence, at either of these two levels (a and b) of salinity on growth.

The appetite of a fish (also called 'voluntary food intake', Lepkovsky, 1948; Brett, 1971) can be defined as food intake during a unit of time when food supply is not limiting. Appetite has, in the past, been measured under two categories of feeding régime, restricted and unrestricted rations. A restricted ration régime typically involves feeding the fish to satiation over a pre-determined limited period of time and then removing the remaining food. Pandian (1967) fed Ophiocephalus striatus and Megalops cyprinoides with an excess of live prawns (Metapenaeus monoceros) over a period of 10 minutes, after which uneaten prawns were removed and weighed. An unrestricted régime is typified by the procedure of Brown (1946b), who fed brown trout (Salmo trutta) six days weekly on a slight excess of minced meat and liver, removing residual food three times weekly, so that there was effectively always an available surplus of food.

It is assumed in the present study that restricted

food intake provides a valid measure of appetite. The results of Kinne (1960) suggest that restricted food intake is proportional to unrestricted food intake. Relatively little work has been done on the effects of salinity on appetite (Kinne, 1960; Nelson, 1968; Vallet et al., 1970; Otto, 1971; Peters and Boyd, 1972).

In this study, the term absorption efficiency is used in the same sense as that used by Gerking (1952): "'Absorption efficiency' is equivalent to the term 'coefficient of digestibility' which is commonly used in standard physiology and nutrition work. Coefficient of digestibility is objected to on the grounds that it is always expressed in per cent (not a coefficient) and that digestion does not always necessarily result in absorption."

The same working definition was quoted by Pandian (1967a). Absorption efficiency was calculated in the present study by the formula $p = \frac{I-E}{I}$, where I is food intake or ingestion and E is faecal output or egestion. To facilitate the use of the value p in later conversion efficiency calculations it was expressed as a fraction of unity rather than as a percentage. Absorption efficiency was calculated in terms of total nitrogen as well as energy and total dry matter.

Only one paper (Brocksen and Cole, 1972) has been noted which deals with the effect of salinity on absorption efficiency in fishes, and this one paper quotes an unusually low figure for energy absorption efficiency (72.0%), even at the optimum salinity.

Winberg (1956) cites three orders of conversion efficiency used by Ivlev (1945):

K_1 = the relationship between the total energy content of food and the energy equivalent of gain in weight.

K_2 = the relationship between the 'physiologically useful' energy of the food and the energy equivalent of gain in weight.

K_3 = the relationship between growth and that part of the physiologically useful energy which remains after deducting what is required for 'internal and external work'.

Following the notation used by Winberg, 1956, Ivlev, 1961, and Paloheimo and Dickie (1965, 1966a, 1966b),

$$K_1 = \frac{\Delta W}{R\Delta t}$$

$$K_2 = \frac{\Delta W}{pR\Delta t}$$

$$K_3 = \frac{\Delta W}{pR\Delta t} - T$$

where W is weight

R is rate of feeding

t is time

T is total metabolic rate

p is a correction factor which converts consumed rations to absorbed or assimilated rations.

K_1 is equivalent to the total efficiency of Warren and Davis (1967) and the gross efficiency of most other workers.

K_2 is equivalent to the net efficiency of Pandian (1967a) and Kelso (1972)and the gross efficiency of Brown (1946b, 1946c, 1957).

K_3 is equivalent to the partial growth efficiency of Warren and Davis (1967) and the net efficiency of Brown (1946b, 1946c, 1957), Brett et al. (1969) and Brett (1971b).

Following Kleiber (1961), Warren and Davis (1967) quoted yet another measure of efficiency, partial maintenance efficiency:

$$E_{pm} = \frac{L_p}{I_p}$$

where L_p = tissue loss prevented

I_p = part of ration preventing loss.

This expression quantifies the efficiency with which an animal utilises rations at or below the maintenance level to maintain its tissues or to prevent them from being catabolized. It seems to have found little, if any, application in fish growth studies.

In K_3, T can be considered equivalent to the maintenance requirement. Brown (1946b) defines maintenance requirement as the amount of food which must be eaten by the animal to prevent loss of weight. Dawes (1930-1931) and Pentelow (1939) employ a similar definition: the energy equivalent of average rations consumed per unit time during a prolonged period of no growth. Smith (1963) defines it as the rate of depletion of stored material during a period of starvation. Brett et al. (1969) have shown that, where stable growth rates have been established, the maintenance requirement can be interpolated from the curve of growth rate against ration level.

The maintenance requirement concept is problematical.

Brown (1946b) has indicated that maintenance requirement shows adaptation to a decrease in food consumption. Paloheimo and Dickie (1966a) reported that the level of metabolism falls from 'active' to 'standard' with an alteration of ration from excess to maintenance. Brown (1957) recorded that, on a restricted diet, values for net efficiency of more than 100% were obtained in her brown trout growth experiments (Brown, 1946a,b,c).

This seems likely to have been a consequence of difficulties in obtaining a valid estimate of maintenance requirement. Perhaps because of these difficulties, K_3 has been infrequently employed. The maintenance requirement interpolation method of Brett et al. (1969) may provide the most acceptable estimate, and these workers used this method to calculate K_3.

K_1 and K_2 have been employed more frequently than K_3, but K_2 has seldom been calculated exactly according to the definition of Ivlev (1945) because of the technical difficulty of estimating the amount of energy dissipated in the form of nitrogenous excretory matter. Davies (1963, 1964), working with Carassius auratus, determined the total energy lost in excretion and defaecation by evaporating to dryness the entire contents of experimental aquaria and determining the calorific value of the residue. A larger number of workers have calculated K_2 with a correction for faecal matter alone (e.g. Brown, 1946b,c; Pandian, 1967a,b,c; Kelso, 1972). According to Winberg (1956) this does not introduce a large error, as faecal matter accounts for 97% of non-assimilated food energy.

K_1, K_2 and K_3 have all been expressed, by various workers, in terms of weight, protein and energy. Efficiency measurements will differ according to the basis of calculation (Winberg, 1956). The degree of discrepancy between weight, protein and energy conversion efficiencies will vary with the difference between the composition of food and fish.

Many workers, including Dawes (1930), Pentelow (1939), Brown (1946b,c), Kinne (1960, 1962), Johnson (1966), Otto (1971), Brett (1971b) and Peters and Boyd (1972) have calculated conversion efficiency on the basis of weight. Fewer, including Kelso (1972) have used energy conversion figures. Gerking (1952, 1954, 1955) measured nitrogen conversion efficiency. Pandian (1967a,b,c) took account of food both as an energy source and as a raw material by calculating both energy and nitrogen conversion efficiencies.

Conversion efficiency has often been employed as an indicator of the effect of external and internal factors on the bio-energetic physiology of fishes. A change in metabolic rate is assumed to be reflected in an increase or a decrease of food conversion efficiency. The greater the proportion of absorbed food necessary for other processes, the smaller is the proportion available for storage as growth. Kinne (1962) stated that conversion efficiency was a very sensitive indicator of the effect of salinity on the metabolism of Cyprinodon macularius, especially in the juvenile fish, before the situation becomes complicated by the synthesis of reproductive material.

A small number of workers have studied the effects of salinity on conversion efficiency in fishes (Kinne, 1960, 1962; Vallet et al., 1970; Otto, 1971; Brocksen and Cole, 1972; Peters and Boyd, 1972). A few others have recorded the effect of salinity on growth, which allows us - as long as food intake is also noted - to make an inference about conversion efficiency (Gibson and Hirst, 1955; Canagaratnam, 1959,1966; Chervinski, 1961).

The validity of studying food intake and conversion efficiency concurrently depends - in the type of experimental design employed in this study - on defining whether there is an interaction between these two variables. Paloheimo and Dickie (1966b), after re-analyzing the data of several earlier workers, concluded that there was an inverse relationship between ration level and conversion efficiency. Several more recent workers have not been in agreement with this conclusion (Pandian, 1967c; Brett et al., 1969; Brett, 1971; Gerking, 1971; Kelso, 1972; Pandian and Raghumuraman, 1972).

Study of the depletion of energy reserves during starvation can be used to supplement information obtained by the study of conversion efficiency. Energy loss during starvation can be assumed to be related to the amount of food energy used in processes other than growth in the feeding animal. It is therefore inversely related to conversion efficiency. Peters and Boyd (1972) found that the rate of weight loss in the hogchoker, Trinectes maculatus, was related to salinity in a comparable but inverse fashion to conversion efficiency. In the present study, the effect

of salinity on weight loss was studied, and the lipid and water contents of certain tissues were monitored simultaneously as indicators of the depletion of energy reserves during starvation. Love (1970) stated that 'while a steady depletion of lipid characterizes subsistence without food in almost all cases, the lipid content cannot necessarily be taken as a reliable index of nutritional status.' This is true of fish caught in the wild, but with fish of the same age, physiological condition and previous nutritional history kept in controlled conditions in the aquarium, lipid content is more likely to provide a reasonable comparative index of the rate of utilization of stored reserves.

As well as weight and some aspects of chemical composition, the effect of starvation on the weight:length relationship was quantified in terms of the condition factor, calculated by the formula of Brown (1957), which is the one generally used for salmonids.

$$CF = \frac{100W}{L^3}$$

where W = wt in g

and L = length in cm (fork length).

Western (1969, 1971) has compared the structure of the gut in the marine teleost Enophrys bubalis and the closely-related freshwater species Cottus gobio. In a comparison of this sort, where genotype, type of food and amount of food all differ, it is difficult

to draw any conclusions as to the effect of salinity, *per se*, on gut histology. A more useful approach seems to be to examine the gut of a euryhaline species adapted to different salinities but otherwise maintained under identical conditions. Virabhadrachari (1961) studied the effects of salinity on the gut (together with other organs implicated in osmoregulation) of individuals of the euryhaline species *Etroplus maculatus* acclimatised to fresh water, sea water and intermediate saline concentrations.

The subject of the present study, *Salmo gairdneri*, is also a euryhaline freshwater fish. The histology and gross anatomy of the gut of this species has been described by Weinreb and Bilstad (1955). The intestine has been studied at the electron microscope level by Yamamoto (1966). Those aspects of the histological structure of the alimentary canal of the rainbow trout which are particularly relevant to the present study are illustrated in Plate 1.

Observations have been made, in the present work, on the influence of starvation, as well as salinity, on the gut of the rainbow trout to determine whether there is any interaction between the effects of salinity and starvation. The consequences of the spawning migration in anadromous salmonids include both starvation and changes in salinity. The effects of the spawning migration have been described by Gulland (1898), Greene (1919, 1926) and Robertson *et al*. (1961). But, as Robertson *et al*. (1961, 1963) have demonstrated, important hormonal changes also take place during this part of the life-cycle; the present study cannot claim to simulate the salinity change and starvation effects of the spawning migration.

2. MATERIALS AND METHODS

2.1. Stock maintenance

In October to December of the years 1970, 1971 and 1972, a stock of 150-200 0+ year-group rainbow trout Salmo gairdneri Richardson was obtained from Howietoun and Northern Fisheries, Bannockburn, Stirling. These trout had a mean length of about 14.0 cm and a mean weight of about 40.0 g. When required, replacement stock of the same year-group and size were obtainable, throughout the year, from the same hatchery.

The stock fish were maintained in running copper-free mains fresh water (i.e. water drawn off iron mains in plastic piping) in a cylindrical glass-reinforced plastic (G.R.P.) tank, containing a volume of approximately 2000 l (diam. 185 cm x depth 75 cm). They were fed at a ration level (approximately 10% per week on a dry weight basis) which was sufficient to maintain a slow, stable growth rate. In order to monitor growth rate, samples of 20 trout were weighed monthly and the ration level was adjusted when necessary. There was an annual temperature variation in the stock tank of between about $4^{o}C$ and about $15.5^{o}C$.

The diet consisted of Beta Floating Trout Pellets, supplied by Cooper Nutrition Products Ltd., Witham, Essex, who specified the following crude analysis:

	%
protein	37.0
oil	4.0
ash	11.5
fibre	5.0
moisture	10.0
carbohydrate (by difference)	32.5

For experimental work, exact analyses were carried out of certain components of the specific batches of pellets used.

The ingredients include: fish meal, shrimp meal, liver meal, meat meal, unextracted dried yeast, distillers solubles, grass meal, cereal by-products, soya bean meal, animal and vegetable oils.

An analysis of aquarium water was carried out on 1st June, 1973 by a commercial analyst (R.R. Tatlock and Thomson, Bath Street, Glasgow):

Substance	Concentration ($mg.l^{-1}$)
total dissolved solids	40.0
total alkalinity as $CaCO_3$	14.0
chloride	7.0
free ammonia	0.007
albuminoid ammonia	0.063
nitrous nitrogen	none
nitric nitrogen	trace
sodium	2.6
potassium	0.3
iron	0.12
copper	0.04
lead	0.01
free chlorine	0.04
pH	7.0

2.2. Salinity: food intake and conversion efficiency

Rainbow trout studied in experiments in which salinity and temperature were controlled were held in rectangular G.R.P. tanks of 250 l capacity (100 cm x 100 cm x 25 cm) covered with $\frac{1}{4}$ in. mesh semi-rigid nylon netting (Netlon). In specific experiments, 10-20 trout were randomly assigned to each tank. The fish were not individually identified. The tanks were located in a controlled temperature room set to give a water temperature of $10.0^{\circ}C \pm 0.5^{\circ}C$. Oxygen concentration was maintained at near-saturation levels by compressed-air aeration, and monitored with a Mackereth Oxygen Electrode. A 12 h photoperiod was maintained by an automatic time-switch.

Sea water, initially obtained from Dunstaffnage Marine Laboratory, Oban, was drawn off the aquarium's recirculating filtered seawater system. Copper-free fresh water was drawn off the mains. Intermediate salinities were prepared by mixing water from these two sources in measured proportions. Salinity in the experimental tanks was monitored regularly with a salinity/temperature bridge (Type MC5 National Institute of Oceanography), which was calibrated against silver nitrate chloridimetric titrations. Salinity was maintained within a range of $\pm$ 0.5 p.p.t. Three times weekly, half the water in each experimental tank was siphoned off and replaced with water of the appropriate salinity and temperature.

Conversion efficiency (K_1 as defined earlier) was measured over several periods of 14 days in three categories

of trout: (1) 0+ trout 50-95 g approx., (2) 0+ trout 80-155 g approx., (3) 1+ trout 115-155 g approx. Food intake was measured in categories (2) and (3).

2.2.1. Acclimatization: Individuals to be used in experiments were removed from the stock tank and transferred to the experimental tanks described above. In these tanks, they were held in fresh water at the experimental temperature for 14 days to allow temperature acclimation before the salinity was altered. From fresh water, trout were transferred to salinities of 7.5 p.p.t. and 15.0 p.p.t. in weekly steps of 7.5 p.p.t., and from 15.0 p.p.t. to either 28.0 p.p.t. or 32.5 p.p.t. in one further step. They were then maintained at these salinities for 14 days before the commencement of an experiment.

2.2.2. Feeding: The diet consisted of Coopers' Beta Floating Pellets Size 5 (maximum dimension 3.5-5.0 mm). This diet was chosen because:

1) Residual pellets float and can readily be recovered from the water surface.

2) The pellets have a sufficiently high water-stability to ensure that there is no appreciable loss of constituent material during the feeding period.

3) Properties 1) and 2) combine to minimise fouling of the tank by uneaten food particles.

Many of the problems noted by earlier workers are thus circumvented. Brown (1946c) estimated the dry weight

of a fresh meat diet from the dry weight of sub-samples and siphoned off residual food particles for direct dry weight determinations. Brown (1957) drew attention to problems inherent in this method.

1) The fish break up the food and eject small unrecoverable particles.

2) The absorption of water by uneaten food makes it necessary to use dry weight measurements.

In the present method, pellets were sieved before use to remove undersized food fragments. During certain experiments, a predetermined daily ration was fed. In others, the trout were fed once daily 6 days weekly to satiation in order to determine 'appetite'. A weighed excess of pellets was provided and the fish were permitted to feed for 30 minutes from about 0930. Food intake was then measured by calculating the difference in weight between supplied food and residual food. As the pellets were of uniform weight, residual weight could be estimated accurately from the number of pellets uneaten.

2.2.3. Weighing and measuring: Before being weighed and measured, the trout were starved for 48-72 h to allow the gut to be emptied. Fish to be weighed and measured were anaesthetized in a 1:15,000 solution of MS-222 Sandoz (meta-aminobenzoic acid ethylester) in water of the appropriate salinity. Length, to the nearest 1.0 mm, was measured from the extreme anterior end of the fish to the shortest caudal fin ray (fork length). Weight was measured to the nearest 0.1 g after removing excess surface water with damp absorbent

paper. In most cases, experimental fish were weighed fortnightly.

2.3. Salinity: absorption efficiency

Food absorption efficiency was measured at four salinities: fresh water, 7.5 p.p.t., 15.0 p.p.t. and 32.5 p.p.t. The experimental fishes had already been maintained at these salinities for at least 100 days. 18-20 rainbow trout (mean weight 130 g approx.) were held in each of four 250 l static aerated tanks at a temperature of $10^{\circ}C \pm 0.5^{\circ}C$ and fed on Coopers' Floating Pellets at a ration level of about 10% per week on a dry weight basis.

Immediately before the experimental period, the trout were starved for 72 h and the tanks were carefully cleaned to remove any remaining faecal material. Thereafter, the trout were fed daily for 2 weeks. Faeces were collected on alternate days by siphoning them off, together with a minimal volume of water. An almost complete recovery of faeces is possible, as egested material is surrounded by a layer of mucus. The faeces were concentrated on a pre-weighed glass fibre filter disc by suction filtration and washed with a small volume of distilled water to remove residual salt water. Faecal recoveries were continued until 72 h after the last feeding to ensure as complete a recovery as possible.

The dry weight of faeces was determined by drying the samples to constant weight at $80^{\circ}C$. Aliquots of faeces from each salinity and samples of food were analyzed for

both energy content and total nitrogen by the methods described in detail elsewhere in this thesis (pp. 22-24). Determinations were made in triplicate. Faecal ash content determinations were made in duplicate for each salinity, to allow a correction to be made for any extraneous inorganic matter present in faeces from 7.5 p.p.t., 15.0 p.p.t. and 32.5 p.p.t. tanks.

Absorption efficiency was calculated for dry matter, energy and nitrogen, according to the formula $p = \frac{I - F}{I}$, where p is absorption efficiency as a fraction of unity, I is food intake and F is faecal output.

2.4. Sodium chloride dietary supplementation

Experimental conditions differed in several respects from those maintained in the standard growth tanks described above.

20 rainbow trout of the 0+ year-group with a mean starting weight of about 34.0 g and a mean starting length of about 14.0 cm were randomly allotted to each of three 84 l G.R.P. tanks. A continuous flow of 'copper-free' mains water was passed through the tanks at mains temperature, which varied from 4.2 to 9.9°C [$\bar{x}$ = 6.25] over the experimental period of 14 weeks. The lighting régime was not under automatic control, but a photo-period was maintained of as close as possible to 9 h in each 24.

NaCl supplements of 5.0% and 8.5% were added to Coopers' 'Growers' slow-sinking' pellets by the manufacturers, according to our specifications. A control batch of pellets

without NaCl supplementation but of otherwise identical composition was also prepared.

The trout in one tank were fed on control pellets while those in the two other tanks were fed on 5.0% and 8.5% respectively. A predetermined ration was provided, the ration being adjusted fortnightly to give a range of ration levels between 4 and 40% per week on a dry weight basis. Appetite on the three different diets was compared by daily feeding over one 14-day period to a measured satiation level.

In calculating conversion efficiency and food intake, a correction was applied for the diluent effect of the NaCl supplement and for differences in water content.

2.5. Salinity: fasting

Two separate starvation experiments were carried out under the standard experimental conditions described for the growth experiments (salinities 0.0, 7.5, 15.0, 32.5 p.p.t.; temp. 10°C; 250 l tanks).

At commencement, there were 15-20 individuals (mean weight (i) about 80.0 g (ii) about 130.0 g) in each tank. These trout had been maintained on a controlled diet at the experimental salinities for at least 120 days prior to the experiment. All fish were weighed and measured (on day 0) 72 h after their last meal. Further weight and length measurements were made at intervals during starvation, up to day 46.

On days 0, 20 and 46, five individuals were sampled from each salinity for lipid and water content determinations. In one experiment (130.0 g) the individuals in 32.5 p.p.t. sea water were sacrificed on day 35, as they were showing signs of distress.

The sampled trout were killed by a sharp blow on the head. In one experiment (130.0 g) an epaxial muscle sample of 1-2 g wet weight was excised from each side of each individual immediately anterior to the dorsal fin. This sample was free of red muscle, external myocommata and skin. The whole liver was excised and all bile was drained from the gall bladder by slitting it with a scalpel. In the other experiment (80.0 g) the gut and visceral adipose deposits were sampled. The alimentary canal was transected at the oesophagus immediately posterior to the pericardium and at the anus and removed, together with the attached adipose deposits. The liver and spleen were discarded. The alimentary canal was split longitudinally and washed in 0.9% saline to remove mucus and any remaining ingested matter. Tissue samples were weighed and immediately deep-frozen at -20°C. Water content was later determined by vacuum freeze-drying. The lipid content of freeze-dried samples was estimated by n-hexane extraction (Groves, 1970) according to the method described in detail later in this section.

2.6. Keep-net: Airthrey Loch

Until aquarium accommodation became available, some observations were made on trout maintained in a keep-net in Airthrey Loch for later comparison with fish kept under controlled conditions. 150 rainbow trout of the 0+ year-group, with an approximate mean length of 15.0 cm and an approximate mean weight of 44.0 g, were transferred to a cuboidal net enclosure located in Airthrey Loch, University of Stirling. The net, which enclosed a water volume of 4000 l (2 m x 2 m x 1 m) was constructed from 0.25 in mesh semi-rigid nylon netting (Netlon) on an aluminium alloy framework. The net was suspended above the loch bottom on four vertical posts.

The trout were fed 5 times weekly over a period from 3rd December 1971 to 22nd April 1972, on floating pellets of the type described earlier. A daily ration of 100 g was adhered to throughout.

Samples of 10 fish were taken at intervals (usually fortnightly) for weighing and measuring. The sampled fish were measured to the nearest 1.0 mm and weighed to the nearest 0.5 g. To permit a calculation of total weight, a record was kept of the weight of any fish which died or which was removed without being replaced. Surface temperature was recorded at the time of each feeding.

2.7. Chemical analyses

2.7.1. Freeze-drying: Samples were dried to constant weight at -40°C in an Edwards High Vacuum Model EF_2 freeze-drier. All samples were pre-frozen for at least 12 h at -20°C. Dried samples were allowed to attain room temperature in a silica gel desiccator.

2.7.2. Homogenisation: Material for nitrogen content, energy content and ash content determinations was finely ground in a Glen Creston rotary hammer mill to help ensure homogeneity in the small samples (20-40 mg) used in energy and nitrogen determinations. Before being milled, whole fish were deep-frozen, ground in a domestic-type mincer and freeze-dried, while faecal samples were oven-dried at 80°C. Food pellets, on the other hand, were milled without any pre-treatment as they are already friable and have a low water content. All pulverised samples were stored over silica gel in vacuum desiccators.

2.7.3. Calorimetry: The energy content of samples of fish, food and faeces was estimated by combustion in a miniature bomb calorimeter (Phillipson, 1964), supplied by Gentry and Wiegert Instruments Inc., Aiken, South Carolina. The bomb was calibrated against benzoic acid (Thermochemical Standard Grade) which has a calorific value of 26.455 $J.mg^{-1}$. In the course of calibration, 13 pellets of benzoic acid, weighing from 1.0 to 21.0 mg, were combusted. The calibration curves obtained are presented in Figures (22) and (23) (Appendix 6).

Determinations were carried out on duplicated (fish) or triplicated (food and faeces) 9.9-25.5 mg pellets, prepared from freeze-dried, homogenized samples, and stored until combustion in silica gel desiccators.

2.7.4. Lipid extraction: Total lipid was estimated by extraction in n-hexane, using a modification of the method employed, and rigorously tested, by Groves (1970). Pre-weighed, freeze-dried whole tissue samples (epaxial muscle, liver, gut) were extracted three times in 10 times their own volume of n-hexane in sealed beakers. Each extraction took place over 24 h, with periodical agitation. After the third 24 h extraction period, the n-hexane solvent was decanted and the fat-free sample air-dried for 12 h at room temperature to allow the bulk of the remaining solvent to evaporate. Each sample was then dried for 24 h at 80°C, cooled in a desiccator and weighed. This technique has the advantage that a large number of extractions can be performed concurrently, and the added advantage for some work that protein and ash determinations can readily be carried out on the extracted samples.

2.7.5. Nitrogen: The nitrogen content of fish, food and faeces was determined by the micro-Kjeldahl method as described by Niederl and Niederl (1942).

Duplicated (fish) or triplicated (food and faeces) samples of 30.0-40.0 mg of dry powdered material were digested by boiling for 30 minutes in 1.0 ml of nitrogen-free concentrated sulphuric acid (S.G. 1.835) with approximately

0.075 g of catalyst mixture, composed of potassium sulphate (K_2SO_4), copper sulphate ($CuSO_4$) and mercuric sulphate ($HgSO_4$) in the proportions 1.0:3.0:0.2.

The digest was then cooled and quantitatively transferred, with several washings of distilled water, into the distillation apparatus, and neutralized with 10.0 ml of 30% sodium hydroxide, containing 5% of sodium thiosulphate to break down any mercury-ammonia complexes that may have been formed.

The ammonia which was distilled over was collected in exactly 25.0 or exactly 30.0 ml of 0.01 M hydrochloric acid containing a trace of methyl red indicator. After boiling for five minutes to expel dissolved carbon dioxide, the excess of HCl was determined by titration against 0.01 M NaOH. A duplicated blank was carried through the whole procedure and a blank correction was applied to the titration results. The quantity of ammonia, and hence of nitrogen, contained in the sample was calculated from the volume of HCl neutralised by the distillate.

Niederl and Niederl (1942) used urea to illustrate the chemical reactions involved in the method:

i	Digestion	$H_2NCONH_2 \xrightarrow[H_2SO_4]{\Delta} (NH_4)_2SO_4$
ii	Distillation	$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + Na_2SO_4$
iii	Titration	$NH_3 + HCl \longrightarrow NH_4Cl$

2.8. Histology

The trout sampled for histological study belonged to the 0+ year-group, were sexually immature and had a mean length of about 23.0 cm and a mean weight of about 140.0 g. They had been maintained, for at least 120 days, in four salinities: fresh water, 7.5 p.p.t., 15.0 p.p.t., and 32.5 p.p.t. During this period, the water temperature had been 10 $\pm$ 0.5°C. All fish were fed at the same measured daily rate which was below satiation level for all treatments. 72 h before initial samples were taken, feeding was terminated to allow the gut contents to be voided. 3-4 trout from each salinity were then sampled for histological observation. The remaining individuals were starved for a total of 48 days and samples of 3-4 trout from each salinity were then sampled for histological observation. The remaining individuals were starved for a total of 48 days and samples of 3-4 trout from each salinity were then removed.

Fish included in the samples were killed by a sharp blow on the head, measured and weighed. The following parts of the alimentary canal were excised for histological examination:

a) oesophagus and anterior region of cardiac stomach

b) central region of cardiac stomach

c) intestine: a 5 mm length beginning 1 cm posterior to the last pyloric caecum.

d) rectum: a 5 mm length beginning 1 cm anterior to the anal opening.

All tissue samples were fixed immediately post-mortem in 0.9% neutral formol-saline. After fixation, the samples were dehydrated in graded alcohols and embedded in paraffin wax (M.P. 56°C) according to the schedule recorded in Appendix 7.

The embedded tissues were sectioned at 5 μm on a rotary microtome. Gut areas (b), (c) and (d) were studied in transverse section, area (a) in longitudinal section. Staining and histochemical methods employed included haematoxylin and eosin, periodic-acid-Schiff (PAS) with light green counterstain, Lison's (1954) alcian blue-chlorantine fast red (AB). PAS reacts with neutral muco-polysaccharides. AB stains acid or sulphated mucopoly-saccharides. Before being stained, the sections were dewaxed in xylol and taken to water (Appendix 7). The staining methods are described in detail in Appendix 7.

Histological measurements were made with an eyepiece graticule. The external diameter (and hence the radius) of each section examined was estimated by taking the mean of the maximum and minimum diameters. The mean depth of the columnar epithelium in the intestine and rectum was estimated from a sample of 8-10 randomly selected cells in each section. An estimate of mucus (goblet) cell density in each intestine and rectum section was made from the number of such cells in 10 randomly-selected 136 μm lengths of columnar epithelium. Other measurements were made in quadruplicate, with observations at intervals of approxi-mately 90°.

Where required, total gut cross-sectional area (including lumen) was estimated from the formula:

$$A = \pi r^2$$

and total muscle cross-sectional area from the formula:

$$M = \pi r^2 - \pi (r - m_1 - m_2)^2$$

where A is total cross-sectional area,

M is muscle cross-sectional area,

r is the external radius of the section,

m_1 is the depth of the circular muscle layer

and m_2 is the depth of the longitudinal muscle layer.

To allow direct comparisons to be made between sections from individuals of differing sizes, a relationship between cross-sectional area and fish length was used. It is assumed that

$$A \propto L^2$$

$$\therefore A = k_1 L^2$$

$$\therefore k_1 = \frac{A}{L^2}.$$

Similarly, it is assumed that

$$M \propto L^2$$

$$\therefore M = k_2 L^2$$

$$\therefore k_2 = \frac{M}{L^2}$$

where L is fish length

k_1 is a constant

k_2 is a constant

Changes in k_1 and k_2 in response to salinity and starvation can be directly compared, even among individuals of different size.

3. RESULTS

3.1. Effects of salinity on food intake

To take account of the large discrepancy between the water contents of the trout and their pelleted diet, food intake was expressed in terms of per cent dry weight per week ($\frac{\text{dry weight food}}{\text{estimated dry wt fish}} \times 100$). A week was selected as the time unit, as the fish were fed 6 days weekly, making a week the shortest repeated time cycle.

3.1.1. Fluctuations in food intake: There was a marked day-to-day fluctuation in food intake. This is illustrated by data from Experiment (3) (Figs. 1a,b,c,d). For simplicity, this figure shows absolute food intake. Each day of non-feeding is signified by a break in the record. There was no clear tendency for a day of non-feeding to be followed by increased food intake on the succeeding day.

3.1.2. Acute effects of salinity on food intake: At each abrupt change in salinity there was a decrease in growth rate by comparison with the control (Fig. 2a,b). A comparison of weekly % weight increase and weekly food intake (Fig. 3a,b) indicates that this decrease can be explained mainly by a decrease in food intake after each salinity change. The greatest decrease in food intake and growth rate occurred after the increase of salinity from 15.0 p.p.t. to 28.0 p.p.t. The recovery of food intake and growth rate to pre-change levels occurred within 14 days.

FIGURE 1. Day-to-day fluctuations in the absolute food intake (g) over a period of 40d of groups of rainbow trout maintained in four different salinities, (a) fresh water, (b) 7.5 p.p.t., (c) 15.0 p.p.t., and (d) 32.5 p.p.t.

n the

over a

f rainbow

different

er,

p.p.t.,

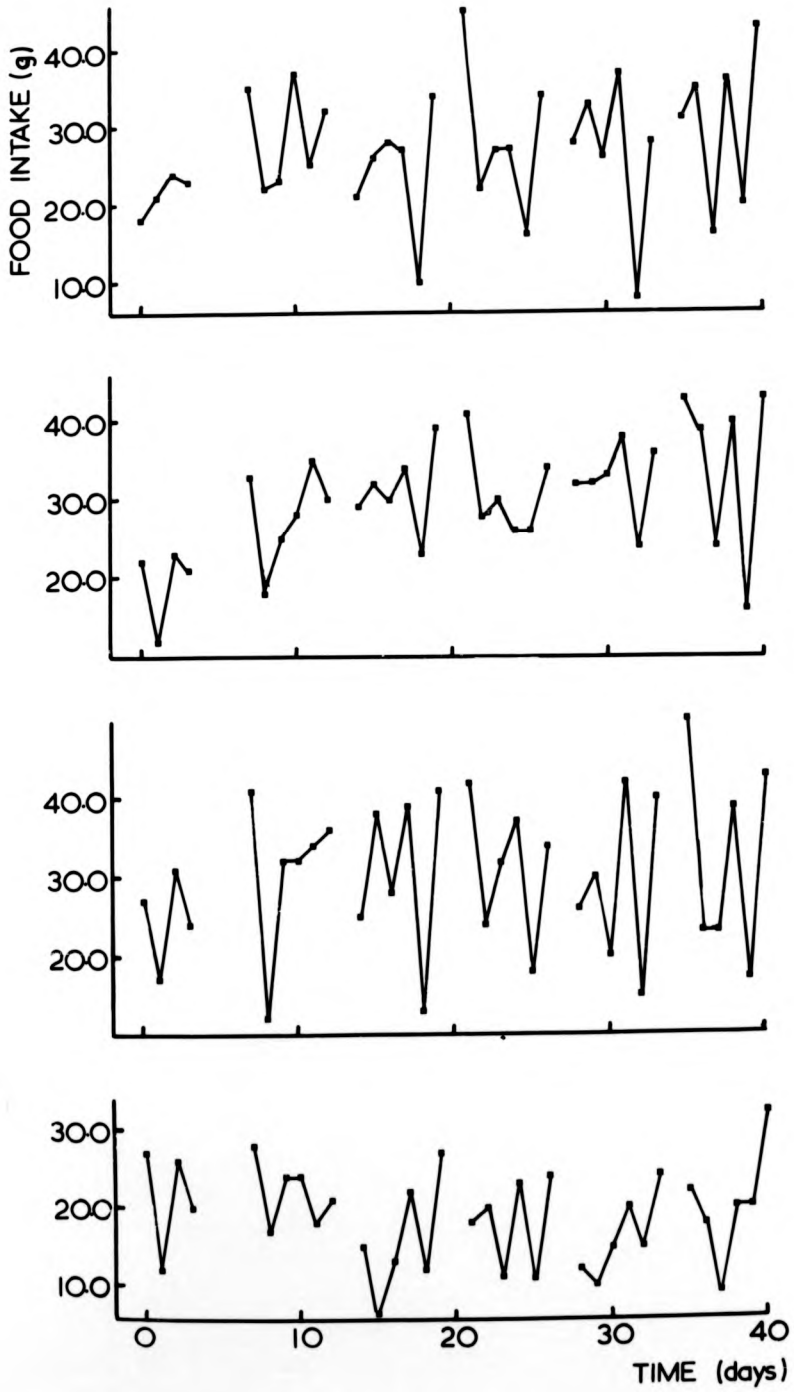


FIGURE 2. The acute effect of salinity on rate of weight increase, expressed as mean weight relative to a mean commencing weight of 1.00. At each of the points marked by an arrow, a water change was carried out. In the case of the experimental group, the salinity of the medium was abruptly changed to that indicated above the arrow, while the control group were kept in fresh water.

y on
pressed
a mean
At
y an
arried
xperi-
of the
to
row,
e kept

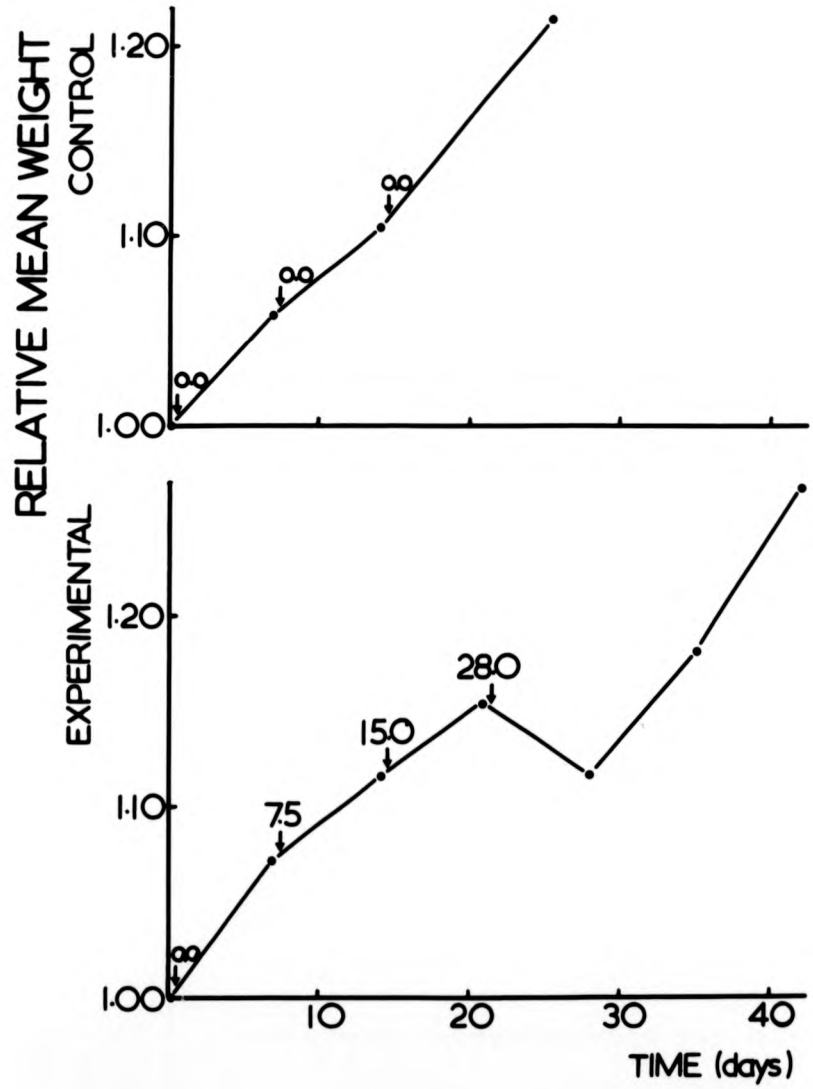
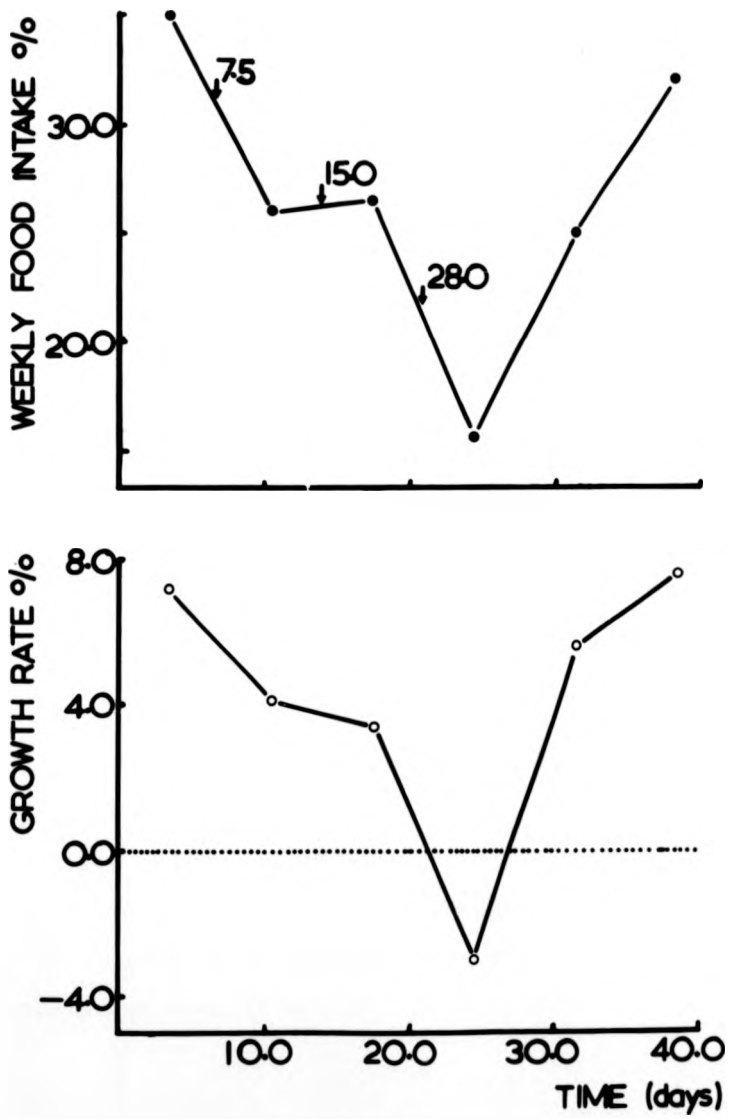


FIGURE 3. The relationship between the acute effects of salinity on (a) weekly food intake and (b) growth rate (% weekly weight increase). Alterations of salinity are indicated as in Fig. 2.

acute

ekly

te (%



3.1.3. Chronic effects of salinity on food intake: As stated in the previous section, three separate experiments were carried out to determine the effect of salinity on appetite in the acclimatized fish. The results of these three experiments are summarised in Table 1 and Appendix 1. Food intake tends towards a maximum at intermediate salinities. The combined results of the three experiments are presented in Fig. 4. Table 1 summarises the statistical significance of differences between food intakes at the five different salinities investigated ('Student's' t-test).

	0	7.5	15.0	28.0
7.5	n.s.	-	-	-
15.0	0.05	n.s.	-	-
28.0	0.05	n.s.	n.s.	-
32.5	0.01	0.05	0.01	0.01

Table 1. A summary of the statistical significance of differences between food intakes in the five salinities investigated.

Food intake was significantly higher in salinities of 15.0 p.p.t. and 28.0 p.p.t. than in fresh water. Food intake in 7.5 p.p.t. was intermediate between fresh water and 15.0 p.p.t., and was significantly different from neither. In a salinity of 32.5 p.p.t., food intake was significantly lower than at any of the other four salinities.

FIGURE 4. Weekly food intake (mean % dry weight ± s.e.) *versus* salinity (p.p.t.) in acclimatised fish.

weight ±

) in

3.2. Effects of salinity on food absorption

Absorption efficiency was calculated in terms of total dry matter, energy and total nitrogen. In all cases, there was a negative linear relationship between salinity and absorption efficiency (Fig. 5). As the method employed in estimating absorption efficiency entailed pooling the individual faecal samples, it is difficult to give an indication of variability for each of the points on which the regressions were based. The standard errors of the nitrogen and energy content determinations implicated in two of the curves are indicated elsewhere in this thesis (Appendix 2).

3.2.1. Dry Matter: Dry matter absorption efficiency fell from 0.89 in fresh water to 0.80 in 32.5 p.p.t. sea water (where y is salinity in parts per thousand and x is absorption efficiency as a fraction of unity, $y = 0.8957 - 0.0028x$). There was a significant negative correlation between salinity and dry matter absorption efficiency ($r = -0.9883$; $d.f. = 2$; $p < 0.05$).

3.2.2. Energy: When absorption efficiency was calculated on the basis of calorific value, a slightly higher value was obtained. Energy absorption efficiency fell from 0.91 in fresh water to 0.83 in 32.5 p.p.t. ($y = 0.9186 - 0.0027x$). There is again a significant negative correlation between salinity and absorption efficiency ($r = 0.9775$; $d.f. = 2$; $p < 0.05$).

FIGURE 5. (a) Food absorption efficiency *versus* salinity (p.p.t.)

● ..... dry matter ($y = 0.8957 - 0.0028x$)

o ..... energy ($y = 0.9186 - 0.0027x$)

▲ ..... total nitrogen ($y = 0.9584 - 0.0019x$)

(b) The relationship between faecal nitrogen content (as % of corrected dry matter [mean ± s.e.]) and salinity ($y = 2.869x^{0.049}$).

cy versus

.8957 - 0.0028x)

.9186 - 0.0027x)

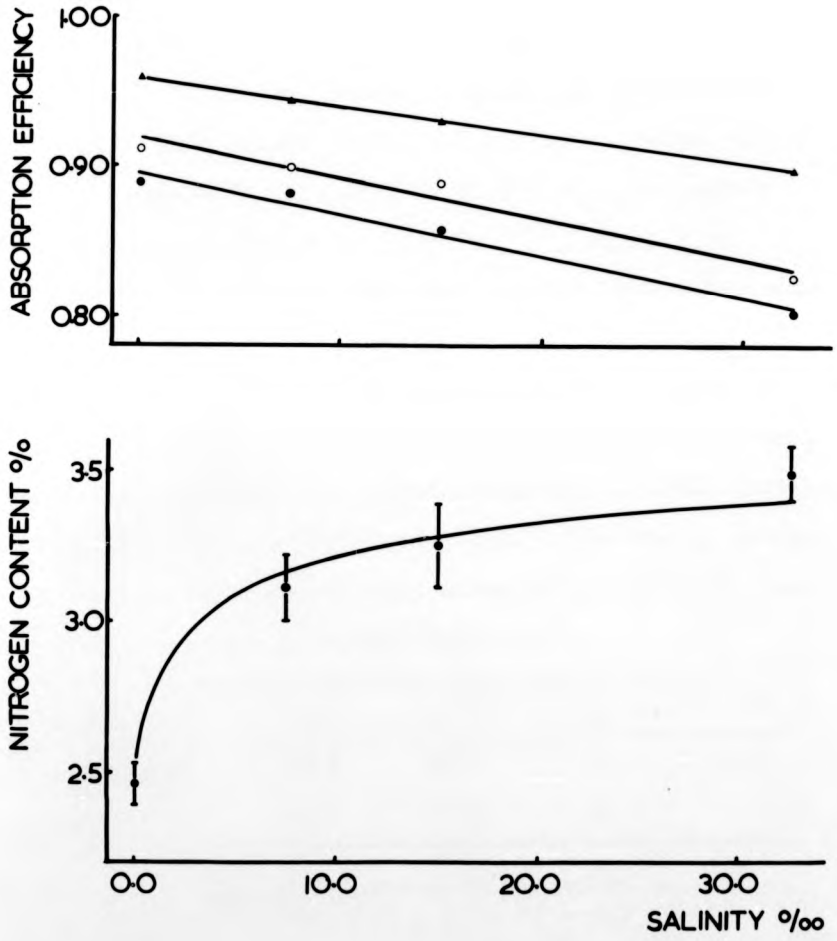
y = 0.9584 -

0.0019x)

faecal

rrected dry

linity



3.2.3. Total Nitrogen: Total nitrogen is absorbed considerably more efficiently than energy. The efficiency with which this component of the food is absorbed varies from 0.96 in fresh water to 0.90 in 32.5 p.p.t. sea water ($y = 0.9584 - 0.0019x$). There is again a significant negative correlation between salinity and absorption efficiency ($r = 0.9767$; d.f. = 2; $p < 0.05$).

It was found that faecal nitrogen content increased exponentially with salinity, from 2.46% of corrected dry weight in fresh water to 3.49% in 32.5 p.p.t. sea water, according to the equation $y = 2.869x^{0.049}$, where x is salinity (p.p.t.) and y is nitrogen content (%). There is a significant positive correlation between $\log_{10}x$ and $\log_{10}y$. ($r = 0.9957$; d.f. = 2; $p < 0.05$). The curve in Fig. 5b is drawn from the regression equation, and the vertical bars represent the standard errors of triplicated micro-Kjeldahl nitrogen determinations. The energy content of the faeces is not appreciably affected by salinity, but ash content increases markedly (Table 2).

Salinity (p.p.t.)	0.0	7.5	15.0	32.5
Energy content %	15.6	16.7	15.2	17.5
Ash content %	18.3	24.4	22.2	27.0

Table 2. Energy and ash contents of faeces in salinities between 0.0 and 32.5 p.p.t. as % dry weight.

3.3. Effects of salinity on food conversion efficiency

$K_1(\frac{\Delta W}{R\Delta t})$ and $K_2\ (\frac{\Delta W}{pR\Delta t})$ were estimated in terms of dry weight, energy and total nitrogen. Conversion of wet weight was not calculated because of the large difference which exists between the water content of fish (73.6%)and food pellets (7.6%). Conversion efficiency was calculated for three groups of fishes (mean weights given to the nearest 5 g)

(1) 0+ year-group, 50-95 g

(2) 0+ year-group, 80-155 g

(3) 1+ year-group, 115-155 g.

In the case of Group (3), conversion efficiency values were obtained at only 3 salinities, 0.0 p.p.t., 7.5 p.p.t., and 28.0 p.p.t. Rather than presenting the results for this group in graphical form, they are therefore presented, in terms of dry weight alone, in the form of a table (Table 3).

Salinity (p.p.t.)	K_1 (dry matter)	s.e.
0.0	0.12	0.03
7.5	0.15	0.03
28.0	0.16	0.02

Table 3. The effect of salinity on conversion efficiency in rainbow trout of the 1+ year-group (115-155 g).

The mean K_1 exhibited by Group (3) was significantly lower than that of trout one year younger but of similar weight ($t = 2.5476$; d.f. = 28; $p < 0.05$). There was no significant correlation between K_1 and salinity ($r = 0.3000$; d.f. = 7; $p > 0.1$).

In both other groups ((1) and (2)), K_1 (dry matter) was found to be little affected by salinity over the range from 0.0 p.p.t. to 28.0 p.p.t. At 32.5 p.p.t., however, $\bar{K}_1$ was significantly lower than $\bar{K}_1$ at the other four salinities.

Group (1) ($t = 3.5336$; d.f. = 18; $p < 0.01$)

Group (2) ($t = 2.1875$; d.f. = 22; $p < 0.05$).

Because of this phenomenon of a sudden decline in conversion efficiency, the salinity range 28.0 p.p.t. to 32.5 p.p.t. was attributed with a curve of a different gradient, intersecting the 0.0 to 28.0 p.p.t. curve where the latter curve has $x = 28.0$.

3.3.1. Dry matter conversion efficiency.

(a) K_1

(i) Group (1) 50-95 g

In this group K_1 fell from 0.24 at 0.0 p.p.t. to 0.18 at 28.0 p.p.t. and 0.14 at 32.5 p.p.t. (Fig. 6a). There was a significant negative correlation between salinity and K_1 over the range 0.0-28.0 p.p.t. ($r = -0.5624$; d.f. = 16; $p < 0.05$). The regression coefficient for the 0.0 to 28.0 p.p.t. line was 0.0020 and that for the 28.0 to 32.5 p.p.t. line was 0.0093. K_1 in fresh water was higher (0.26) than in the case of the larger individuals (Group (2)). This difference was, however, not significant ($t = 1.1484$; d.f. = 9; $p > 0.1$).

(ii) Group (2) 80-155 g

K_1 remained relatively unchanged, at about 0.20, from

FIGURE 6. The relationship between conversion efficiency (K_1 and K_2) and salinity (p.p.t.) in rainbow trout of Group 1 (50 - 95 g). (a) dry matter, (b) energy, (c) total nitrogen.

● ..... K_1

o ..... K_2

version

alinity

Group 1

en.

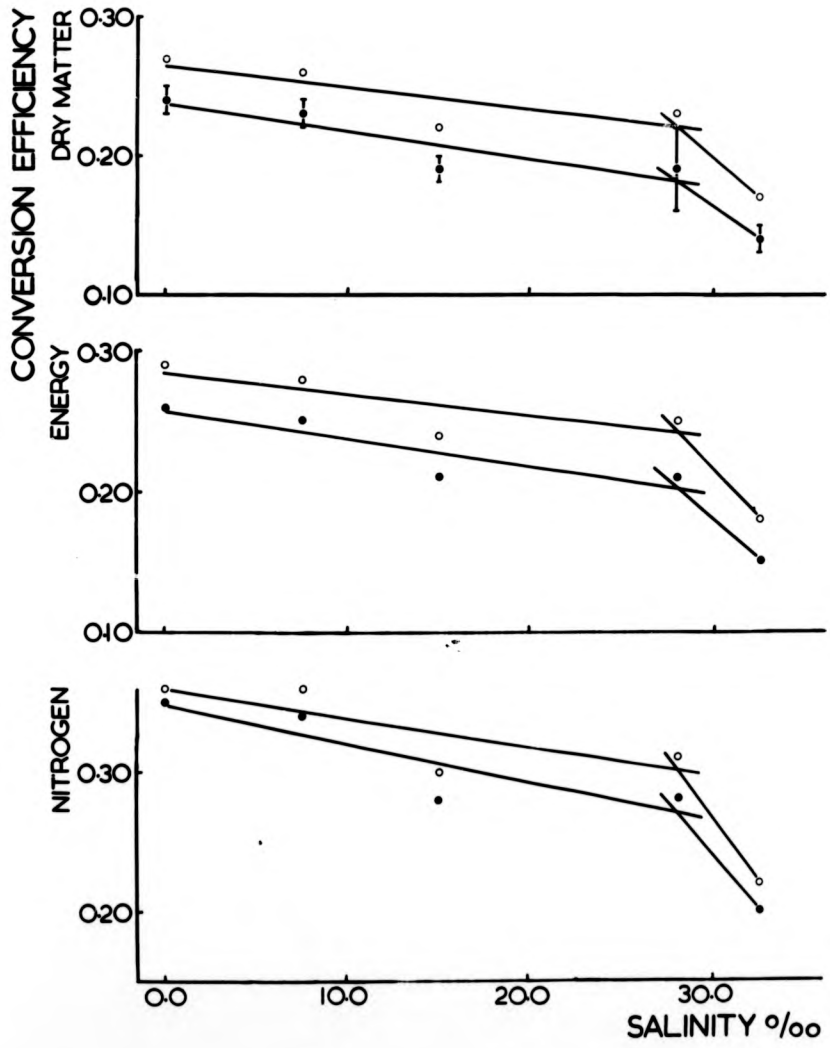


FIGURE 7. The relationship between conversion efficiency (K_1 and K_2) and salinity (p.p.t.) in rainbow trout of Group 2 (80 - 155 g). (a) dry matter, (b) energy, (c) total nitrogen.

● ..... K_1

o ..... K_2

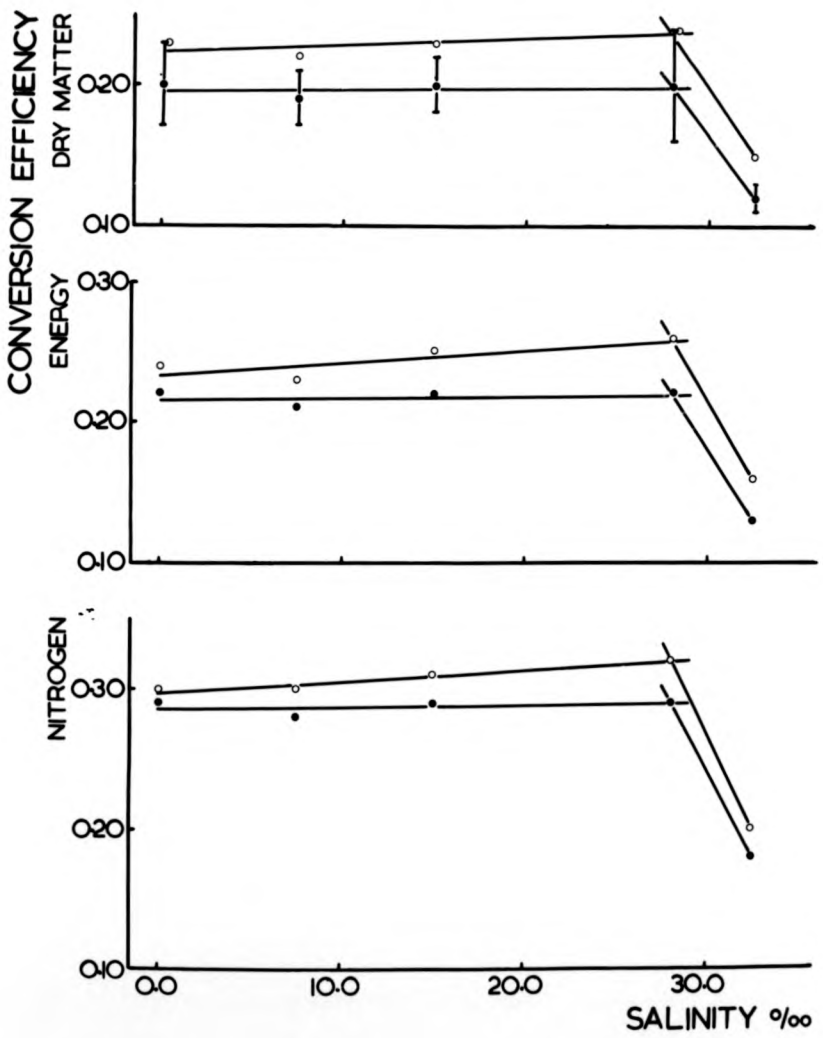
nversion

salinity

f Group 2

er,

ogen.



0.0 to 28.0 p.p.t., but fell to 0.12 in 32.5 p.p.t. sea water (Fig. 7a). There was no significant correlation between salinity and conversion efficiency K_1 over the range 0.0 to 28.0 p.p.t. ($r = 0.0720$; d.f. = 22; $p > 0.1$). The positive gradient over this range was negligible (regression coefficient = 0.0001), but there was a steep decline from 28.0 to 32.5 p.p.t. (regression coefficient = 0.0176). There was no significant difference between the conversion efficiency gradients exhibited by Groups (1) and (2) over the range from 0.0 to 28.0 p.p.t. ($t = 1.5333$; d.f. = 35; $p > 0.05$).

(b) K_2

In all calculations of K_2, p for the appropriate salinity was obtained from absorption efficiency experiments. As p for 28.0 p.p.t. was not directly determined, the required value was interpolated from the absorption efficiency/salinity curve (Fig. 5a) for either dry weight, energy or nitrogen. K_2 was derived, at each salinity, from the mean value of K_1.

(i) Group (1)

As could be predicted when absorption is not totally efficient, K_2 was slightly higher than K_1 at all salinities, varying from 0.27 at 0.0 p.p.t. to 0.22 at 28.0 p.p.t. and 0.17 at 32.5 p.p.t. ($\bar{K}_2 - \bar{K}_1 = 0.032$) (Fig. 6a). Because of the measured decrease in absorption efficiency with salinity, K_2 diverges increasingly from K_1 over the range 0.0 to 28.0 p.p.t. The regression coefficient of K_1 is -0.0020 while that of K_2 is -0.0016.

(ii) Group (2)

K_2 varies from 0.22 at 0.0 p.p.t. to 0.24 at 28.0 p.p.t. and 0.15 at 32.5 p.p.t., is higher than K_1 ($\bar{K}_2-\bar{K}_1$ = 0.0322), and diverges from it as salinity increases (Fig. 7a). From 0.0 to 28.0 p.p.t., the regression coefficient for K_1 is 0.0001 while the equivalent value for K_2 is 0.0005.

3.3.2. Energy conversion efficiency

Energy conversion efficiency was derived from the means of the dry weight conversion efficiency by the use of determinations of the energy content of whole fish and food (Appendix 3). In neither group of fish was energy conversion efficiency (K_1) significantly different from dry weight conversion efficiencies.

Group (1) (50-95 g) $t = 0.7142$; d.f. = 8; $p > 0.1$.

Group (2) (80-155 g) $t = 0.7929$; d.f. = 8; $p > 0.1$.

This result is to be expected in the light of the similarity between the calorific value of dry food (20.76 $\pm$ s.e. 0.52) and whole dry fish (22.77 $\pm$ 0.21).

(a) K_1

(i) Group (1)

In this group, K_1 was estimated to vary from 0.26 at 0.0 p.p.t. to 0.20 at 28.0 p.p.t. and 0.15 at 32.5 p.p.t. The regression equation of K_1 (energy), $y = 0.2578-0.0020x$ was very similar to the equation of K_1 (dry weight), $y = 0.2378-0.0020x$ (Fig. 6b).

(ii) Group (2)

K_1 remained at about 0.22 from 0.0 to 28.0 p.p.t.,

falling to 0.13 at 32.5 p.p.t. As stated above, energy conversion efficiency was not significantly different from dry weight conversion efficiency, and the regression equation of K_1 (energy), y = 0 2762 + 0.0001x was again closely similar to the equation of K_1 (dry weight), y = 0.1962 + 0.0001x (Fig. 7b).

(b) K_2

(i) Group (1)

Energy conversion K_2 varied from 0.28 at 0.0 p.p.t. to 0.24 at 28.0 p.p.t. and 0.18 at 32.5 p.p.t., was higher than K_1 ($\bar{K}_2$-$\bar{K}_1$ = 0.032), and diverged slightly from it as salinity increased. The regression coefficients for K_1 and K_2 were -0.0020 and -0.0016 respectively (Fig. 6b).

(ii) Group (2)

K_2 varied from 0.23 at 0.0 p.p.t. to 0.26 at 28.0 p.p.t. and 0.16 at 32.5 p.p.t. The pattern of vertical translation above ($\bar{K}_2$-$\bar{K}_1$ = 0.028) and divergence from K_1 is again present (Fig. 7b). The regression coefficient of K_1 is 0.0001 while that of K_2 is 0.0009.

3.3.3. Nitrogen conversion efficiency

Nitrogen conversion efficiency was estimated from the means of dry weight conversion efficiency by the use of nitrogen content figures for food and whole fish presented elsewhere (Appendix 3). In both groups of trout (1) and (2), nitrogen conversion efficiency (K_1) was significantly higher than both dry weight and energy conversion efficiency.

Group (1) $t = 3.2421$; d.f. = 13; $p < 0.01$

Group (2) $t = 3.4246$; d.f. = 13; $p < 0.01$

This result is due to the higher nitrogen content of the fish than the food. The nitrogen content of whole rainbow trout was estimated from a sample of 10 individuals to be 10.19 $\pm$ s.e. 0.21% of total dry weight, while the nitrogen content of the food pellets was 6.97 $\pm$ s.e. 0.15% dry weight, which indicates that, for a given conversion of dry matter, there is a higher conversion of nitrogenous matter.

(a) K_1

(i) Group (1)

K_1 varies from 0.35 at 0.0 p.p.t. to 0.27 at 28.0 p.p.t. and 0.20 at 32.5 p.p.t. As already noted, K_1 (nitrogen) is significantly higher than K_1 (dry weight). For K_1 (nitrogen), from 0.0 to 28.0 p.p.t., $y = 0.3479 - 0.0028x$ (Fig. 6c).

(ii) Group (2)

K_1 remains at 0.29 from 0.0 to 28.0 p.p.t. and falls to 0.18 at 32.5 p.p.t. K_1 (nitrogen) is again significantly higher than K_1 (dry weight). For K_1 (nitrogen) (Fig. 7c) from 0.0 to 28.0 p.p.t., $y = 0.2862 + 0.0001x$.

(b) K_2

(i) Group (1)

K_2 varies from 0.36 at 0.0 p.p.t. to 0.30 at 28.0 p.p.t. and 0.22 at 32.5 p.p.t. The translation of K_2 above K_1 ($\bar{K}_2 - \bar{K}_1 = 0.0200$) is less than for both dry weight and energy, because of the higher absorption

efficiency of nitrogen at all salinities. As before, the K_2 curve diverges from that for K_1 (Fig. 6c). For K_2, the regression coefficient is 0.0008, while for K_1 it is 0.0001.

(ii) Group (2)

K_2 in this case varies from 0.30 at 0.0 p.p.t. to 0.32 at 28.0 p.p.t. and 0.20 at 32.5 p.p.t. ($\bar{K}_2-\bar{K}_1 = 0.020$). The K_2 curve diverges from the curve of K_1, the respective regression coefficients being 0.0008 and 0.0001 (Fig. 7c).

3.4. Effects of ration on food conversion efficiency

As results to be presented later will indicate, sodium chloride dietary supplementation was found to have no effect on conversion efficiency. The results of dietary supplementation experiments were therefore pooled to provide information on the effects of ration level on conversion efficiency.

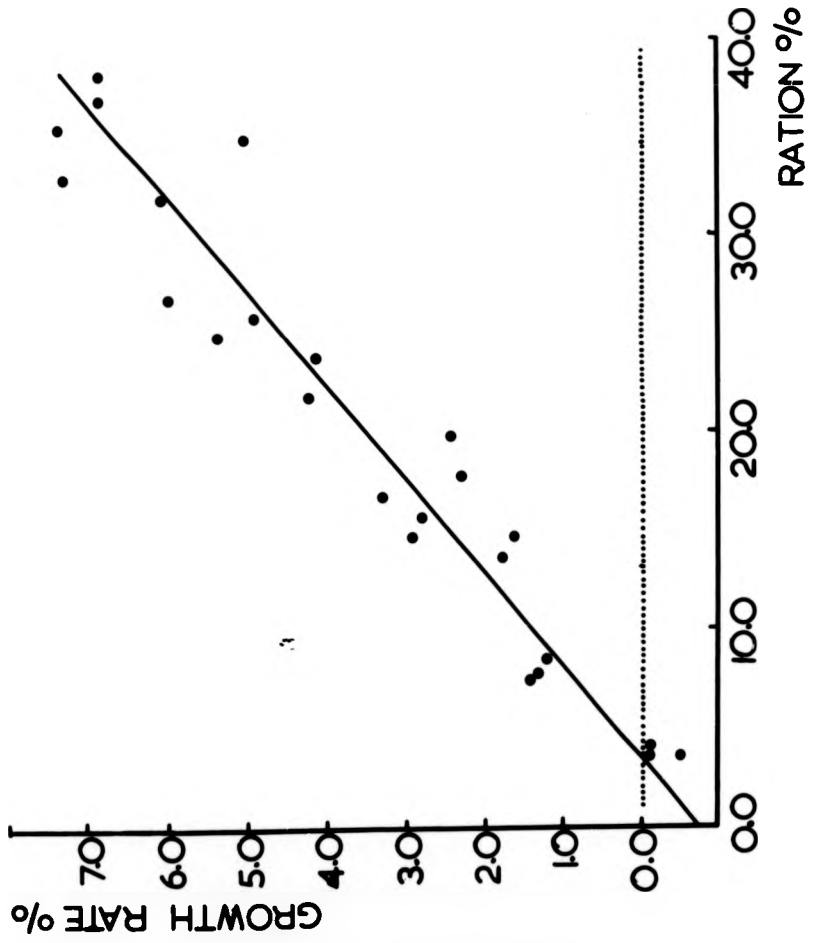
Weight increase (% increase in dry weight per week) was plotted against ration (on a % dry weight basis), corrected for the diluent effect of added NaCl (Fig. 8). A straight line relationship was obtained over the range between rations of 3.82 and 38.23% ($y = 0.2115x-0.7699$). The lowest ration led to a loss of weight. It is possible to interpolate the maintenance ration (i.e. the ration which would produce no change in weight) from the regression line.

When $y = 0$, $0.2115x = 0.7699$

$\therefore \quad x = 3.64$

FIGURE 8. The relationship between growth rate (as % increase in dry weight per week) and ration (on a % dry weight basis). ($y = 0.2115x - 0.7699$).

growth rate
ght per
dry weight
.7699).



The interpolated maintenance ration is thus 3.64% per week.

It should also be possible to estimate the rate of weight loss during starvation by extrapolating to the intercept on the y axis.

When $x = 0$, $y = -0.7699$

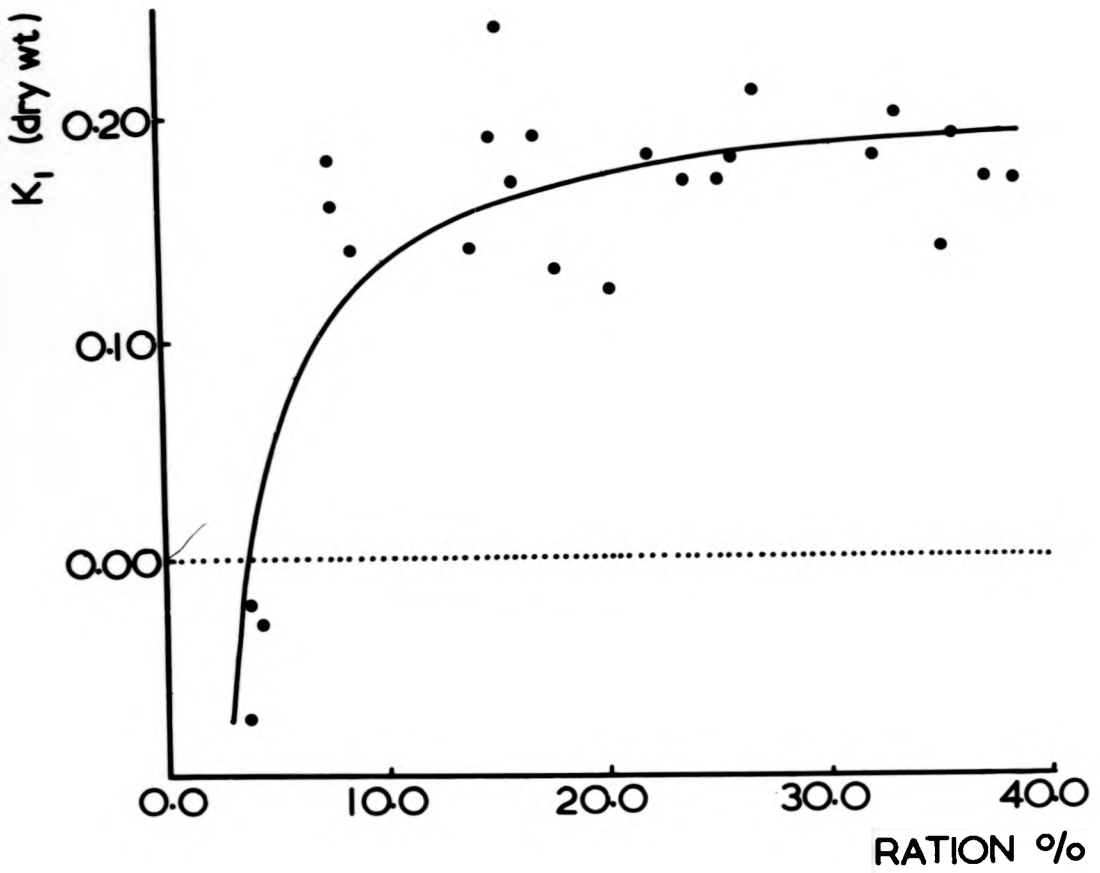
Thus, fish of this size, under similar conditions, might be expected to lose 0.7699% of their dry weight per week, at least during the first 2 weeks of food deprivation. This estimate is in good agreement with results of starvation experiments to be described later in this thesis.

Conversion efficiency (K_1 dry weight) was lower than the comparable value found with floating pellets in the salinity/conversion efficiency experiments (0.18 at a ration of 25% per week, as compared with about 0.20). This may be due, at least in part, to differences in the composition of the foods (energy content: 17.87 $J.mg^{-1}$ compared with 20.76 $J.mg^{-1}$; nitrogen content: 6.40% of dry weight compared with 6.97%) but, as already described, experimental conditions were not identical.

It is apparent from Fig. 8 that there must be a close relationship between K_1 and % ration. There is no correlation between K_1 and either absolute ration or body weight. K_1 (dry weight) was plotted against % ration (Fig. 9). The equation describing this relationship can be derived from the equation relating % weight increase (y) and % ration (x).

FIGURE 9. The relationship between K_1 (dry matter) and weekly ration as % of dry weight. ($y = 0.2115 - \frac{0.7699}{x}$)

FIGURE 9. The relationship between K_1 (dry matter) and weekly ration as % of dry weight. ($y = 0.2115 - \frac{0.7699}{x}$)


K_1 (dry wt.)
0.20
0.10
0.00
0.0
10.0
20.0
30.0
40.0
RATION %

$$K_1 = \frac{y}{x}$$

but $y = 0.2115x - 0.7699$

$$\therefore \quad K_1 = \frac{0.2115x - 0.7699}{x}$$

$$= 0.2115 - \frac{0.7699}{x}$$

The observed values of K_1 show a good fit with this equation. K_1 rises from 0.00 at 3.64% dry weight per week to 0.13 at 10.0% and 0.19 at 38.0%.

It has been shown by Winberg (1956), Paloheimo and Dickie (1966a) and earlier workers, that it is possible to estimate metabolic rate from measurements of ration and growth rate. T (total metabolic rate) was in this case estimated from the relationship between K_1 and ration (x).

where T is total metabolism

k_1 is a constant converting ration as % dry weight per week to joules per kg live weight per hour

x is ration (% dry weight per week)

p is energy absorption efficiency (fresh water)

K_1 is conversion efficiency (dry weight)

$k_1 = 276.55$

$p = 0.91$

$T\ (J.kg^{-1}.h^{-1}) = k_1 x\ (0.91 - K_1)$

but $K_1 = 0.2115 - \frac{0.7699}{x}$

$$\therefore \quad T = k_1 x(0.91 - 0.2115 + \frac{0.7699}{x})$$

$$= (0.91 - 0.2115)k_1 x + 0.7699k_1$$

$$= 0.6985k_1 x + 0.7699\ k_1$$

$$T\ (J.kg^{-1}.h^{-1}) = 193.17x + 212.92$$

where k_2 is a constant relating joules to mg)$_2$[1 mg O_2 = 3.42 cal (Brody 1945) = 14.34 joules]

$$T(\text{mg } O_2 kg^{-1}.h^{-1}) = 193.17\ k_2 x + 212.92\ k_2$$

$$k_2 = \frac{1}{14.34} = 0.06974$$

$$\therefore T\ (\text{mg } O_2\ kg^{-1}.h^{-1}) = 13.47x + 14.85$$

Estimated oxygen consumption rises linearly from 63.88 mg O_2 $kg^{-1}.h^{-1}$ at a ration of 3.64% per week (weight maintenance) to 526.71 mg O_2 $kg^{-1}.h^{-1}$ at a ration of 38.0% per week (twice-daily satiation). Estimated oxygen consumption therefore increases by 8.25 times for an increase in % food intake by 10.5 times.

3.5. Effects of dietary sodium chloride on food intake and conversion efficiency

The effects on food intake of feeding a dietary sodium chloride supplement are summarized in Table 4.

SALT SUPPLEMENT (as % total wt)	WEEKLY FOOD INTAKE (as % dry weight)	ADJUSTED WEEKLY FOOD INTAKE (corrected for added NaCl)	CONVERSION EFFICIENCY (K_1)
0.0	28.81	26.73	0.16
4.9	27.20	25.78	0.18
8.5	27.60	25.07	0.17

Table 4. The effect of dietary sodium chloride on food intake and conversion efficiency.

Food intake is expressed as a percentage of fish dry weight. The fish were fed to satiation twice daily for 14 days, during which time the mean water temperature was 4.23°C.

Food intake observations were adjusted to take account of the diluent effect of the added sodium chloride. (A given weight of food containing salt has a lower nutrient content than the same weight of food without salt). Despite two feeding sessions per day instead of one, and the somewhat smaller fish, % food intake was similar to that recorded in the controlled-temperature 10°C experimental tanks (mean about 26% per week compared with about 23%), probably because of the lower water temperature. It can be seen from Table 4 that food intake in rainbow trout, under the particular experimental conditions described, was unaffected by increases in dietary salt of up to 8.5% of total ration.

Table 4 presents mean conversion efficiencies (K_1) calculated on the basis of corrected dry weight for seven 14-day periods on rations of between 8% and 38% dry weight per week. One 14-day period, during which the ration was fixed at a level very close to weight maintenance, was not included in the computation.

Mean conversion efficiency (K_1), like appetite, changes little with sodium chloride supplementation. There was no statistically significant variation. There was also no indication that altering the ration influenced the effect, if any, of sodium chloride supplementation on conversion efficiency.

3.6. Effects of salinity on fasting rainbow trout

3.6.1. Weight loss during fasting: The effects of salinity on rate of weight loss were studied in two size ranges of trout, in two separate experiments. Experiment 1

was carried out with groups of initially 18-20 trout with group mean weights of 100-155 g and Experiment 2 with groups of 13-16 trout with group mean weights of 80-95 g. These weight ranges correspond with groups (2) and (1) respectively of the individuals studied in the earlier experiments on the effects of salinity on food intake and conversion efficiency.

Experiment 1 (100-155 g): To facilitate direct comparison between treatments, loss of weight was measured in relation to a mean weight, at the beginning of the starvation period, of 1.00.

Trout in fresh water, 7.5 p.p.t. and 15.0 p.p.t. lost weight at approximately equal rates, attaining a relative weight of 0.89 after 48 days of food deprivation (Fig. 10). After about 35 days of fasting, the trout maintained in 32.5 p.p.t. sea water began to show signs of distress. These fish were sacrificed on or before day 37, by which time they had attained a considerably lower relative weight (0.76) than those in the other three salinities. At the previous weighing, on day 19, the trout in 32.5 p.p.t. sea water had not shown any sign of more rapid weight loss.

With the exception of the 32.5 p.p.t./37 day point, it was found that, over the period from 7 to 48 days, rate of weight loss could be adequately described by a straight line with the equation $y = 0.9794 - 0.0019x$ (Fig. 10). Between days 0 and 7, weight loss appeared to be somewhat more rapid, with a gradient of 0.0048.

Experiment 2 (80-95 g): In this experiment, only two weighings were made during the deprivation period, but, in

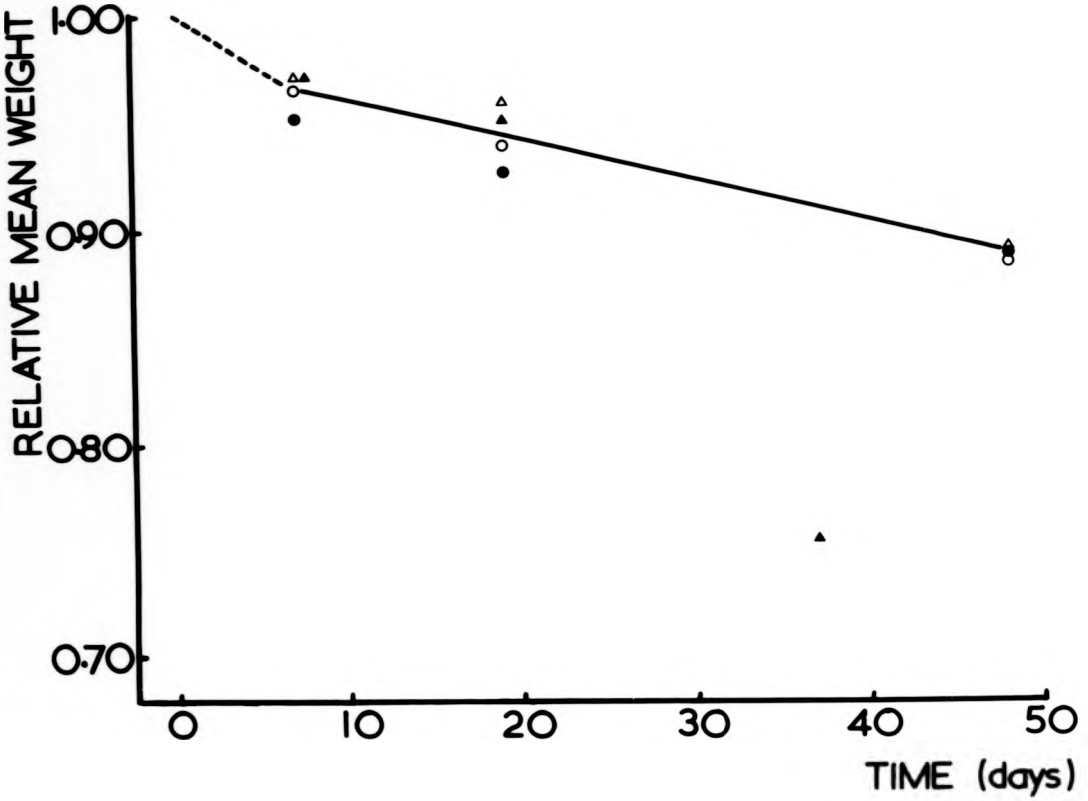
FIGURE 10. Relative live weight loss in rainbow trout of 100 - 155 g maintained without food in four different salinities. ($y = 0.9794 - 0.0019x$).

○ ..... fresh water

● ..... 7.5 p.p.t.

△ ..... 15.0 p.p.t.

▲ ..... 32.5 p.p.t.


RELATIVE MEAN WEIGHT
1·00
0·90
0·80
0·70
0
10
20
30
40
50
TIME (days)

the light of the results from Experiment 1, it was thought that, although the results might well be describable by a straight line intersecting the y axis at 1.00, it would be more valid to assume a change of gradient at x = 7 days. A regression was therefore calculated on the basis of the 23 and 48 day weighings and extrapolated to x = 7 days (Fig. 11). On day 23, the result for 32.5 p.p.t. fell within the 95% confidence limits of the remaining three salinities but on day 48 the relative mean weight of fish in 32.5 p.p.t. sea water fell outwith these limits. The calculated regression line therefore includes the 32.5 p.p.t. result at 23 days, but excludes it at 48 days. The mean relative weight of trout in fresh water, 7.5 p.p.t. and 15.0 p.p.t. fell to about 0.85 in 48 days, according to the equation $y = 0.9825 - 0.0028x$, whereas that of trout maintained in 32.5 p.p.t. sea water fell to 0.77. Especially if the results of Experiment 1 are considered, it cannot be assumed that fish in 32.5 p.p.t. lose weight more rapidly than fish in the other salinities throughout starvation. It is clear, however, that over the period from days 20 to 48 rainbow trout in 32.5 p.p.t. sea water lose weight considerably more rapidly than those in the other three treatments. The fish in Experiment 2 (i.e. the smaller fish) showed a significantly greater rate of relative weight loss than those in Experiment 1 ($t = 3.0000$; d.f. = 14; $p < 0.01$).

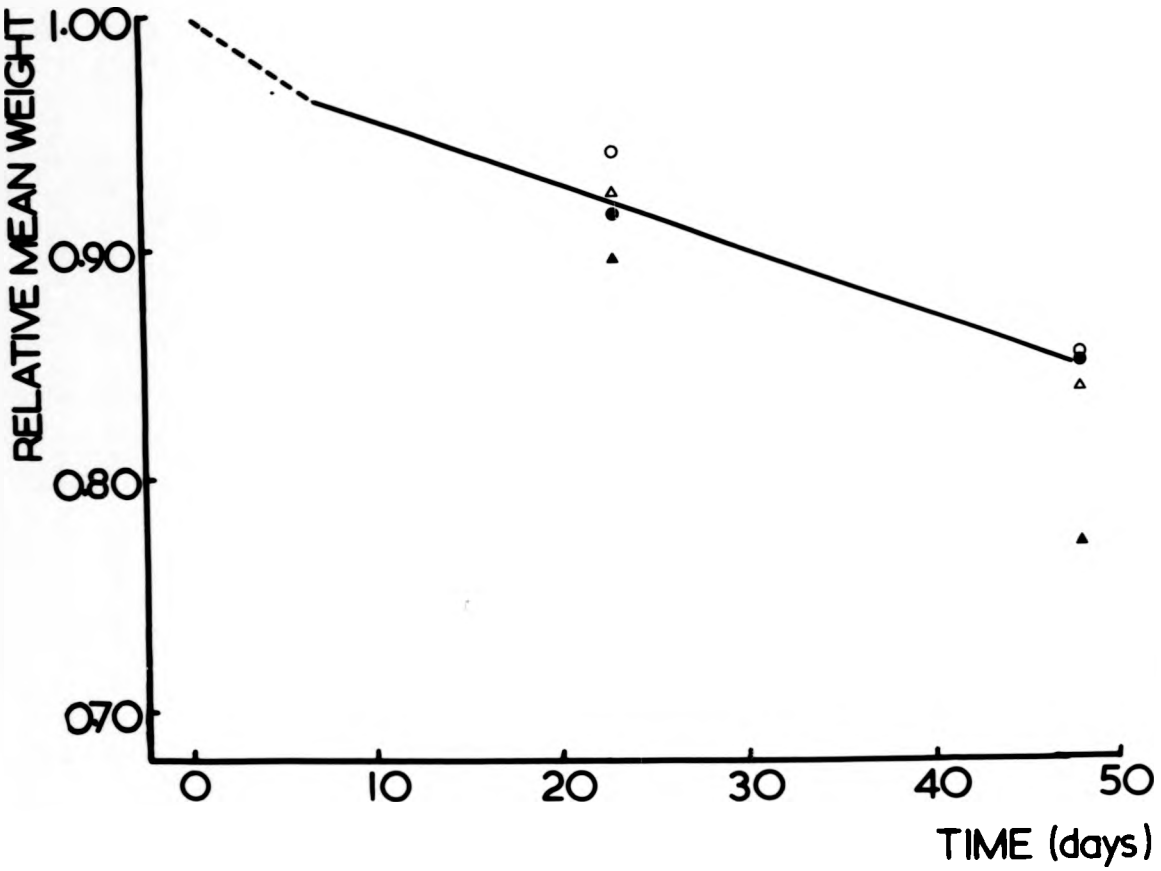
FIGURE 11. Relative live weight loss in rainbow trout of 80 - 95 g maintained without food in four different salinities ($y = 0.9825 - 0.0028x$)

○ ..... fresh water

● ..... 7.5 p.p.t.

△ ..... 15.0 p.p.t.

▲ ..... 32.5 p.p.t.


RELATIVE MEAN WEIGHT
1.00
0.90
0.80
0.70
0
10
20
30
40
50
TIME (days)

3.6.2. Decrease in condition factor during fasting:
Condition factor, $CF = \frac{100W}{L^3}$, where W is weight (g) and L is fork length (cm) (Brown 1957). Condition factor was calculated for each individual in starvation experiment (2) at each weighing, and plotted against time (Fig. 12). There is a significant decrease in mean condition factor, from about 1.15 to about 0.96, with time of depletion. ($r = -0.9760$; d.f. = 10; $p < 0.001$). Over the period studied, the decrease was linear. Condition factor tended to decrease more rapidly in trout maintained in 32.5 p.p.t. sea water than in trout maintained in water of the three remaining salinities, but this tendency was not statistically significant. (The significance test employed was based on individual determinations of condition factor and gave $t = 0.5384$; d.f. = 35; $p > 0.05$). A common regression equation (Fig. 12) was calculated for all four salinities, $y = 1.1538 - 0.0040x$.

3.6.3. Lipid and water content of certain tissues before and during fasting:
(a) White epaxial muscle: Extracted lipids accounted for an overall mean of 0.62% of the total wet weight of white muscle tissue in the unstarved fish, which fell to an overall mean of 0.47% in fish which had been starved for 48 days. ($t = 2.1877$; d.f. = 33; $p < 0.05$). There was no significant difference between salinity treatments either before or during starvation.

In the same tissue samples, water content increased from an overall mean of 77.7% to an overall mean of 78.8%

3.6.2. Decrease in condition factor during fasting: Condition factor, $CF = \frac{100W}{L^3}$, where W is weight (g) and L is fork length (cm) (Brown 1957). Condition factor was calculated for each individual in starvation experiment (2) at each weighing, and plotted against time (Fig. 12). There is a significant decrease in mean condition factor, from about 1.15 to about 0.96, with time of depletion. ($r = -0.9760$; d.f. = 10; $p < 0.001$). Over the period studied, the decrease was linear. Condition factor tended to decrease more rapidly in trout maintained in 32.5 p.p.t. sea water than in trout maintained in water of the three remaining salinities, but this tendency was not statistically significant. (The significance test employed was based on individual determinations of condition factor and gave $t = 0.5384$; d.f. = 35; $p > 0.05$). A common regression equation (Fig. 12) was calculated for all four salinities, $y = 1.1538 - 0.0040x$.

3.6.3. Lipid and water content of certain tissues before and during fasting:

(a) White epaxial muscle: Extracted lipids accounted for an overall mean of 0.62% of the total wet weight of white muscle tissue in the unstarved fish, which fell to an overall mean of 0.47% in fish which had been starved for 48 days. ($t = 2.1877$; d.f. = 33; $p < 0.05$). There was no significant difference between salinity treatments either before or during starvation.

In the same tissue samples, water content increased from an overall mean of 77.7% to an overall mean of 78.8%

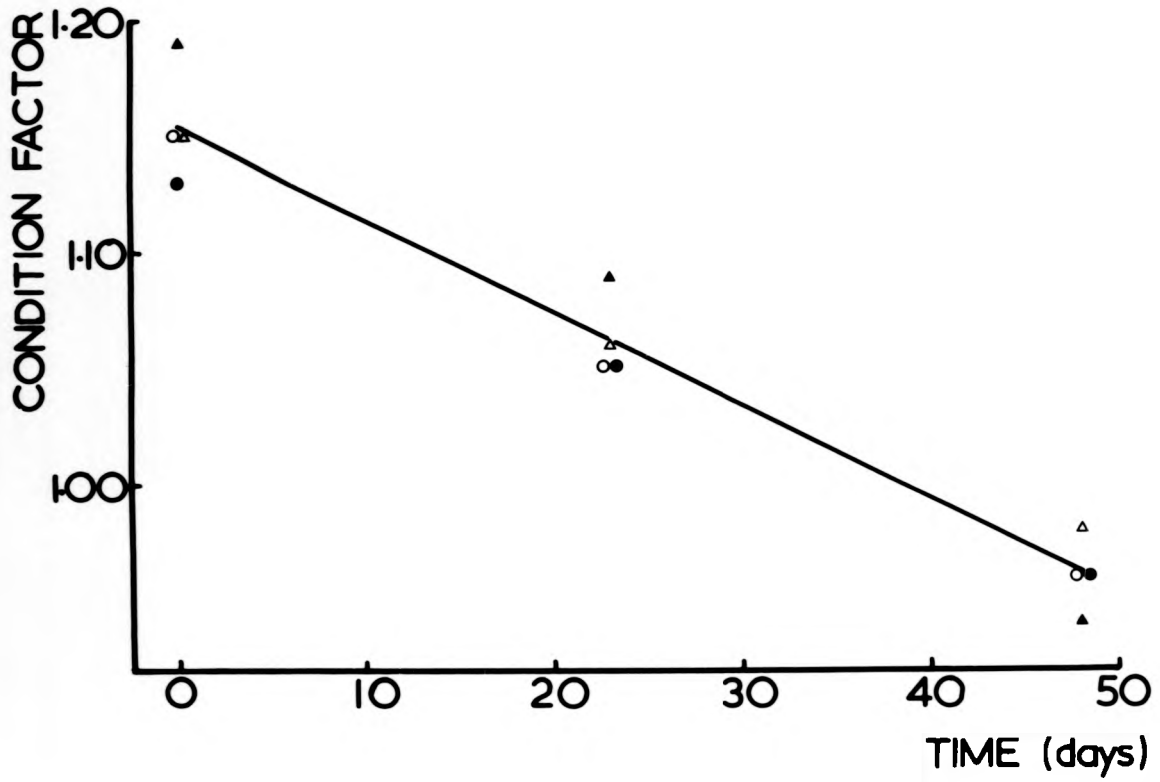
FIGURE 12. The relationship between condition factor ($\frac{100\ W}{L^3}$) and duration of fasting, at various salinities, in rainbow trout of 80 - 95 g.
($y = 1.1538 - 0.0040x$)

○ ..... fresh water

● ..... 7.5 p.p.t.

△ ..... 15.0 p.p.t.

▲ ..... 32.5 p.p.t.


CONDITION FACTOR
1·20
1·10
1·00
0
10
20
30
40
50
TIME (days)

during 48 days of starvation. With the exception of the individuals maintained in 32.5 p.p.t. sea water, there was again no significant difference between treatments. In the case of the individuals kept at 32.5 p.p.t. epaxial muscle water content fell from 78.3% on day 19 to 74.1% on day 37, when the fish in this salinity were beginning to show signs of distress and had to be sacrificed. This decrease was significant ($t = 3.4719$; $d.f. = 8$; $p < 0.05$).

The pooled results from individuals maintained in fresh water, 7.5 p.p.t. and 15.0 p.p.t. indicate that there is a statistically significant negative correlation between water content and extracted lipid ($r = -0.64$; $d.f. = 45$; $p < 0.001$) (Fig. 15b).

(b) Liver: There was no apparent trend for the weight of the liver, either in absolute or in relative terms, to change during 48 days of starvation. Extracted lipids, however, fell from an overall mean of 1.83% to an overall mean of 1.18% after 19 days of starvation. This decrease is significant ($t = 6.6420$; $d.f. = 38$; $p < 0.001$). Between days 19 and 48, there was a relative increase to an overall mean of 1.35% ($p > 0.05$) (Fig. 14b). Salinity did not affect extracted lipid estimates before or during starvation.

Liver water content increased slightly (but not significantly) during starvation, from about 75.0% to about 75.5%, but the liver water content of individuals kept in 32.5 p.p.t. sea water decreased from 75.0% on day 19 to 74.0% on day 37 ($p > 0.05$). In the case of the liver, there was no correlation between extracted lipid and

FIGURE 13. (a) n-hexane extracted lipid as % of wet weight in epaxial white muscle during fasting at various salinities (mean ± s.e.)

(b) water content of epaxial white muscle during fasting at various salinities (mean ± s.e.)

○ ..... fresh water

● ..... 7.5 p.p.t.

△ ..... 15.0 p.p.t.

▲ ..... 32.5 p.p.t.

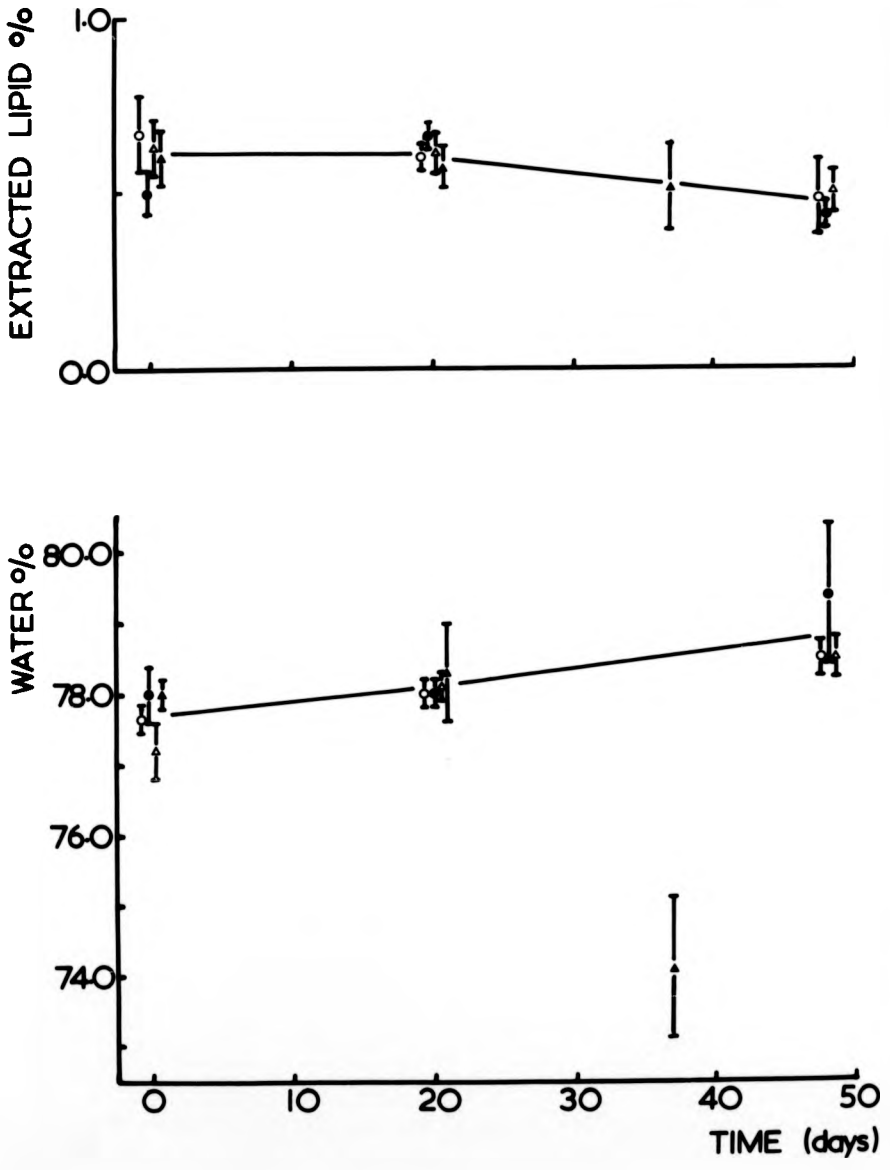

EXTRACTED LIPID %
1·0
0·0
WATER %
80·0
78·0
76·0
74·0
0
10
20
30
40
50
TIME (days)

FIGURE 14. (a) n-hexane extracted lipid as % of wet weight of liver during fasting at various salinities (mean ± s.e.)

(b) water content of the liver during fasting at various salinities (mean ± s.e.)

○ ..... fresh water

● ..... 7.5 p.p.t.

△ ..... 15.0 p.p.t.

▲ ..... 32.5 p.p.t.

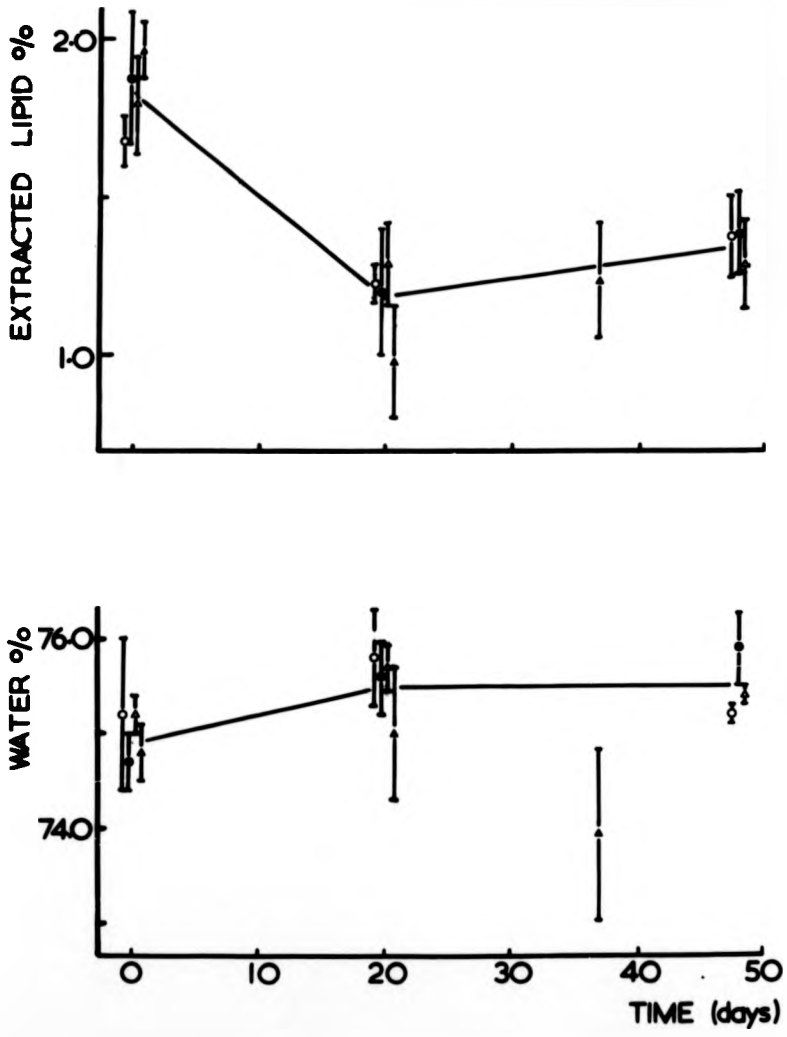
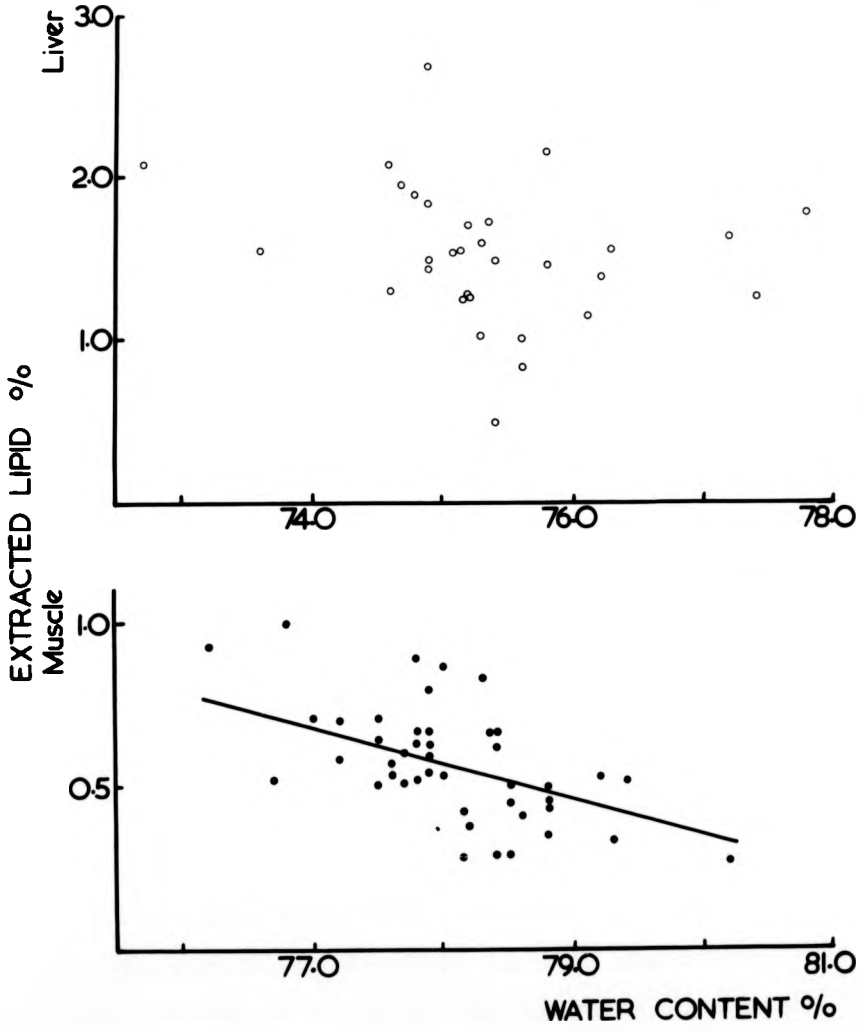

EXTRACTED LIPID %
2·0
1·0
WATER %
76·0
74·0
0
10
20
30
40
50
TIME (days)

FIGURE 15. n-hexane extracted lipid as % of total wet weight versus water content as %. (a) liver, (b) epaxial white muscle.


Liver
3.0
2.0
1.0
74.0
76.0
78.0
EXTRACTED LIPID %
Muscle
1.0
0.5
77.0
79.0
81.0
WATER CONTENT %

water content ($r = 0.21$; d.f. = 45; $p > 0.05$) (Fig. 15a). As in cod (Love, 1958), the gall bladder became noticeably distended during starvation and the bile assumed a denser coloration than in normally-fed trout.

(c) Visceral adipose deposits: In starvation experiment 2 it was planned to study the depletion of visceral adipose deposits during starvation at different salinities. In practice, however, it was found that the initial deposits in the individuals used in this experiment were not at a sufficiently high level to make this study useful. Some pilot observations, carried out on rainbow trout which had either been starved or had been fed at several ration levels over a long period, had indicated that lipid contents of up to 40.6% of total gut weight could be expected. At the beginning of starvation experiment 2, however, the mean lipid store amounted to only 4.37 $\pm$ s.e. 1.20. Lipid content was determined as a percentage of the total fresh weight of the alimentary canal, together with attached adipose deposits. The liver and spleen were detached before weighing and extraction. On the basis of the preliminary observations, in which considerable variation had been observed in visceral fat deposits in response to feeding régime, it had been hypothesized that, as in brown trout Salmo trutta (Swift, 1955), these deposits would constitute important reserves of energy during depletion and would be utilised in preference to lipids contained in other tissues including liver and muscle.

There was a very highly significant correlation

between extracted lipid and water content (both expressed as % of total fresh weight) in the alimentary canal and the associated adipose deposits ($r = -0.99$; d.f. = 60; $p < 0.001$: $y = 94.42 - 1.16x$ where x is water content % and y is extracted lipid %) (Fig. 16). These computations were based on the combined results of the preliminary observations, of starvation experiment (2) and of determinations carried out on rainbow trout sampled from a commercially-operated fish farm at Otter Ferry, Argyllshire. Results from these three sources showed a high degree of homogeneity. There appears to be a tendency for the rate of decrease in lipid content with increase in water content to decline when lipid content reaches about 2.0% (water content = 79.5% approx.).

3.7. Keep-net; Airthrey Loch

In Fig. 17a,b mean length, mean weight and mean temperature are plotted against time in days. Mean temperature during the course of the period of study varied between 3.12°C and 10.40°C. There was a tendency for the variance about sample mean weights to increase with time. This phenomenon was much more conspicuous in controlled salinity/growth experiments, in which the growth of complete experimental populations was studied, and can be explained as the result of sustained individual differences in growth rate caused either by behavioural traits or differences in the physiological capacity for growth. Increase in both weight and length, as far as could be

FIGURE 16. The relationship between extracted lipid (as % of total wet weight) and water content in the alimentary canal and associated adipose deposits ($y = 94.42 - 1.16x$)

● .....Aquarium

o .....Otter Ferry

EXTRACTED LIPID %
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
WATER CONTENT %

resolved in this series of observations, were unaffected by temperature and could be expressed by straight lines. It could be expected that this would not have been the case if an unlimited amount of food had been available; the same absolute ration was, however, provided throughout the period.

Mean length was related to time by the equation $y = 0.03x + 14.78$ (where y is length in cm and x is time in days). Weight was related to time by the equation $y = 0.25x + 40.94$ (y is weight in g).

Total live weight production over the growth period was estimated from the weight/time regression, correction being made for mortalities recorded during the 140 days over which observations had been made. Dry weight conversion efficiency (K_1) was estimated on the basis of water content analyses reported elsewhere in this thesis (Appendix 3).

$y = 0.25x + 40.94$

$\therefore$ y at time 0 = 40.94 g

where N is population no., N at time 0 = 150

$\therefore$ estimated total wt at time 0 = 6141 g

y at time 140 = 75.94

N at time 140 = 134

$\therefore$ estimated total wt at time 140 = 75.94 x 134

= 10,176 g

wt of mortalities and removals = 783 g

$\therefore$ Total live weight increase = 10,176 + 783 - 6141 g

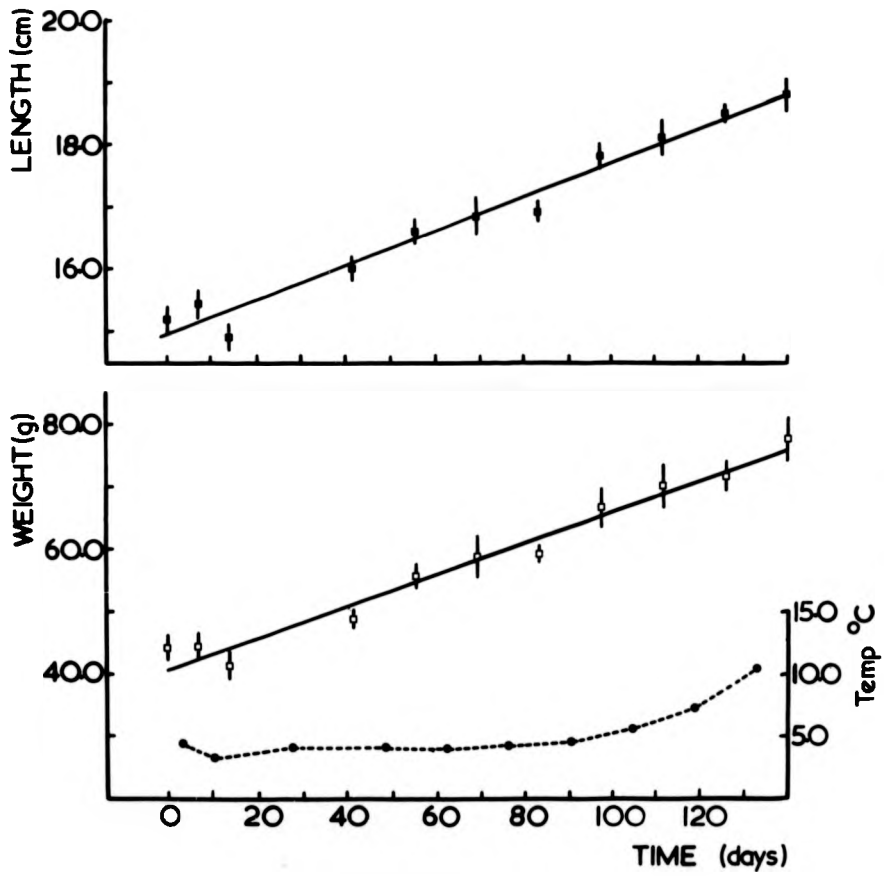
= 4818 g

Total food intake = 9,500 g

$\therefore$ K_1 live weight = 0.51

K_1 dry weight = 0.15

FIGURE 17. Growth of rainbow trout maintained in a net enclosure in Airthrey Loch, Stirling. (a) length (mean ± s.e.) (b) weight (mean ± s.e.).


LENGTH (cm)
200
180
160
WEIGHT (g)
800
600
400
150
100
50
°C
Temp
0
20
40
60
80
100
120
TIME (days)

3.8. Histology

3.8.1. Salinity

(a) Intestine.

Between 0.0 and 15.0 p.p.t. there was a positive correlation between salinity and intestinal cross-sectional area ($\frac{\pi r^2}{L^2}$). This correlation was not statistically significant at the 95% level. Between 15.0 p.p.t. and 32.6 p.p.t. there appeared to be no further increase in cross-sectional area (Fig. 18). Between 0.0 and 15.0 p.p.t. there was also a significant positive correlation ($r = 0.6457$; $d.f. = 9$; $p < 0.05$) between salinity and the height of the villi which was not sustained over the range 15.0 p.p.t. to 32.6 p.p.t. (Fig. 19).

The thickness of none of the tissue layers measured, including the columnar epithelium and the tunica propria [both of which were said by Virabhadrachari (1961) to have increased in depth in Etroplus maculatus adapted to higher salinities] appeared to be influenced by salinity (Table 5). As far as could be seen at the light-microscope level, the depth of the microvilli was also unaffected by salinity. In certain of the trout acclimatized to 15.0 p.p.t. and 32.6 p.p.t. there was a high incidence of deep depressions in the columnar epithelium (Plate 5). These depressions were entirely lined with a 'brush border' of micro-villi. Some of those occupying only one cell-width had a mucus cell at the base.

Salinity has a marked effect on the density of the mucus cells. There is a significant ($r = -0.9190$; $d.f. = 13$; $p < 0.001$) negative correlation between salinity

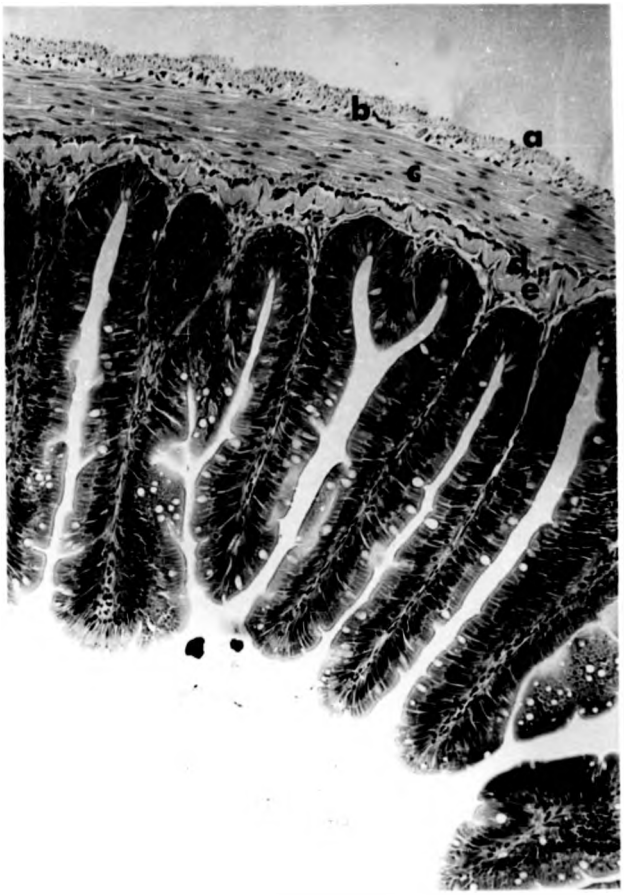


PLATE 1. Transverse section of intestine showing general structure.

(a) serosa
(b) longitudinal muscle layer
(c) circular muscle layer
(d) stratum granulosum
(e) stratum compactum
(f) tunica propria
(g) mucosa

Haematoxylin and eosin. x 40

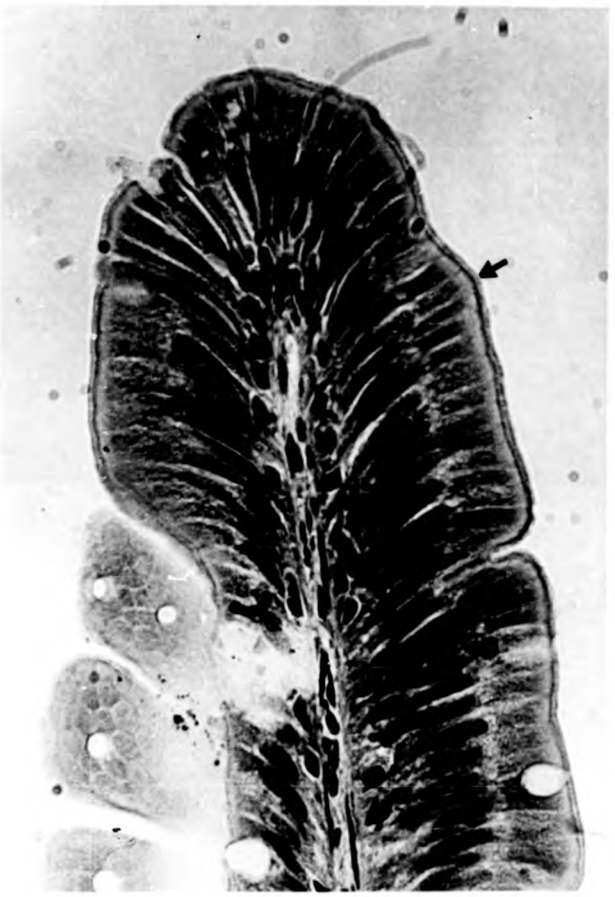


PLATE 2. Transverse section of intestine showing striated border of columnar epithelial cells (arrowed).
Haematoxylin and eosin. x 170.

PLATE 3. Transverse section of intestine illustrating a typical density of goblet cells in the mucosa of rainbow trout maintained in fresh water.
Alcian blue/chlorantine fast red. x 40.

PLATE 4. Transverse section of intestine illustrating a typical density of goblet cells in the mucosa of rainbow trout maintained in 32.5 p.p.t. sea water.
Alcian blue/chlorantine fast red. x 40.

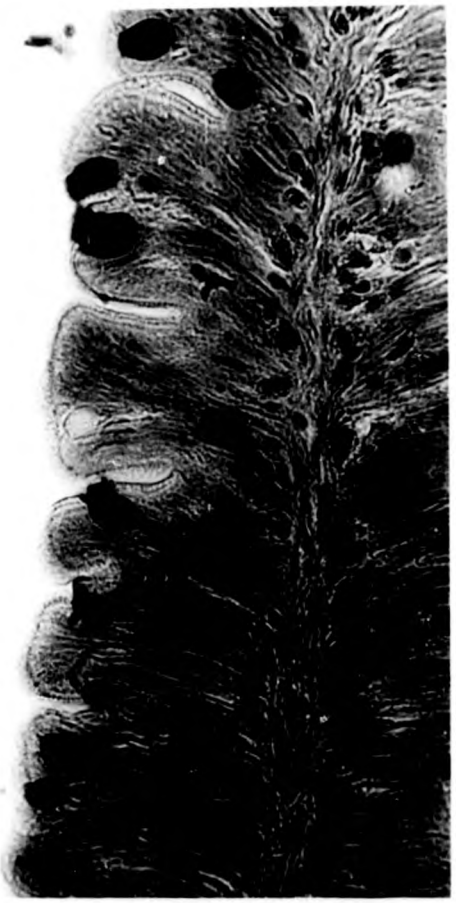


PLATE 5. Transverse section of intestine showing the pits developed in the mucosa of rainbow trout maintained in 32.5 p.p.t. sea water. Alcian blue/chlorantine fast red. x 170.

PLATE 6. Transverse section of the rectum showing the greater prevalence and size of goblet cells on the outer edge of the spiral septum. Haematoxylin and eosin. x 40.

Part of gut	Salinity	Feeding	Height of Columnar Epithelium	Depth of Muscle Layers	Depth of Stratum Compactum	Intra-Villus Tunica Propria
intestine	F.W.	fed	45.10±1.45	105.83±14.88	15.73±2.21	12.33±2.83
		starved	44.54±2.41	110.75±1.75	21.82±0.75	13.83±2.17
	7.5%	fed	46.67±1.18	96.62±6.67	15.78±0.74	11.62±0.75
		starved	50.89±3.02	103.42±13.42	18.13±0.75	13.88±4.97
	15.0%	fed	41.77±1.17	98.60±4.00	17.85±2.03	7.44±0.41
		starved	42.17±1.15	100.58±13.57	17.85±2.25	8.22±1.14
	32.6%	fed	43.52±0.50	81.81±14.55	13.18±1.65	8.46±1.95
		starved	33.89±4.91	82.83±18.13	14.73±2.04	8.22±1.13
rectum	F.W.	fed	41.00±1.69	68.22±3.82	8.50±1.43	8.29±1.32
		starved	38.28±0.81	68.00±13.04	11.90±1.47	11.62±1.50
	32.6%	fed	39.82±1.86	59.92±4.94	8.82±1.09	10.20±1.51
		starved	32.54±1.25	77.82±28.23	10.20±1.30	7.37±2.21

Table 5. The effect of salinity on some alimentary canal tissue dimension in fed and fasting trout (measurements in μm).

FIGURE 18. Effect of salinity on the total cross-sectional area (relative to the square of fish length) of intestine and rectum in fed and unfed rainbow trout (mean ± s.e.).

● ..... intestine fed

○ .....intestine unfed

■ ..... rectum fed

□ ..... rectum unfed

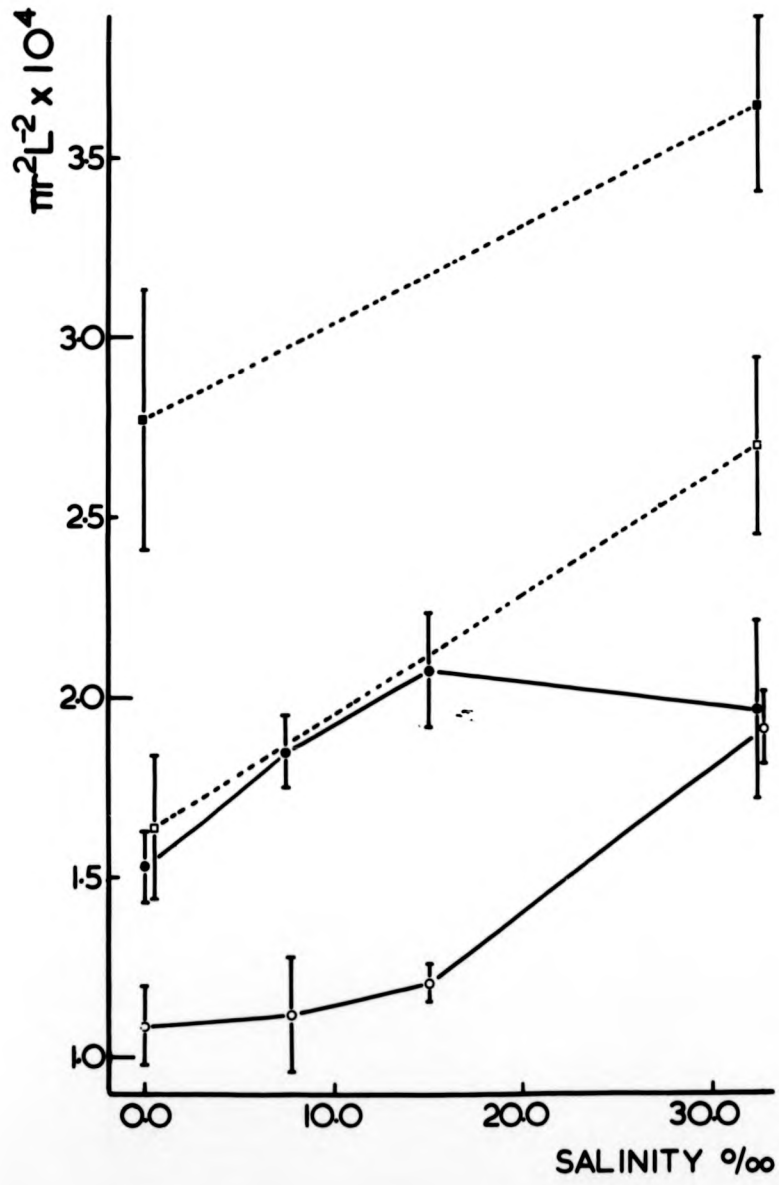

$m^2L^{-2} \times 10^4$
3.5
3.0
2.5
2.0
1.5
1.0
0.0
10.0
20.0
30.0
SALINITY °/oo

FIGURE 19. Effect of salinity on the height of the villi (relative to fish length) in the intestine of fed and unfed trout.

●..... fed

○..... unfed

rout.

HEIGHT OF VILLI. L⁻¹ x 10⁴
300
250
200
150
0.0
10.0
20.0
30.0
SALINITY ‰

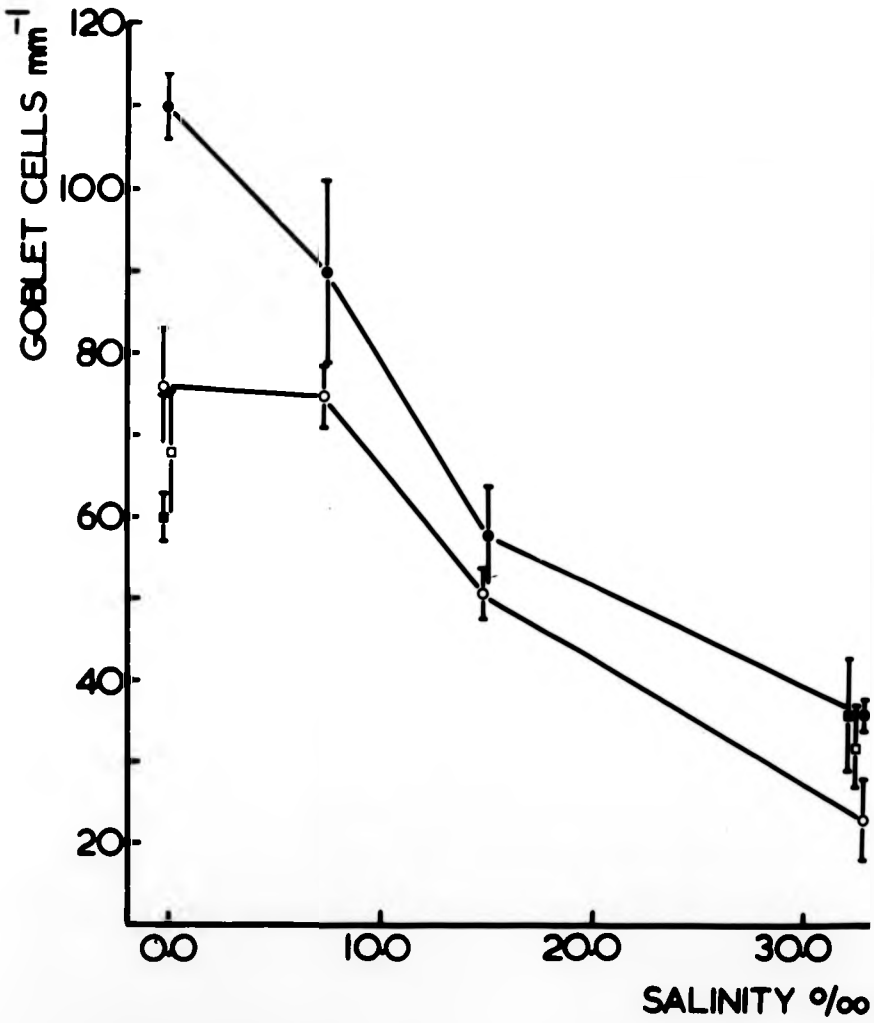
FIGURE 20. Effect of salinity on the density of distribution of mucus cells in sections of the intestine and rectum (mean ± s.e.).

● ..... intestine fed

○ ..... intestine unfed

■ ..... rectum fed

□ ..... rectum unfed


GOBLET CELLS mm⁻¹
120
100
80
60
40
20
0.0
100
200
300
SALINITY 0/00

and number of mucus cells sectioned per 1000 µm. In trout maintained in fresh water, there was a mean density of 110 mucus cells per 1000 µm whereas in trout acclimatized to sea water (32.6 p.p.t.) there was a mean density of only 36 per 1000 µm. Trout acclimatized to the two intermediate salinities had intermediate mucus cell densities (Fig. 20, Plates 3 and 4).

(b) Rectum

The rectum was examined in detail only in the trout maintained in fresh water and in 32.6 p.p.t. sea water. In this part of the alimentary canal, as in the intestine, the total cross-sectional area is greater in the higher salinity (Fig. 18). No figure comparable with 'height of villi' in the intestine can be obtained for the rectum because of its specialised structure. A muscular annulo-spiral septum (Burnstock, 1959) (Plate 6) protrudes into the lumen. However, in cases where responses to salinity could be directly compared, intestine and rectum were similar. The thickness of the tissue layers measured was uninfluenced by salinity (Table 5). The mean density of mucus cells was 60 per 1000 µm in fresh water and 37 per 1000 µm in sea water (32.6 p.p.t.) (Fig. 20). In both salinities, mucus cells were more numerous and larger on the distal edges of the annulo-spiral septum than in other parts of the rectal epithelium.

(c) Oesophagus and cardiac stomach

The oesophagus and stomach were not visibly affected by changes in salinity. The depth of those layers which

are also present in the intestine and the rectum, as well as the depth of the gastric gland cell layer were unaltered. The saccular mucus cells of the posterior oesophagus and the columnar mucus cells of the cardiac stomach show no change which is attributable to salinity.

3.8.2. Fasting

(a) Intestine

In fresh water, 7.5 p.p.t. and 15.0 p.p.t., there was a marked decrease in intestinal cross-sectional area after 48 days of food deprivation. This decrease was not observed to occur in fish kept in sea water (32.5 p.p.t.) (Fig. 20). The height of the villi showed a similar trend towards a somewhat less marked decrease in fish acclimatized to 32.5 p.p.t. sea water than in fish acclimatized to lower salinities and to fresh water (Fig. 19). There was a decrease in muscle cross-sectional area after 48 days of starvation, again in all salinities except 32.5 p.p.t. The thickness of the muscle layers, however, shows a slight increase during fasting (Table 5). The stratum compactum also increases in thickness in starved trout; the stratum compactum is a dense layer of collagen shown by Burnstock (1959) to have the effect of restricting the extensibility of the gut. These instances of an increase in the depth of a tissue layer suggest that much of the decrease in the total cross-sectional area of the intestine and the cross-sectional area of the muscular layer are due to contraction rather than to tissue depletion. There was no indication of any increase in extracellular

space after starvation. In fresh water, 7.5 p.p.t. and 15.0 p.p.t., the height of the columnar epithelial cells was unaffected by starvation (Table 5). In sea water (32.5 p.p.t.), on the other hand, a considerable decrease was recorded in mean epithelial cell height. The thickness of other tissue layers showed no measurable change after 48 days of starvation.

b) Rectum

The effects of starvation on the rectum are similar to the effects on the intestine.

(c) Oesophagus and cardiac stomach

A 48-day period of starvation had little visible effect on the histological structure of the posterior oesophagus and the cardiac stomach. The stomach showed a tendency to decrease in total cross-sectional area, but the depth of those tissue layers examined, including the columnar epithelial cells and the cardiac gland cells, was unchanged. The height of the rugae did not show any change which could be related to starvation.

4. DISCUSSION

4,1. Effects of salinity on food intake

4.1.1. Chronic effects: In rainbow trout of 100-150 g, acclimatised to various salinities, food intake was significantly higher at intermediate salinities of 15.0 p.p.t. and 28.0 p.p.t. than at salinities of 0.0 p.p.t. and 32.5 p.p.t. A notably sharp decline in intake was apparent between 28.0 p.p.t. and 32.5 p.p.t. Otto's (1971) data for Oncorhynchus kisutch indicate a comparable sharp depression of food intake at salinities of above 10.0 p.p.t. Throughout the course of pre-smolt development, this species appears to show food intake optima at salinities of 5.0 - 10.0 p.p.t.

Cyprinodon macularius Baird and Girard (Kinne, 1960) consumed the maximum amount of Enchytraeus in a salinity of 35.0 p.p.t., less in 15.0 p.p.t. and still less in 0.0 p.p.t. Although Kinne did not quantify intake in 55.0 p.p.t., he stated tentatively that intake at this salinity was lower than at 35.0 p.p.t.

Nelson (1968) observed that the three-spined stickleback Culaea inconstans fed more actively in 20.0% sea water than in fresh water. In this species, there was some feeding up to a salinity of 60.0% sea water, but no feeding in 70.0% sea water [100.0% s.w. = 35.0 p.p.t. salinity (Nelson, 1968)].

Despite high mortality (30% and 10% respectively) at salinities of 5.0 and 12.0 p.p.t., there seems to be little effect of salinity, between the limits 5.0 and

37.5 p.p.t., on the food intake of Mugil auratus and Mugil capito (Vallet et al., 1970). Similarly, the food intake of the estuarine flatfish Trinectes maculatus Bloch and Schneider does not differ at salinities of 0.0, 15.0 and 30.0 p.p.t. (Peters and Boyd, 1972).

It is not easy to explain the chronic effects of salinity on food intake in physiological terms and none of the authors quoted has attempted to do so From the small amount of data that is available, it can be seen that there is considerable inter-specific variability in the food intake response of fishes to salinity. The salinity of maximum intake must depend initially on the degree of euryhalinity shown by a particular species and, irrespective of euryhalinity, on the particular narrow or broad range of salinities to which it is adapted by genotype or by previous history.

Two types of mechanism have been implicated in the control of food intake in fishes:

(1) control of mass or volume by gastric stretch receptors (Lepkovsky, 1948; Paintal, 1954; Brett, 1971a)

(2) control of chemical energy content (or nutrient value) (Mayer, 1955; Rozin and Mayer, 1961).

Although Rozin and Mayer (1961) state that goldfish increased their total food intake when their diet was diluted with inert material (kaolin), recent work by Brett and Higgs (1970), Brett (1971a) and Wallace (1973) suggests that the more important control is the rate of evacuation of the stomach.

In discussing the effect of salinity on food intake

in the fully-acclimatized fish, we should therefore consider the possibility that stomach evacuation rate is affected by salinity. This could occur in either of two ways:
(1) increased rate of clearance without an increase in digestion rate.
(2) increased rate of clearance related to an increase in digestion rate.

Results presented earlier in this thesis may favour the first mechanism. (a) There is a decrease in absorption efficiency with salinity. (b) There is an increase in intestinal volume with salinity due probably to increased osmoregulatory water intake, which may increase evacuation rate by increasing the fluidity of the gut contents.

It is of interest that Bell and Razig (1973) have shown that the osmotic concentration (which was experimentally manipulated by NaCl and $NaHCO_3$ dosage) of the calf's abomasal contents has a significant acceleratory effect on the rate of abomasal emptying.

But the second mechanism (2) cannot be discounted. Fish (1960) stated that gastric hydrochloric acid production was very low in fasting perch (Perca fluviatilis) on first feeding, unless Cl^- ions were added to the diet in the form of NaCl. This suggests that, in the perch at least, dietary chloride may be a necessary supplement to chloride absorbed from a chloride-poor external medium. The effect of total ionic concentration, or the concentration of specific ions on digestive enzyme activity has not been studied, but it has been shown that sodium chloride enhances alkaline phosphatase activity in the hind-gut mucosa of

rainbow trout (Utida, 1967) and of the euryhaline eel Anguilla japonica and several other freshwater and marine species (Utida and Isono, 1967). This effect seems more likely to be related to the osmoregulatory than to the alimentary functions of the hind-gut.

4.1.2. Acute effects: Food intake was found to be adversely affected by a sudden increase in salinity. This may have been a result of factors other than the ability of the alimentary canal to process food e.g. psychological effects on behaviour patterns or physiological effects on activity levels. Houston (1959) reported that transfer into sea water from fresh water produced a marked depression in the cruising speed of chum salmon (Oncorhynchus keta), with almost complete recovery after 30 h.

4.1.3. Fluctuations in daily food intake: The existence of a marked fluctuation in the food intake of various species of fishes has been noted several times in the literature (Pandian, 1967a; Ishiwata, 1969; Brett, 1971a; Solomon and Brafield, 1972; Wallace, 1973). The finding of the present study that food intake is not necessarily higher after a longer period of starvation is in agreement with the conclusions of Ishiwata (1969) and Brett (1971), the latter of whom found that, in Oncorhynchus nerka, appetite increases in sigmoid fashion with starvation, approaching a plateau after 25-30 hours.

4.2. Effects of salinity on food absorption efficiency

There was a significant linear decrease in dry matter, energy and total nitrogen absorption efficiency with salinity between the salinities of fresh water and 32.5 p.p.t. Brocksen and Cole (1972) noted that the energy absorption efficiency of corvina (Cynoscion xanthulus) fed on shrimp and squid decreased on either side of an optimum salinity, from 72% in 37.0 p.p.t. to 66% in 28.0 p.p.t. and 59% in 45.0 p.p.t. These figures are markedly lower than those found by other workers in similar 'natural food' systems (e.g. Pandian, 1967a; Wallace 1973) who reported no values below 86%.

The fresh water absorption efficiencies measured in the present study are closely comparable with those found, for various species, by other authors. For energy, p = 0.91 (91%) and for total nitrogen p = 0.96 (96%). Some previous results are presented in table form for comparison (Table 6).

In the present study, the absorption of total nitrogen, rather than protein nitrogen was measured because
(a) there is likely to be little indigestible nitrogenous material, e.g. chitin, in the consumed food in comparison with those cases in which live arthropod food material was utilised (e.g. Gerking, 1952, 1955; Pandian, 1967a),
(b) digestible or soluble non-protein nitrogen compounds (e.g. amino acids, ammonium salts) may be nutritionally important (Maynard and Loosli, 1962), and
(c) where both total nitrogen and protein nitrogen absorption efficiencies are calculated, there is little difference between them, even in the case of natural feeding (Pandian, 1967a).

Author	Species	Food	Absorption Efficiency energy	protein N	total N
Gerking, 1952	Lepomis megalotis megalotis	Tenebrio molitor (mealworm)	-	97%	-
"	Lepomis cyanellus	"	-	96%	-
Gerking, 1955	Lepomis macrochirus	"	-	97%	-
Davies, 1964	Carassius auratus	Enchytraeus albidus (whiteworm)	92-94%	-	-
Pandian, 1967a	Megalops cyprinoides	Metapenaeus monoceros (prawn)	86-98%	95-99%	92-98%
"	Ophiocephalus striatus	"	87-93%	96-99%	93-97%
Birkett, 1969	Perca fluviatilis	Lumbricus sp. (earthworm)	-	-	0.963
"	Pleuronectes platessa	Arenicola marina (lugworm)	-	-	0.918
"	Solea vulgaris	"	-	-	0.850
"	yearling P. platessa	Artemia salina (brine shrimp)	-	-	0.960
Beamish, 1972	Micropterus salmoides	Nototropis atherinoides (emerald shiner)	90%	96%	-
Brocksen and Cole, 1972	Cynoscion xanthulus	shrimp and squid	59-72%	-	-
Kelso, 1972	Stizostedion vitreum vitreum	Gammarus lacustris	82%	-	-
"	"	Oronectes virilis (crayfish)	84%	-	-
"	"	Nototropis atherinoides	98%	-	-
"	"	Perca flavescens	97%	-	-
Solomon and Brafield, 1972	Perca fluviatilis	Gammarus pulex	84-87%	-	-
Wallace, 1973	Blennius pholis	squid	96%	-	-

TABLE 6. A survey of absorption efficiency results from a range of fish species and diets.

Faeces were collected every second day. Davies (1964) has indicated that over periods considerably longer than this (21 days), and at higher temperatures (12.5 and 21.5°C as opposed to 10.0°C) dissipation of energy by micro-organisms was negligible. In the case of nitrogen absorption efficiency measurements, the egestion of metabolic faecal nitrogen (i.e. mucus, digestive enzymes, gut epithelial cells) constitutes a source of error which is difficult, although not impossible (Ogino et al., 1973) to quantify. According to Maynard and Loosli (1962), however, the error is small (about 0.1% of total dry ingested food in mammals).

It is possible that some of the increase in faecal nitrogen content with salinity (from 2.46% of dry weight in fresh water to 3.49% in 32.5 p.p.t. sea water) may be due to an increase in metabolic faecal nitrogen production; but it seems likely that most of it results from a real decrease in absorption.

In relation to experiments where food intake was allowed to vary in response to salinity, it is important to note that absorption efficiency appears to be little affected by ration size (Gerking, 1952; Pandian, 1967c; Beamish, 1972; Kelso, 1972; Wallace, 1973).

The increase in the ash content of the faeces with salinity can be related to the osmoregulatory function of the intestine, although there may be a small contribution from solutes diffusing into the faeces from the medium. Shehadeh and Gordon (1969) have shown that the faecal mucus

of rainbow trout in sea water contains a precipitate of calcium and magnesium carbonates. This precipitate was visible in the intestinal mucus defaecated by fasting trout in sea water.

The observed decrease in absorption efficiency with salinity may be related in some way to osmotic and ionic regulatory processes in the intestine. It has been reported (Conte et al., 1973) that protein synthesis in Artemia salina (brine shrimp) nauplii is inhibited by elevated concentrations (> 0.25 M) of sodium chloride in the external medium. The authors suggested that there was competition between protein synthesis and osmotic and ionic regulation for available energy (in the form of ATP). It is recognised that alkaline phosphatase is implicated both in protein absorption (Tuba and Dickie, 1955) and body fluid regulation (Utida, 1967; Utida and Isono, 1967).

4.3. Effects of salinity on food conversion efficiency

Canagaratnam (1959) tabulated the sizes attained by several species of fish which are found in both salt and fresh water, and demonstrated the larger mean size of the marine inhabitants. Under natural freshwater and saline conditions, however, salinity is confounded with such variables as temperature and - probably an even more potent factor - food supply.

In the present study, temperature and diet were controlled. In cases where the fish were fed to satiation,

food intake, as indicated by results described earlier, did not vary sufficiently to have a measurable effect on conversion efficiency.

There is no evidence for an optimization of conversion efficiency (K_1 or K_2) at salinities above that of fresh water. In rainbow trout of the 0+ year-group from 50-95 g in weight (Group 1), K_1 decreases with salinity over the whole range of salinities from 0.0 to 32.5 p.p.t., falling most sharply between 28.0 and 32.5 p.p.t. In individuals of the 0+ year-group from 80 to 155 g in weight (Group 2), K_1 remains constant over the salinity range 0.0 - 28.0 p.p.t., but falls sharply and significantly between 28.0 and 32.5 p.p.t. This relatively abrupt drop in conversion efficiency between 28.0 and 32.5 p.p.t. in both groups is coupled with a drop in appetite over the same salinity range. Interesting in this regard is Madan Mohan Rao's (1971) result that only at 30.0 p.p.t. did size affect the relationship between oxygen consumption and salinity. There is, however, no evidence from the present study that conversion efficiency at 32.5 p.p.t. was affected by body weight. For Group 1, K_1 = 0.14, and for Group 2, K_1 = 0.12.

In Group 3, which was made up of trout of the 1+ year-group, the results do not include the full range of salinities. Those results that are available indicate that there was no significant correlation between conversion efficiency and salinity over the range from 0.0 to 28.0 p.p.t. The mean conversion efficiency (K_1 dry weight) is, however, significantly lower than that of the other groups.

Although the fish comprising this group were of similar weight to those in Group 2, they were one year older (1+ year-group). There is a tendency for the growth capacities of fishes to decline with size, age, and sexual maturity (Brown, 1957). The males in this group had well-developed gonads, and visual observation indicated that more aggression was displayed among this group than among younger and less mature fishes of the same size.

The presence of a significant negative correlation between salinity and conversion efficiency in Group 1 fish, compared with the absence of correlation in Group 2, may bear some relation to the results of Parry (1958,60) and Conte and Wagner (1965) who found that the salinity tolerance of three salmonid species (Salmo salar, Salmo trutta, Salmo gairdneri) increases with size. Two explanatory hypotheses (Parry, 1960) seem plausible; (1) that there is a decrease in the surface/volume ratio with size, (2) that there is an increase in the absolute capabilities of osmotic and ionic regulatory mechanism with size.

The general level of dry weight conversion efficiency (K_1 = 0.20) is within the range quoted by the feed manufacturers, and compares favourably with results obtained in studies using 'natural' food materials. Energy conversion efficiency is slightly higher and nitrogen conversion is significantly higher ($p < 0.01$). Mean K_1 for nitrogen is about 0.30, which approaches the upper end of the range of values reported by Pandian (1967) for Megalops cyprinoides and Ophiocephalus striatus fed on live prawns Metapenaeus

monoceros. In the former species, K_1 (nitrogen) fell from 0.34 (34.2%) in a 1.4 g individual to 0.20 (19.5%) in a 149.6 g individual, while, in the latter species, K_1 fell from 0.36 (36.1%) in a 1.9 g fish to 0.14 (14.0%) in a 123.8 fish.

In the case of bluegill sunfish Lepomis macrochirus (Gerking, 1971) fed on Tenebrio molitor larvae (mealworms), optimum nitrogen conversion efficiency varied from about 0.39 (39%) for a 13.9 g individual to 0.10 (10%) for an 85.2 g individual. In the present study, the size range was limited, but there was a tendency for K_1 to be slightly (but not significantly) higher in the 50-95 g range than in the overlapping 80-155 g range. In terms of physiological age (which, in fish, is related within any one species to size rather than to chronological age) a given weight range in the case of rainbow trout will not be equivalent to the same absolute weight range in species of different growth rate or different maximum size such as those studied by Pandian (1967) and Gerking (1971).

In the present study, because of the high efficiency with which all measured food components were absorbed, K_2 was always only slightly higher than K_1. The very small difference in gradient between the curves of K_1 and K_2 versus salinity illustrates the relatively minor effect on conversion efficiency of the statistically significant effect of salinity on absorption efficiency.

Like the effect of salinity on food intake, the effect of salinity on conversion efficiency varies much from species to species. Canagaratnam (1959) measured the

growth rate of 0+ coho, chum and sockeye salmon and adult goldfish in fresh water and in water of several salinities (6.0, 12.0 and 18.0 p.p.t.) under aquarium conditions. The salmon fingerlings exhibited a faster growth rate in salt water, reaching a maximum at 12.0 p.p.t. Goldfish maintained at salinities of 0.0 and 6.0 p.p.t. showed no difference in growth rate. The salmon were fed at a rate of 10.0% of body weight per day and, assuming that the ration was consumed equally in all salinities, an increase in growth rate would appear to result from an increase in food conversion efficiency.

The results of Otto (1971), who also worked with pre-smolt coho salmon (Oncorhynchus kisutch), are in partial agreement with those of Canagaratnam (1959). Otto found that the highest conversion efficiencies (K_1) were observed at salinities of 5.0 and 10.0 p.p.t. and that, during most of the year, conversion efficiency was lowest at 15.0 p.p.t. As the fish increased in size, approaching the stage at which their seaward migration would take place, the conversion efficiency exhibited by individuals in fresh water became relatively lower in comparison with those exhibited by individuals in intermediate salinities.

Saunders and Henderson (1969a,b,c) found, on the other hand, that Atlantic salmon Salmo salar held in fresh water beyond the smolt stage grew at similar or higher rates than individuals of similar size and physiological states transferred into the brackish water or full strength sea water to which they were now adapted. In parr of this species, growth rate was not markedly

affected by salinity, up to 22.0 p.p.t., where growth was reduced. Fry grew most rapidly in a salinity of 6.0 p.p.t.

Like Canagaratnam (1959), Gibson and Hirst (1955) studied growth rate (in the guppy Lebistes reticulatus) without calculating conversion efficiency. Unlike Canagaratnam, however, they did not indicate the amount of food supplied to their experimental animals, making it impossible to make a reliable estimate of the effect of salinity on conversion efficiency. They found that the guppy grew more rapidly (at all temperatures investigated) in 25% sea water than in fresh water. Individuals maintained in 50% sea water at 20°C showed an even greater rate of growth, but the authors admit that this could have been an artefact of their experimental design; one pregnant female guppy had been placed in each of a series of tanks kept at 20, 23, 25, 30 and 35°C, and their families maintained at these same temperatures in fresh water and 25% sea water. Two families were maintained in 50% sea water at 20 and 25°C. Differences in experimental treatment are therefore confounded with differences in genotype.

Kinne (1960) found that, at 30°C, Cyprinodon macularius showed its maximum conversion efficiency (K_1 dry weight) at 15.0 p.p.t., and conversion efficiency decreased in the order 15.0, 35.0, 0.0 p.p.t. Mean conversion efficiencies at 30°C were 0.14 (14.4%) in 15.0 p.p.t., 0.11 (10.6%) in 35.0 p.p.t. and 0.09 (8.8%) in fresh water. Food intake was permitted to vary, producing maximum growth rates in 35.0 and 55.0 p.p.t. at temperatures of 25, 30 and 35°C and in fresh water at 15° and 20°C, showing a marked inter-

action between salinity and temperature.

Later, Kinne (1962) reported that Cyprinodon macularius from eggs hatched in the spawning salinity had higher growth rates and conversion efficiencies than individuals from eggs transferred 3-6 hours after insemination into a different salinity. The author related his results to known and hypothetical post-fertilization changes which may contribute to the establishment of 'irreversible non-genetic adaptations' to salinity. Differences in growth rate were maintained after sexual maturity but, because of variation in reproductive performance with salinity (a larger number of eggs were produced in 15.0 and 35.0 p.p.t. than in 0.0 p.p.t.), conversion efficiency was more difficult to monitor.

The ability of Cyprinodon macularius to tolerate transfer into different salinities decreases with increasing age. This contrasts with the situation in those salmonid species which have been examined (Parry, 1958, 1960; Conte and Wagner, 1965; Otto, 1971).

Tilapia mossambica fry were maintained by Canagaratnam (1966) in salinities of 0, 25, 50, 75 and 100% sea water (100% = 35.0 p.p.t.); growth rates (on a 10% daily ration) were maximal and closely similar in 50 and 75% sea water, followed by the growth rates in 100%, 25% and fresh water in that order. Chervinski (1961), on the other hand, found that there was no significant difference between the growth rates, in fresh water and 25% and 50% sea water, of the closely related species

Tilapia nilotica.

The conversion efficiency exhibited by sargo Anisotremus davidsoni sampled from the Salton Sea (salinity = 37.0 p.p.t.) in California has been shown by Brocksen and Cole (1972) to be highest at a salinity of 33 p.p.t., falling rapidly on either side of this salinity. As these authors point out, the sargo is an oceanic species, and is adapted to salinities of this order.

Peters and Boyd (1972) have indicated that the migratory pattern of the hogchoker Trinectes maculatus paradoxically brings this species into environmental conditions where growth rate and conversion efficiency are below the optimum. As in Cyprinodon macularius (Kinne, 1960, 1962) the food conversion efficiency of this species is affected by a marked temperature-salinity interaction. At 15°C, K_1 varies from 0.21 at 0.0, through 0.41 at 15.0 p.p.t. to 0.47 at 30.0 p.p.t. The corresponding figures for 25°C and 35°C respectively are 0.23, 0.25 and 0.27 and 0.25, 0.20 and 0.18.

Vallet et al. (1970) maintained young Mugil auratus and Mugil capito for 11 days at 16°C in salinities of 5.0, 12.0, 20.0 and 37.5 p.p.t. A ration close to maintenance in 37.5 p.p.t. (3.3 g dry wt per 100 g live fish weight per day) produced weight increments of 3.0 and 2.4% in 20.0 and 12.0 p.p.t. respectively, and a weight loss of 6.2% in 5.0 p.p.t. Conversion efficiencies (K_1) at the two salinities where weight change was positive (12.0 and 20.0 p.p.t.) were 0.11 (10.7%) and 0.09 (9.0%)

respectively. These salinities are close to the salinity at which Mediterranean mullet are usually found during their fastest-growing phase.

As indicated in the introduction to this thesis, the major physiological determinant of conversion efficiency is metabolic energy expenditure. The higher the proportion of food energy dissipated in processes other than growth, the lower is food conversion efficiency. As is to be expected, therefore, most of such discussion as there has been on the effect of salinity has centred on the effect of salinity on metabolic energy demands. Conversion efficiency is assumed to be highest where the energy requirements of osmotic and ionic regulations are least. Paloheimo and Dickie (1966a,b) reanalyzed Kinne's (1960, 1962) data to provide more information on its metabolic implications. These authors demonstrated that temperature affected only the rate of energy turnover, whereas salinity affected the partitioning of energy between growth and catabolism. They also calculated that, in low salinities, small Cyprinodon macularius were less able than larger individuals to maintain their conversion efficiency.

The results summarised above, together with the results of the present study, indicate that there is little basis for assuming that food conversion efficiency is necessarily highest at the isosmotic point. There is evidence that the effect of salinity on conversion efficiency is profoundly modified by non-genetic adaptation (Kinne, 1962), genetic adaptation to the salinity of the

normal habitat (Brocksen and Cole, 1972), ontogeny and growth (especially in the case of migratory species) (Otto, 1971) and temperature (Kinne, 1960; Peters and Boyd, 1971). There are also cases (Chervinski, 1961, and the present study) where salinity, between fairly wide limits, may have little or no effect on conversion efficiency, at least over a certain size range; perhaps these could be said to be the species best adapted for euryhalinity in that they show the greatest measure of independence from environmental salinity.

Respirometric studies of the effect of salinity on O_2 consumption bear out the evidence of the conversion efficiency results that it is not always the case that metabolic requirements are least in an isosmotic external medium. These studies also demonstrate that there may be little support for the conclusion of Canagaratnam (1959) that fishes of a given species grow faster in sea water because of 'the higher osmotic content of the medium.' There is certainly no evidence that marine fishes in sea water have metabolic requirements any different from those of freshwater fishes in fresh water. Winberg (1956) combined the results of earlier workers in equations which summarise the metabolic rate/weight relationship in marine and freshwater fishes:

> 'for marine fish, $Q = 0.321\ W^{0.79}$
> for freshwater fish, $Q = 0.297\ W^{0.81}$
>
> It is hardly necessary to prove once again that there is not the slightest reason to ascribe any significance to the small differences between the parameters of these two equations, which summarize the results of much work done by many authors, working independently at different times, and in different countries.

> The question of the relative metabolic rate of marine and freshwater fishes, which has excited a vast amount of discussion, can be regarded as solved. The average level of metabolism is the same in marine and freshwater fish.'

There is also evidence to indicate that the O_2 consumption rate of a number of euryhaline species may be unaffected by salinity. Winberg (1956) cites some earlier workers who failed to demonstrate any significant long-term changes in O_2 consumption at different salinities (Raffy, 1932; Keys, 1931; Schlieper, 1936; Maloeuf, 1937; Markova, 1949), although there was usually a pre-acclimation change. This highlights the importance of specifying whether an experiment is carried out on acclimated or non-acclimated individuals. Raffy (1933, 1955) working with Pleuronectes platessa and Gordon et al. (1965) working with Periophthalmus sobrinus also found that salinity had no effect on O_2 consumption. When the euryhaline freshwater teleost Etroplus maculatus was suddenly transferred from fresh water to salinities of 9.7, 19.4 and 32.4 p.p.t., there was no significant change in O_2 consumption (Parvatheswararao, 1965).

In some cases, a higher O_2 consumption was found in sea water than in water of lower salinities and fresh water. Job (1959) found that the marine catfish Plotozus anguillaris had a higher respiratory rate in sea water than in water of lower salinities. Hickman (1959) recorded that when both Platichthys stellatus and Citharichthys stigmaeus were transferred to fresh water from sea water there was a long-term decrease in O_2 consumption.

In the case of corvina (Cynoscion xanthulus) from the Salton Sea, California, Brocksen and Cole (1972) showed that O_2 consumption at either 29.0 p.p.t. or 45.0 p.p.t. was higher than that at the salinity (37.0 p.p.t.) to which the fish is accustomed.

Job (1969) measured the routine metabolism of Tilapia mossambica in fresh water and at salinities of 12.5 and 30.5 p.p.t. and stated that 5 g fish tended to have their minimum O_2 consumption at 12.5 p.p.t., whereas 80 g fish tended to have their maximum at this salinity.

Muir and Niimi (1972) studied the effect of salinity on the O_2 consumption of the euryhaline Hawaiian reef fish, Kuhlia sandvicensis. A 30 g individual of this species had an active (maximum sustained swimming speed) oxygen consumption about 12% higher in water of 32.0 p.p.t. than in fresh water, whereas standard oxygen consumption was only about 6% higher in sea water. In neither case, however, was the difference significant.

It is noteworthy that, in the only two species in which respiratory rate was found to be markedly lower at or near the isosmotic point, the respirometry was carried out at an enforced high level of activity. Farmer and Beamish (1969) measured the oxygen consumption rate of Tilapia nilotica for a range of swimming speeds (30, 40 and 50 cm sec^{-1} in fish of 60 - 120 g) in salinities of 0.0, 7.5, 11.6, 22.5 and 30.0 p.p.t. Respiratory rates at 0.0, 7.5 and 22.5 p.p.t. were approximately equal. The minimum respiratory rate occurred at 11.6 p.p.t. and the

maximum at 30.0 p.p.t. It was concluded that the amount of energy required for osmoregulation was minimal in the near absence of an osmotic gradient (11.6 p.p.t.) and maximal when the osmotic gradient was at its highest (30.0 p.p.t.). Farmer and Beamish (1969) estimated that, at a salinity of 30.0 p.p.t., 29% of the total metabolism and, at 0.0, 7.5 and 22.5 p.p.t., 19% of the total metabolism was involved in ionic and osmotic regulation.

Madan Mohan Rao (1968, 1971) stated that the minimum oxygen consumption of rainbow trout, Salmo gairdneri (23 - 196 g) occurred at a salinity of 7.5 p.p.t. This salinity is approximately isosmotic with the plasma of the fish (Madan Mohan Rao, 1969). The highest oxygen consumption was measured at 30.0 p.p.t. Intermediate, and approximately equal, values were obtained in fresh water and 15.0 p.p.t. Taking the 7.5 p.p.t. value as the base-line, the author estimated that the energy requirements of ionic and osmotic constitute about 20% of the total metabolism in fresh water and 15.0 p.p.t., and 27% in 30.0 p.p.t. As Madan Mohan Rao (1968) pointed out, the increase in respiratory rate was not proportional to the increase in osmotic gradient.

When oxygen consumption was extrapolated from active to standard, in the studies of both Madan Mohan Rao (1968, 1971) and Farmer and Beamish (1969), the difference between respiration rates at different salinities was considerably less marked. Standard rate may be more applicable to both field and fish-farm conditions than the high levels

of activity on which the conclusions of these two studies are based (Young et al., 1972; Holliday et al., 1974)

Chervinski's results (1961) indicate that, in the case of Tilapia nilotica, there was no measurable effect of salinity (fresh water, 25% and 50% seawater), through conversion efficiency, on growth.

It should be possible to calculate expected conversion efficiencies in Salmo gairdneri on the basis of Madan Mohan Rao's (1968) metabolic estimates. It has been shown that metabolic rate can be estimated from growth rate (Winberg, 1956; Paloheimo and Dickie, 1965) by the following equation:

$$T = R - \frac{\Delta W}{\Delta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where T is total metabolic rate

R is energy equivalent of consumed rations per unit time

W is energy equivalent of weight

t is time.

Conversion efficiency (K_1) has already been defined as

$$K_1 = \frac{\Delta W}{R \Delta t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

from equation 1,

$$\frac{\Delta W}{\Delta t} = R - T$$

$$\therefore \quad K_1 = \frac{R - T}{R}$$

$$= 1 - \frac{T}{R}$$

From the metabolic figures of Madan Mohan Rao (1968)

if at 7.5 p.p.t. $K_1 = 1 - \frac{T}{R}$

then at 0.0 p.p.t. $K_1 = 1 - \frac{1.25\ T}{R}$

at 15.0 p.p.t. $K_1 = 1 - \frac{1.25\ T}{R}$

and at 30.0 p.p.t. $K_1 = 1 - \frac{1.37\ T}{R}$

If, like Madan Mohan Rao (1968) we take K_1 at 7.5 p.p.t. as the base-line, then, for 0+ rainbow trout 90-155 g mean weight, for a given ration at 7.5 p.p.t.,

K_1 (energy) = 0.22

i.e. $1 - \frac{T}{R}$ = 0.22

$\therefore \frac{T}{R}$ = 0.78

$\therefore$ at 0.0 p.p.t. and 15.0 p.p.t., $K_1 = 1 - \frac{1.25\ T}{R}$

$= 1 - 0.98$

$= 0.02$

and at 30.0 p.p.t. $K_1 = 1 - \frac{1.37\ T}{R}$

$= -0.07$ (i.e. sub-maintenance)

The results from active respirometry and the results from growth experiments in which the trout were at a routine level of activity are thus patently not in good agreement. It appears from our conversion efficiency results with Salmo gairdneri at different salinities that the energy requirements of osmoregulation, at least in individuals above about 80 g in weight, were insufficient to have an important effect on conversion until 28.0 - 32.5 p.p.t., when there was a sudden decrease in K_1.

In smaller individuals there seemed to be a gradual decline over the salinity range 0.0 - 28.0 p.p.t., which did not, however, seem to be simply related to the osmotic gradient.

There is evidence that passive mechanisms may be important in osmotic and ionic regulation in fishes. Gordon (1963, 1964) found that total and transintegumentary Cl^- exchanges in the rainbow trout were not proportional to the osmotic gradient and concluded that permeability changes - not necessarily involving significantly increased energy expenditure - may constitute an important part of the process of adaptation to a change in salinity. Motais et al. (1966) review evidence for an 'exchange diffusion effect' and changes in integumentary permeability in response to changes in external salinity in fishes and other aquatic animals.

The osmoregulatory energy requirements at different salinities should not be regarded as the only possible mechanism by which salinity can affect conversion efficiency. Oxygen concentration is inversely related to the concentration of other solutes. Several instances are cited in Warren and Davis (1967) (Hermann et al., 1962; Stewart, 1962; Fisher, 1963) of a decline in growth rate with a slight decrease in water oxygen concentration, but this decline was largely a result of depressed food intake and only below 4 $mg.l^{-1}$ was a decrease in conversion efficiency noted (at $20^{o}C$, in sea water, oxygen saturation = 7.5 $mg.l^{-1}$).

Spontaneous activity influences conversion. Kinne

(1960) recorded the spontaneous activity of Cyprinodon macularius at different salinities and reported that there was no significant effect of salinity on spontaneous activity levels. Holliday (1969) suggested that the higher specific gravity of sea water may reduce the amount of energy required by a fish to maintain its position in the vertical plane. This saving of energy may be quantitatively more important to larvae than to adults with fully-developed swim-bladders. It cannot be ruled out that the process of conversion of absorbed nutrients to body substance is affected at the biochemical level by a change in the concentration or turnover of ions or water in different environmental salinities (Conte et al., 1973).

Salinities of up to 28.0 p.p.t., which are likely to be encountered frequently by trout farmed under sea-loch and estuarine conditions, have no significant effect on conversion efficiency in rainbow trout of the size range examined, while salinities of above 28.0 p.p.t. appear to have a detrimental effect. From the point of view of conversion efficiency, taken in isolation, there seems, therefore, to be no advantage in producing rainbow trout in sea water rather than fresh water. It may be, however, that the increased food intake observed at salinities of between 7.5 and 28.0 p.p.t. could be exploited to increase the throughput of a rainbow trout production unit and, hence, to allow more efficient use of capital equipment.

Further laboratory experiments are necessary on the effects of temperature and fish size on the relationship

between salinity and growth. It might also be instructive to investigate the effects on growth of adding fresh water to the pelleted diet in salinities above 28.0 p.p.t. This may have a sparing effect on the trout's osmoregulatory load in these salinities. To test the applicability of the present results to the commercial situation, pilot-scale farm experiments would be necessary.

4.4. Effects of food intake on conversion efficiency

Over the range of food intakes studied, there was a linear relationship between growth rate (% increase per week) and ration (% dry weight per week). This suggests that there was no discernible tendency for absorption or assimilation to be adversely affected by the level of intake. A similarly linear relationship between growth and intake has been noted by other workers in the case of both nitrogen (Birkett, 1969; Gerking, 1971) and total food (Warren and Davis, 1967; Brett, 1969; Brett, 1971b). This relationship persists even at food intake levels which allow loss of weight (or nitrogen, Gerking, 1971), making it possible, as has been done in this study (Fig. 8) to interpolate the maintenance ration (Brett, 1969, 1971b).

From their reanalysis of the data of a number of other workers (Dawes, 1930-31a,b; Surber, 1935; Pentelow, 1939; Tunison *et al.*, 1939; Phillips *et al.*, 1940; Gerking, 1952; Karzinkin, 1952; Baldwin, 1956; Hatanaka *et al.*, 1956a; Menzel, 1960; Kinne, 1960, 1962),

Paloheimo and Dickie (1966b) made the generalization that conversion efficiency and ration are inversely related. Brown (1946b, 1957) also indicated that conversion efficiency was at a maximum at near-maintenance food intake levels.

Brett et al. (1969) considered that the data used by Paloheimo and Dickie (1966b), to show that conversion efficiency decreased when rations were increased, were unsuitable because most of them were obtained from fishes fed at supra-optimal rates. In the present study, it was found that conversion efficiency increased rapidly from the maintenance ration up to a ration of about 15% body weight per week and then increased less rapidly up to the highest ration provided (38% per week), according to the equation $y = 0.2115 - \frac{0.7699}{x}$, where y is conversion efficiency and x is ration (Fig. 9). According to this equation, conversion efficiency would approach a maximum of 0.2115, but in practice, because of the constraints of maximum food intake and the rates of digestion, absorption and assimilation, this value would not be attained. Pandian and Raghumuraman (1972) found that conversion efficiency in Tilapia mossambica increased to a maximum (24%) at a ration of 58 mg food g^{-1} fish d^{-1}, then fell precipitously to 4% at 65 mg $g^{-1}d^{-1}$, which was the maximum intake attained under the existing environmental conditions.

The results obtained by most other recent workers on laboratory-fed fishes support the conclusion of the present

study that, between broad limits, conversion efficiency increases with food intake. Gerking (1971) found, in the case of bluegill sunfish Lepomis macrochirus, that K_1 was related to ration (x) by an equation of the same form as that determined for rainbow trout in this study, i.e. $K_1 = \frac{a}{x} + b$. On theoretical grounds, this equation would be expected to describe the K_1/ration relationship in all cases where there is a rectilinear relationship between growth rate and ration. The values attributable to the parameters a and b would depend on experimental conditions. The results of Brett (1971b) suggest that, in Oncorhynchus nerka, the relationship between ration and gross conversion efficiency is of this form, with the parameters of the equation being influenced in this case by the nature of the diet. Pandian (1967c) found a rapid increase in conversion efficiency in Megalops cyprinoides up to an intermediate ration level, above which the rate of increase fell. Pandian and Raghumuraman (1972) obtained similar results for Tilopia mossambica. Kelso (1972) found that a/ efficiency changed little with ration between certain limits, but above a ration of 5.69% of body weight per day, there seemed to be a sudden decline.

Winberg (1956) demonstrated that the equation:

$$T = pR - \frac{\Delta W}{\Delta t}$$

gives an estimate of T which closely approximates values of T obtained by direct respirometry.

T is total metabolic rate,

R is energy equivalent of consumed rations,

p is a correction factor for the conversion of consumed to absorbed rations.

Respiratory measurements are usually carried out over a very short period compared with the life-span of the fish. The close correspondence between T values from growth/food intake and oxygen consumption experiments makes it possible to obtain long-term estimates of T from measurements of growth and food intake under laboratory or field conditions.

In the present study, oxygen uptake has been shown to be related to food intake, under the particular conditions of the experiment, by the equation:

$$T = 13.47x + 14.85$$

where T is total metabolic rate in mg O_2 $kg^{-1}h^{-1}$

and x is ration as % dry weight per week.

From this equation, it was estimated that oxygen uptake increases by 8.25 times, from 64 mg O_2 $kg^{-1}h^{-1}$ to 527 mg O_2 $kg^{-1}h^{-1}$, when food intake increases by 10.5 times from weight maintenance level to maximum food intake level. One of the errors of this method of estimation is that oxygen uptake below the maintenance level is likely to be underestimated because, although weight is being gradually lost, the chemical energy content of unit weight of fish is being reduced by the depletion of energy-rich compounds and an increase in water content. This would have the effect of artefactually increasing the gradient of the estimated oxygen uptake/ration curve. Davis and Warren (1965), working with Cottus perplexus,

found that respiratory energy losses increased by 4 times from maintenance to maximum ration levels. Paloheimo and Dickie (1966a) investigated the relationship between food intake and metabolism in the data of Dawes (1930-31b) Pentelow (1939) and Gerking (1955) and found that there was an increase in metabolic rate of 4-5 times with the increase from maintenance to unrestricted rations. This is equivalent to an increase from 'standard' to 'active' metabolism as measured in terms of oxygen uptake. Warren and Davis (1967) define standard metabolic rate, after Beamish (1964) and Brett (1964) as 'the metabolic rate of an unfed fish whose activity has been projected to the zero level on a graph of the relationship between its metabolic rate and its activity level.' Pandian (1967c) found that, for an increase of about 250% in ration, there was a 175% increase in metabolism. This increase is, proportionately, approximately equivalent to the estimates obtained in the present study, but the absolute increase in this case is considerably higher than that recorded in Pandian's experiments. The estimated rise in metabolic rate, at a mean temperature of 6.25°C, from conversion efficiency measurements is somewhat higher than would be predicted from the oxygen consumption measurements (at 5°C) of Madan Mohan Rao (1968, 1971) on the same species, Salmo gairdneri. The estimated rate at maintenance (64.0 mg O_2 $kg^{-1}h^{-1}$) is, however, close to Madan Mohan Rao's measurement of the standard metabolism of rainbow trout, but, whereas the latter author recorded an increase in metabolism of about 6 times at maximum swimming speed, the

calculated increase in the present study was 8.25 times. A further comparison with both this thesis and the results of Madan Mohan Rao (1968, 1971) is provided by the work of Dickson and Kramer (1971) who reported rises in metabolism from 36 to 384 mg O_2 $kg^{-1}h^{-1}$ at 5°C and from 42 to 468 mg O_2 $kg^{-1}h^{-1}$ at 10°C, i.e. a rise of 10 - 11 times between standard and active metabolism.

Warren and Davis (1967) stated that the four-fold increase in the respiratory rate of Cottus perplexus between maintenance and maximum rations (Davis and Warren, 1965) could be explained almost entirely on the basis of increased SDA (specific dynamic action). SDA is considered to be a reflection of the dissipation of energy by the deamination of proteins for catabolism or for storage as fat. This effect can also be considered to have contributed to the comparable ration-related metabolic rate increases recorded by Paloheimo and Dickie (1966a) and other workers (Muir and Niimi, 1972; Solomon and Brafield, 1972). Beamish (1964) reported that the oxygen consumption rate of fasting brook trout Salvelinus fontinalis and white suckers Catostomus commersoni (when corrected to standard rate) decreased to a minimum within 72 h. Pandian (1967c) attributed the decline in the rate of increase of K_1 with ration to an increase in SDA.

Another factor which must play a part in increasing the metabolic rate as feeding level increases is the amount of energy expended in finding and 'capturing' the food - especially in a 'live food' system. Particle size

is thought to be of major importance in determining the energy cost of feeding (Paloheimo and Dickie, 1966b; Beamish and Dickie, 1967). In the present study, particle size was approximately constant, but the time during which food was made available was directly related to ration, and increased activity due to the presence of food and feeder was thus also directly related to ration. This may have contributed to the increase in metabolic rate (as estimated from the negative acceleration of the rate of increase of K_1 with ration).

4.5. Keep-net: Airthrey Loch

Conversion efficiency K_1, at 0.15, is 25% lower than that recorded for rainbow trout of similar size in the aquarium (0.20). The most probable reason for this is the loss of food material through the net enclosure which was employed, although some lowering of conversion efficiency may be the result of the lower water temperatures (about $4^{\circ}C$) which obtained during much of the experimental period. Atherton and Aitken, 1970, measured optimal growth and food conversion by rainbow trout at a temperature of $12^{\circ}C$ on a low fat diet and $16^{\circ}C$ on a high fat diet.

4.6. Effects of sodium chloride dietary supplementation

The results summarised in Table 4 indicate that, in rainbow trout of 30-60 g, dietary sodium chloride supplementation, at least up to 8.5% of total weight, has no significant effect on either food intake or conversion efficiency (K_1).

Zaugg and McLain (1969) showed that added NaCl, even at a level as low as 1.5%, reduced the growth rate of young (0.55-3.27 g) coho salmon Oncorhynchus kisutch. 6% and 12% salt supplements gave rise to a decrease of approximately 20% in conversion efficiency. A mixture of salts (Instant Ocean) had a similar effect. Salt supplements up to 12% did not, however, affect appetite. Coho salmon fed on a NaCl-supplemented diet had a higher rate of survival on transfer to sea water than those fed on the control diet, and also showed higher gill Na^+K^+-stimulated ATPase activity (Zaugg and McLain, 1969, 1970).

Vallet et al. (1970) found that a mixture of salts (with calcium salts predominating), comprising about 6% of the diet, slightly increased the growth rate of Mugil auratus over a 9-day period by increasing the appetite, but without affecting food conversion efficiency.

The difference between the effects of dietary NaCl on the growth of rainbow trout of about 30-60 g and coho salmon of less than 3.27 g could be explained in terms of either species or size differences. Certain lines of evidence would suggest the hypothesis that dietary NaCl, at least at moderate levels might be beneficial to growth by increasing either appetite or conversion efficiency.

Fish (1960) recorded that the quantity of HCl secreted by the stomach of the perch was very small in starved fish even after their first meal, unless NaCl was introduced into the stomach. A similar result was found with Tilapia. Fish (1960) cites evidence from Krogh (1937) and Wikgren (1953) that feeding aquatic animals depend to a great extent for salts on ions incorporated in the food. Phillips (1959) reported that, the more Ca^{2+} there is in the food, the less is absorbed from the external medium.

It may also be considered that excessive salt-loading may in some cases, by changing the gastric and intestinal environment, have an adverse effect on food intake, digestion or absorption and may even have pathological effects as in birds (Krakower and Goettsch, 1945) and mammals (Meneely et al., 1952).

4.7. Effects of salinity on fasting rainbow trout

4.7.1. Weight loss: In two experiments, it was found that weight loss between days 7 and 48 of food deprivation could be described adequately by a straight line. In the case of trout of 80-95 g mean weight (Experiment 2), the relationship had the equation $y = 0.9825 - 0.0028x$ (where x is time and y is relative weight) and in the case of larger individuals (100-157 g; Experiment 1), the line had the equation $y = 0.9794 - 0.0019x$. From days 0 to 7,

however, the weight loss gradients were estimated at 0.0048 in Experiment 2 and 0.0053 in Experiment 1. The mean weight losses over the whole period in fresh water and in salinities of 7.5 p.p.t. and 15.0 p.p.t. in Experiments 1 and 2 were 11% and 15% respectively. In both experiments, during the period from about 20 to 48 days, trout maintained in 32.5 p.p.t. sea water lost significantly more weight than trout in the other three salinities, the losses in Experiments 1 and 2 being 24% and 23% respectively. From the tissue water results of Experiment 1, there are indications that at least some of the observed decrease in weight may be due to osmotic water loss. Rainbow trout may have difficulty in maintaining osmotic equilibrium in sea water during starvation. It is noteworthy that the greater rate of weight loss in 32.5 p.p.t. does not begin to any significant extent until about 20 days into starvation.

Results presented earlier indicated that conversion efficiency was affected little by salinity between the limits of fresh water and 28.0 p.p.t. but that conversion efficiency in 32.5 p.p.t. sea water was significantly depressed. This finding is corroborated by the results obtained in the food deprivation experiments. Peters and Boyd (1972) also recorded that (in the hogchoker Trinectes maculatus) the relationship between salinity and weight loss during starvation was equivalent to that between conversion efficiency and salinity in the fed fish.

There are two probable explanations for the higher rate of weight loss during the first 7 days of fasting.

(a) A higher routine respiratory rate may continue during the initial period of starvation than during further deprivation (because of a higher standard metabolic rate or a temporarily maintained level of spontaneous and anticipatory behaviour) (b) A small quantity of residual ingested matter may have remained in the gut at the beginning of the experiment. In eels (Anguilla japonica), Inui and Ohshima (1966) found a tendency for a slightly higher rate of weight loss during the first 5-10 days of starvation. They showed also that nitrogen excretion decreased markedly during the first 15 days of starvation and then remained constant during further fasting.

The larger trout (Experiment 1) lost more weight (15%) than the small individuals in Experiment 2 (11%) during the same 48-day period. Bellamy (1968), by contrast, found that smaller piranhas Rooseveltiella nattereri (15-21 g) lost weight less rapidly than larger ones (20-30 g). The rates of weight loss recorded in the present study are in general accord with results published for other species(Love, 1958; Phillips et al., 1960; Creac'h and Serfaty, 1965; Inui and Ohshima, 1966; Kamra, 1966; Wilkins, 1967; Bellamy, 1968; Bumgarner, 1971).

4.7.2. Condition factor: The condition factor gives a quantitative measure of the relative proportions ('fatness', depth, girth) of an individual fish.

Condition factor ($\frac{100\ W(g)}{L^3(cm)}$) was measured for each fish in Experiment 2, during starvation. It decreased from a mean of 1.15 on day 0 to 0.96 on day 48, according to the

equation y = 1.1538 - 0.0040x (y is condition factor; x is time in days). Condition factor tended to decrease more rapidly, but not significantly more rapidly, in 32.5 p.p.t. sea water than in the other three salinities. The more rapid decline in 32.5 p.p.t. can be explained by the more rapid weight loss during starvation in water of this salinity. While weight decreases during fasting, length tends to remain relatively constant in adult fishes. In larvae, however, shrinkage in length, as well as decrease in weight, has been recorded (e.g. Ehrlich, 1972).

Love (1958) found that the weight:length ratio of cod was markedly reduced by starvation. After 14 weeks starvation, the condition index ($\frac{W(g)}{L^3(cm)} \times 1000$) of Pleuronectes platessa (Johnston and Goldspink, 1973) fell from 10.50 to 8.32, a drop of 21%. In the present study, the fall in condition factor was about 17% after approximately 7 weeks fasting.

4.7.3. Lipid and water content of muscle and liver: The trout studied in starvation experiment 1, which had been maintained in the same salinities (fresh water, 7.5 p.p.t., 15.0 p.p.t., and 32.5 p.p.t.) for more than 120 days, exhibited no differences in pre-fasting water or extracted-lipid contents of epaxial white muscle or liver tissue which could be related to salinity. This is important when taken in conjunction with the conversion efficiency results.

Extracted muscle lipid fell significantly during starvation, from 0.62% before starvation to 0.47% in trout

which had been starved for 48 days. There was an accompanying slight increase in water content, giving a statistically significant negative correlation between extracted lipid and water content in fresh water, 7.5 p.p.t. and 15.0 p.p.t. In 32.5 p.p.t., on the other hand, water content fell significantly between days 19 and 37.

In the liver, extracted lipid fell significantly from 1.83% to 1.18% after 19 days starvation, but, between days 19 and 48, increased to 1.35%. This latter increase could have been merely a relative increase; liver lipid may have reached its basal level and, in absolute terms, may have remained constant while other tissue components (e.g. protein) were being depleted. Liver water content increased slightly, but there was not a significant correlation between extracted lipid and water content. In 32.5 p.p.t. sea water, liver water content decreased from a mean of about 75.0% to a mean of about 74.0% between days 19 and 37.

The volume of fluid contained in the gall bladder (which had been drained before drying and processing of the liver) increased during starvation, a feature which Love (1958) noted in the cod, and attributed to the absence of stimulation by food during fasting.

The lipid extraction values are low compared with some of those obtained by other workers in the same or related species. Gras et al. (1967a,b) studied seasonal variation in the composition of the muscle of rainbow trout and found that fat content varied around a median value of about 3% of live weight, falling on occasion to

about 2%. The mean water content was, however, considerably lower than that recorded in the present study, averaging about 74.5%, as opposed to 77.7%, which, taken in conjunction with the correlation demonstrated earlier in this thesis between water content and lipid content, suggests that the difference between the results is not purely one of technique, and that differences in previous nutritional history, or some other factor or factors, must also be considered. The results of Gras et al. (1967a,b) also show a relationship between water and lipid, although this is not made explicit in the text of their publication. This suggests that the muscle of rainbow trout behaves, to some extent at least, like the muscle of 'fatty' fishes, (e.g. Clupea harengus, Scomber scombrus) in which water content shows a strong inverse relationship with lipid content (Love, 1960, 1970).

The results of Swift (1955) show that, in the brown trout Salmo trutta, muscle fat content was about 2.5% during most of the year, while water content was about 78.5%. The king salmon Oncorhynchus kisutch (Greene, 1919), after having spent at least 4 months in fresh water without food, had an average muscle lipid content of 2.2%, varying between 0.7% and 3.7%. Wilkins (1967) records that, after 4 months starvation, the herring, a 'fatty' fish,had a fillet lipid content as low as 0.8%, the mean being 1.42%. According to Johnston and Goldspink (1973), the white muscle of Pleuronectes platessa had a total lipid content of only 0.05%, which fell to 0.02% after 14 weeks starvation. The water content of the muscle meanwhile rose by 6% to about 88%.

4.7.4. Lipid and water content of gut and peritoneal fat deposits: In the gut and the associated visceral adipose deposits, there was a very highly significant inverse relationship between extracted lipid and water content over a wide range of lipid contents (2.0% to 40.6%). This relationship is analogous to the fat/water line described for the carcass of the herring Clupea harengus (Brandes and Dietrich, 1953; Iles and Wood, 1965). In the case of the rainbow trout, the adipose deposits around the alimentary canal constitute the major chemical energy reserve, whereas, in herring and other 'fatty' fish, muscular tissue has this function.

Swift (1955) found that, in the brown trout Salmo trutta, the fat stores around the gut fell from 22% of total gut weight in July to 5% of gut weight in October.

A fat/water regression equation was calculated from Swift's (op.cit.) published grouped data, and is shown below and in Fig. 23 for comparison with the results of the present study and with the results of Iles and Wood (1965). Four separate regression equations are quoted from the results of the latter co-authors, because of slight variation with the stage of sexual maturity which is quantified in terms of Heincke's system, which is in turn described by Iles (1964). In each case, x is water content % and y is fat content %.

Author	Species	Tissue		Equation
present work	S. gairdneri	gut		y = 94.42 - 1.16x
Smith (1955)	S. trutta	gut		y = 92.71 - 1.14x
Iles and Wood (1965)	C. harengus	whole herring	I - III	y = 91.45 - 1.16x
		minus gut and	III/IV-IV/V	y = 90.60 - 1.14x
		gonads	V - VI	y = 88.86 - 1.12x
			VII - II	y = 90.60 - 1.14x

TABLE 7. The lipid/water relationship in the gut of rainbow and brown trout and the carcass of herring.

FIGURE 21. Lipid/water relationships in (a) rainbow trout gut and adipose deposits, (b) brown trout gut and adipose deposits and (c) herring carcass.

——— Salmo gairdneri

·····■·· Salmo trutta

- - - - - Clupea harengus

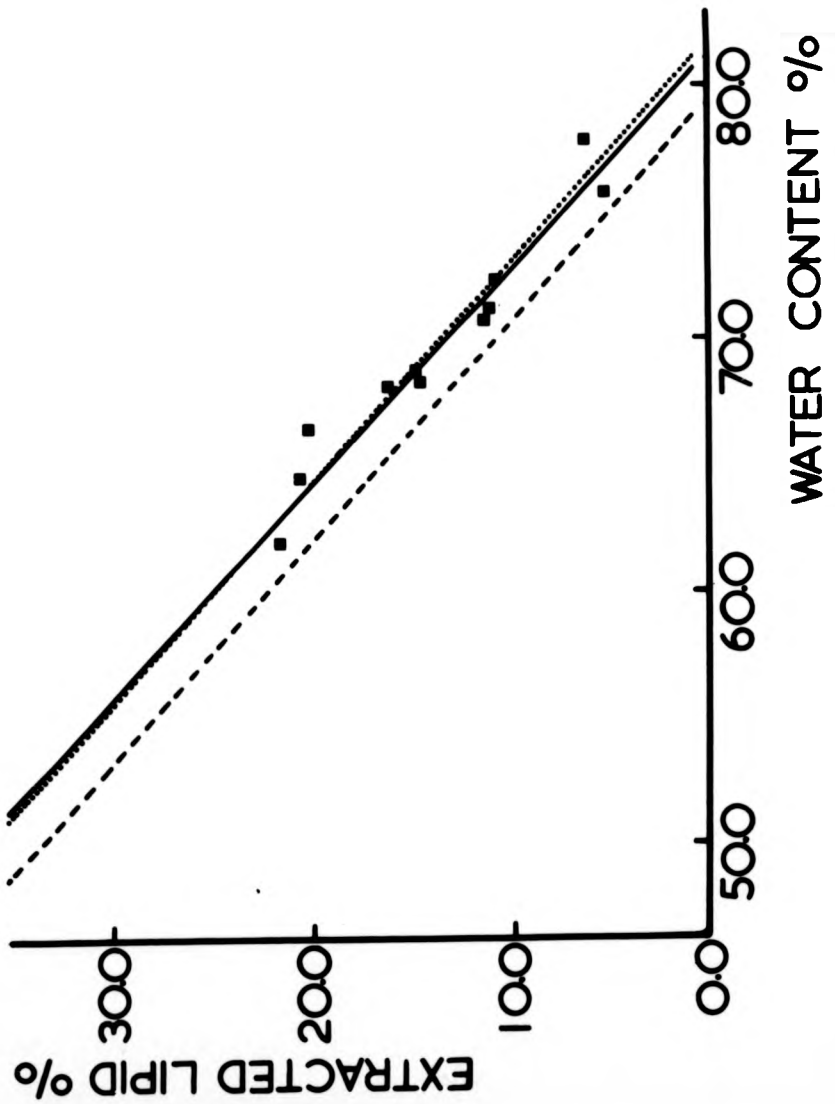
FIGURE 21. Lipid/water relationships in (a) rainbow trout gut and adipose deposits, (b) brown trout gut and adipose deposits and (c) herring carcass.

——— Salmo gairdneri

.....■..... Salmo trutta

- - - - - Clupea harengus

ose
and
ng carcass.



The degree of similarity exhibited by these equations is noteworthy, especially when one considers that, in the two salmonid species discussed, the fat deposits are laid down as more or less discrete masses, whereas in the herring the fat is incorporated in skeletal muscle tissues. Further data from other species would provide useful comparisons. Although there is no significant difference between the gradients of the regressions, the fat/water lines for the herring have a lower intercept on the y axis than the lines for brown trout and rainbow trout. This may be a reflection of the differences in the functions and structures of the tissues involved. Iles and Wood (1965) demonstrated that the fat/water relationship differed significantly from that which would be expected if the accumulation of fat proceeded independently of other flesh components.

Iles and Wood (1965) considered that the relationship between water and lipid in the herring carcass was sufficiently close to justify using water content to estimate fat content. Fig. 18 indicates that the same can be said for the rainbow trout gut, and it is usually more convenient to measure water content directly than it is to measure lipid content.

4.8. Effects of salinity on the histology of the alimentary canal.

The effect of increased salinity on the gut of rainbow trout Salmo gairdneri differs in several respects from

its reported effect on the gut of Etroplus maculatus (Virabhadrachari, 1961). In the latter species, Virabhadrachari (1961) observed an increase in the thickness of the tunica propria and an increase in the height of cells and the size of nuclei in the columnar epithelium. In the rainbow trout, the mean dimensions of these histological components seem to be unaffected by adaptation to different salinities.

Virabhadrachari (1961) noted an increase in the number of mucous cells in the intestine of Etroplus maculatus when it was transferred to sea water. In Salmo gairdneri, by direct contrast, the number of mucous cells decreased with increasing salinity in both the intestine and the rectum.

Greene (1926) recorded that when the king salmon (Oncorhynchus tschawytscha) enters fresh water on its spawning migration, there is an initial increase in the number of mucous cells, followed by their disappearance after some time in fresh water. This 'disappearance' seems likely to be an effect of changes in hormone levels and nutritional status (Robertson et al., 1961) rather than a direct effect of salinity. But the initial increase in mucous cell number on entry into fresh water may be comparable with the effect observed in our study of rainbow trout. Western (1969) found that, in the intestine, mucous cells were fewer in the marine species Enophrys bubalis than in the closely related freshwater species Cottus gobio.

In the rainbow trout rectum, the mucous cells were largest and most numerous, in all salinities, on the apices and on the edges of the annulo-spiral septa. This could be related to the mechanically protective function of mucus. The functions of mucus are generally agreed to include those of chemical and mechanical protection. Its physical properties are such (Hollander, 1954) that it retards diffusion of water and solutes between the external environment and epithelial cells (Ussing, 1960).

Unless individual mucous cells are able substantially to increase mucus production in sea water, it appears that the intestinal and rectal epithelium in rainbow trout adapted to increased salinities may have a reduced surface layer of mucus. A consequence of this could possibly be an increase in the rate of passive diffusion between the contents of the lumen and the epithelial cells. An important mechanism of hypo-osmotic regulation in fishes is active absorption of water (together with monovalent ions) (House and Green, 1963, 1965) from ingested sea water (Smith, 1930; Shehadeh and Gordon, 1969). If a mucous 'barrier' limits the rate of active absorption by retarding passive diffusion towards the cell membrane, a reduction in the mucous layer may be advantageous to fishes adapted to sea water. Even if there is no absolute reduction of the layer of mucus in the intestine, the observed decrease in the density of distribution of mucous cells means that, in increasing salinities, a greater proportion of the epithelium is composed of actively

absorbing columnar epithelial cells. The depressions observed in the epithelium of the majority of the fishes adapted to 15.0 p.p.t. and 32.5 p.p.t. may also have the significance of giving an increased surface for active absorption.

Western (1971) suggested that, if enzymes concerned in active absorption are uniformly arranged on the epithelial membrane, the total amount of enzyme will be proportional to surface area and that, therefore, the greatest concentration of enzyme will occur where the micro-villi are highest and most numerous. He observed that the height of the micro-villi was greater in the marine species Enophrys bubalis than in the freshwater species Cottus gobio. In the rainbow trout, the height of the micro-villi seemed - at the light microscope level - to be unaffected by salinity. Utida (1967) has, however, shown that there was an increase in alkaline phosphatase activity in the intestine of rainbow trout adapted to sea water.

It seems probable that the increase in the total cross-sectional area - and hence the luminal cross-sectional area - of the intestine and rectum is at least partly related to the osmoregulatory function of the intestine. The increase in luminal cross-sectional area as salinity increases may be, in part, a reflection of drinking rate, but may itself have an effect on the food digestion and absorption properties of the intestine. It is notable that only the individuals adapted to 32.5 p.p.t. maintain their intestinal volume during starvation. When

such individuals were dissected, their intestines and rectum were found to contain a large amount of water.

The height of the villi was shown to vary with salinity in a similar way to the cross-sectional area. This may have had its direct cause in the degree of expansion of the intestine rather than in the salinity.

Most previous work on the histology of starvation in the salmonids has dealt with fishes which were not only starving but were also either undergoing a spawning migration or on the point of spawning.

Gulland (1898) reported extensive degeneration of the gastric and intestinal epithelium in migrating Atlantic salmon Salmo salar. Greene (1926) noted even more degenerative changes in the King Salmon (Oncorhynchus tschawytscha). All the tissues of the alimentary canal in this species, including the gastric gland cells, showed atrophic changes. Roberton et al. (1961) compared the histological changes associated with spawning in Pacific salmon (Oncorhynchus sp.), in migratory steelhead trout (Salmo gairdneri) and in non-migratory rainbow trout (S. gairdneri) The stomach of the Pacific salmon showed the most extensive changes, similar to those recorded by Greene (1926). Steelheads showed only minor changes. Rainbow trout showed no observable change. The blood corticosteroid levels in steelhead and rainbow trout were similar, but the pre-spawning feeding habits of the two types differed, rainbow trout feeding normally whereas steelheads fed little and irregularly. Pacific

salmon starve completely during the spawning migration. The authors (Robertson et al., 1961) concluded that feeding rate modifies the catabolic effects of hyper-adrenocorticism.

Later work by Robertson et al. (1963) on the effect of peritoneally-implemented hydrocortisone on starving immature rainbow trout strongly suggested that most of the histological changes seen in spawning salmonids are due to high blood hydrocortisone and cortisone levels and are not caused by starvation alone. The only part of the gut examined by Robertson and his colleagues was the stomach.

The results reported in this thesis confirm that the stomach is virtually unaffected by starvation in the immature rainbow trout and show that other parts of the alimentary canal also present little evidence of degeneration after 48 days of starvation in various salinities. In the stomach, there was no measurable change in the thickness of the tissue layers examined. The only change seen was a tendency towards a decrease in total cross-sectional area. In fresh water, 7.5 p.p.t. and 15.0 p.p.t., the intestine and rectum show a considerable decrease in cross-sectional area. That this is due to elastic contraction rather than atrophy is indicated by a slight increase in the thickness of both the muscle layer and the stratum compactum.

In all salinities, the number and size of mucous cells decreased during starvation, but the only individuals

in which the columnar epithelium was affected by starvation were those in 32.5 p.p.t. sea water, which may suggest that fish subjected to the highest osmotic gradient were most affected by starvation.

5. LITERATURE CITED

ADELMAN, H.M., J.L. BINGHAM and J.L. MAATCH (1955). The effect of starvation upon brook trout of three sizes. Progr. Fish-Cult., 17, 110-112.

ATHERTON, W.D. and A. AITKEN (1970). Growth, nitrogen metabolism and fat metabolism in Salmo gairdneri. Comp. Biochem. Physiol., 36, 719-747.

BALDWIN, N.S. (1956). Food consumption and growth of brook trout at different temperatures. Trans. Am. Fish. Soc., 86, 323-328.

BEAMISH, F.W.H. (1964). Influence of starvation on standard and routine oxygen consumption. Trans. Am. Fish Soc., 93, 103-107.

BEAMISH, F.W.H. (1972). Ration size and digestion in largemouth bass, Micropterus salmoides Lacepede. Can. J. Zool., 50, 153-164.

BEAMISH, F.W.H. and L.M. DICKIE (1967). Metabolism and biological production in fishes. In The Biological Basis of Freshwater Fish Production (Ed. S.D. GERKING), 215-242. Oxford, Blackwell Scientific Publications.

BELL, F.R. and S.A.D. RAZIG (1973). The effect of some molecules and ions on gastric function in the milk-fed calf. J. Physiol., 228, 513-526.

BELLAMY, D. (1968). Metabolism of the red piranha (Rooseveltiella nattereri) in relation to feeding behaviour. Comp. Biochem. Physiol., 25, 343-347.

BIRKETT, L. (1969). The nitrogen balance in plaice, sole and perch. J. Exp. Biol., 50, 375-386.

BRANDES, C.H. and R. DIETRICH (1953). A review of the problem of fat and water content in the edible part of the herring. Fette Seifen, 55, 533-541.

BRETT, J.R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Canada, 21, 1183-1226.

BRETT, J.R. (1971a). Satiation time, appetite and maximum food intake of sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Bd. Canada, 28, 409-415.

BRETT, J.R. (1971b). Growth responses of young sockeye salmon (Oncorhynchus nerka) to different diets and planes of nutrition. J. Fish. Res. Bd. Canada, 28, 1635-1643.

BRETT, J.R. and N.R. GLASS (1973). Metabolic rates and critical swimming speeds of sockeye salmon (Oncorhynchus nerka) in relation to size and temperature. J. Fish. Res. Bd. Canada, 30, 379-387.

BRETT, J.R. and D.A. HIGGS (1970). Effect of temperature on the rate of gastric digestion of fingerling sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Bd. Canada, 26, 2363-2394.

BRETT, J.R., J.E. SHELBOURNE and C.T. SHOOP (1969). Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Bd. Canada, 26, 2363-2394.

BROCKSEN, R.W. and R.E. COLE (1972). Physiological responses of three species of fishes to various salinities. J. Fish. Res. Bd. Canada, 29, 399-405.

BRODY, S. (1945). Bioenergetics and growth: with special reference to the efficiency complex in domestic animals. New York, Reinhold, 1023pp.

BROWN, M.E. (1946a). The growth of brown trout (Salmo trutta Linn.). I. Factors influencing the growth of trout fry. J. Exp. Biol., 22, 118-129.

BROWN, M.E. (1946b). The growth of brown trout. II. The growth of two-year-old trout at a constant temperature of 11.5°C. J. Exp. Biol., 22, 130-144.

BROWN, M.E. (1946c). The growth of brown trout. III. The effect of temperature on the growth of two-year-old trout. J. Exp. Biol., 22, 145-155.

BROWN, M.E. (1957). Experimental studies on growth. In The Physiology of Fishes (Ed. M.E. BROWN) Vol. I, 361-400, New York, Academic Press Inc.

BUMGARNER, D.H. (1971). A technique for controlling trout growth. Progr. Fish-Cult., 33 (1), 41.

BURNSTOCK, G. (1959). The morphology of the gut of the brown trout (Salmo trutta). Quart. J. micr. Sci., 100, 183-198.

BUSNEL, R.G. and A. DRILHON (1944). Modifications de la concentration moleculaire du milieu intérieur de Salmo irideus au cours de la croissance en rapport avec l'adaptation aux changements de salinité. C.R. Soc. Biol., 138, 334-336.

BYRNE, J.M., F.W.H. BEAMISH and R.L. SAUNDERS (1972). Influence of salinity, temperature and exercise on plasma osmolality and ionic concentration in Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Canada, 29, 1217-1220.

CANAGARATNAM, P. (1959). Growth of fishes in different salinities. J. Fish. Res. Bd. Canada, 16, 121-130.

CANAGARATNAM, P. (1966). Growth of Tilapia mossambica Peters in different salinities. Bull. Fish. Res. Sta. Ceylon, 19, 47-50.

CHERVINSKI, J. (1961). Laboratory experiments on the growth of Tilapia nilotica in various saline concentrations. Bamidgen, 13 (1), 8-13.

CONTE, F.P., G.L. PETERSON and R.D. EWING (1973). Larval salt gland of Artemia salina nauplii. Regulation of protein synthesis by environmental salinity. J. Comp. Physiol., 82, 277-289.

CONTE, F.P. and H.H. WAGNER (1965). Development of osmotic and ionic regulation in juvenile steelhead trout (Salmo gairdneri). Comp. Biochem. Physiol., 14, 603-620.

CREAC'H, Y. and C. COURNÈDE (1965). Contribution to the study of enforced starvation in the carp, Cyprinus carpio L.; variations in the amount of water and nitrogen in the tissue. Bull. Soc. Hist. nat. Toulouse, 100, 361-370.

CREAC'H, Y. and A. SERFATY (1965). Proteolysis in the common carp (Cyprinus carpio) during fasting: importance and localisation. C.r. Séanc. Soc. Biol., 159, 483-486.

DAVIES, P.M.C. (1963). Food input and energy extraction efficiency in Carassius auratus. Nature, London, 198, 707.

DAVIES, P.M.C. (1964). The energy relations of Carassius auratus. I. Food input and extraction efficiency at two experimental temperatures. Comp. Biochem. Physiol., 12, 67-80.

DAVIS, G.E. and C.E. WARREN (1965). Trophic relations of a sculpin in laboratory stream communities. J. Wildl. Mgmt., 29, 846-871.

DAWES, B. (1930-31a). Growth and maintenance in the plaice (Pleuronectes platessa L.). Part I. J. mar. biol. Ass. U.K., 17, 103-174.

DAWES, B. (1930-31b). Growth and maintenance in the plaice (Pleuronectes platessa L.). Part II. J. mar. biol. Ass. U.K., 17, 877-975.

DICKSON, I.W. and R.H. KRAMER (1971). Factors influencing scope for activity and active and standard metabolism of rainbow trout. J. Fish. Res. Bd. Canada, 28, 587-596.

EHRLICH, K.F. (1972). Morphometrical, behavioural and chemical changes during growth and starvation of herring and plaice larvae. Ph.D. Thesis. University of Stirling, 1972.

FARMER, G.J. and W.H. BEAMISH (1969). Oxygen consumption of Tilapia nilotica in relation to swimming speed and salinity. J. Fish. Res. Bd. Canada, 26, 2807-2821.

FISH, G.R. (1960). The comparative activity of some digestive enzymes in the alimentary canal of Tilapia and perch. Hydrobiologia, 15, 161-179.

FISHER, R.J. (1963). Influence of oxygen concentration and of its diurnal fluctuations on the growth of juvenile coho salmon. M.S. Thesis. Oregon State University. 48pp.

GARSIDE, E.T. and J.S. TAIT (1958). Preferred temperature of rainbow trout (Salmo gairdneri Richardson) and its unusual relationship to acclimation temperature. Can. J. Zool., 36, 563-567.

GERKING, S.D. (1952). The protein metabolism of the sunfishes of different ages. Physiol. Zool., 25, 358-372.

GERKING, S.D. (1954). The food turnover of a bluegill population. Ecology 35, 490-498.

GERKING, S.D. (1955). Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. Physiol. Zool., 28, 267-282.

GERKING, S.D. (1971). Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiol. Zool., 44, 9-19.

GIBSON, M.B. and B. HIRST (1955). The effect of salinity and temperature on the pre-adult growth of guppies. Copeia, 3, 241-243.

GILLESPIE, A.L. (1898). Changes in the digestive activity of the secretions of the alimentary canal of the salmon in different conditions. Fishery Board for Scotland. Report of investigations on the life history of salmon., 23-35.

GORDON, M.S. (1963). Chloride exchange in rainbow trout adapted to different salinities. Biol. Bull., 124, 45-54.

GORDON, M.S. (1964). Animals in aquatic environments: fishes and amphibians. In Handbook of Physiology, Section 4: Adaptation to the environment (Ed. DILL, B.D., E.F. ADOLPH and C.G. WILBER), 697-713, American Physiological Society, Washington, D.C.

GORDON, M.S., I. BOËTIUS, D.H. EVANS, R. McCARTHY and L.C. OGLESBY (1965). Salinity adaptation in the mudskipper fish. Hvalrad Skr, 48, 85-93.

GRAS, J., R. REYNAUD, L. GAMOTY, J. FREY and J.C. HENRY (1967a). Étude biochimique des poissons: 1. Variations semestrielles de la teneur en eau et an protéines du tissu musculaire de la truite Arc-en-ciel (Salmo gairdnerii Rich). Experientia, 23, 430-431.

GRAS, J., R. REYNAUD, L. GAMOTY, J. FREY and J.C. HENRY (1967b). Étude biochimique des poissons: 2. Variations semestrielles de la teneur en glycogène, en lipides totaux et en minéraux du tissu musculaire de la truite Arc-en-ciel (Salmo gairdnerii Rich). Experientia, 23, 431-432.

GREENE, C.W. (1919). Biochemical changes in the muscle tissue of the king salmon during the fast of the spawning migration. J. Biol. Chem., 39, 435-477.

GREENE, C.W. (1926). The physiology of the spawning salmon. Physiol. Rev., 6, 201-241.

GROVES, T.D.D. (1970). Body composition changes during growth in young sockeye (Oncorhynchus nerka) in fresh water. J. Fish. Res. Bd. Canada, 27, 929-942.

GULLAND, G.L. (1898). The minute structure of the digestive tract of the salmon, and the changes which occur in it in fresh water. Fishery Board for Scotland: Report of investigations on the life history of salmon, 13-22.

HATANAKA, M., M. KOSAKA and Y. SATO (1956). Growth and food consumption in plaice. Part I Limanda yokohamae (Günther) Tokohu J. Agr. Res., 7, 151-162.

HERMANN, R.B., C.E. WARREN and P. DOUDOROFF (1962). Influence of oxygen concentration on the growth of juvenile coho salmon. Trans. Am. Fish. Soc., 91, 155-167.

HICKMAN, C.P. Jr. (1959). The osmoregulatory role of the thyroid gland in the starry flounder, Platichthys stellatus. Can. J. Zool., 37, 997-1060.

HOLLANDER, F. (1954). The two-component mucous barrier. Archs. intern. Med., 93, 107-120.

HOLLIDAY, F.G.T. (1969). The effects of salinity of the eggs and larvae of teleosts. In Fish Physiology (Ed. HOAR, W.S. and D.J. RANDALL) Vol. I, 293-311. Academic Press, New York & London.

HOLLIDAY, F.G.T., P. TYTLER and A.H. YOUNG (1974). Activity levels of trout (Salmo trutta) in Airthrey Loch, Stirling, and Loch Leven, Kinross. Proc. Roy. Soc. Edinb., B, 74, 315-331.

HOUSE, C.R. and K. GREEN (1963). Sodium and water transport across isolated intestine of a marine teleost. Nature, London, 199, 1293-1294.

HOUSE, C.R. and K. GREEN (1965). Ion and water transport in isolated intestine of the marine teleost Cottus scorpio. J. Exp. Biol., 42, 177-189.

HOUSTON, A.H. (1959). Locomotor performance of chum salmon fry (Oncorhynchus keta) during osmoregulatory adaptation to sea water. Can. J. Zool., 37, 591-605.

HOUSTON, A.H. (1961). Influence of size upon the adaptation of steelhead trout and chum salmon (Oncorhynchus keta) to sea water. J. Fish. Res. Bd. Canada, 18, 401-415.

ILES, T.D. (1964). The duration of maturation stages in herring. J. Cons. perm. int. Explor. Mer., 29, 166-188.

ILES, T.D. and R.J. WOOD (1965). The fat/water relationship in North Sea herring (Clupea harengus) and its possible significance. J. mar. biol. Ass. U.K., 45, 353-366.

INUI, Y. and Y. OHSHIMA (1966). Effect of starvation on metabolism and chemical composition of eels. Bull. Jap. Soc. scient. Fish., 32, 492-501.

ISHIWATA, N. (1969). Ecological studies on the feeding of fishes. VII. Frequency of feeding and satiation amount. Bull. Jap. Soc. scient. Fish., 35, 979-984.

IVLEV, V.S. (1945). [The biological productivity of waters.] Fish. Res. Bd. Canada Transl. Ser., 447.

IVLEV, V.S. (1961). Experimental ecology of the feeding of fishes. New Haven, Yale University Press., 302 pp.

JENSEN, K.W. (1966). Saltwater rearing of rainbow trout and salmon in Norway. European Inland Fisheries Advisory Commission. Symp. on Feeding in Trout and Salmon Culture, Belgrade 1966.

JOB, S.V. (1959). The metabolism of Plotozus anguillaris (Bloch) in various concentrations of salt and oxygen in the medium. Proc. Indian Acad. Sci. (B), 50, 260-267.

JOB, S.V. (1960). Growth and calorific approximation in the speckled trout (Salvelinus fontinalis). Indian J. Fish., 7, 129-136.

JOB, S.V. (1969). The respiratory metabolism of Tilapia mossambica. I. The effect of size, temperature and salinity. Marine Biology, 2, 121-126.

JOHNSON, L. (1966). Experimental determination of food consumption of pike, Esox lucius, for growth and maintenance. J. Fish. Res. Bd. Canada, 23, 1495-1505.

JOHNSTON, I.A. and G. GOLDSPINK (1973). Some effects of prolonged starvation on the metabolism of the red and white myotomal muscles of the plaice, Pleuronectes platessa. Marine Biology, 19, 348-353.

KANEKO, T., M. TAKEUCHI, S. ISHII, H. HIGASHI and T. KIKUCHI (1967). Effect of dietary lipids on fish under cultivation. IV. Changes of fatty acid composition in flesh lipids of rainbow trout on non-feeding. Bull. Jap. Soc. scient. Fish., 33, 56-58.

KAMRA, S.K. (1966). Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (Gadus morhua L). J. Fish. Res. Bd. Canada, 23, 975-982.

KARZINKIN, G.S. (1952). [Fundamental processes in the biological production of bodies of water.] Pischepromizdat, Moscow. 342pp. (cited by WINBERG, 1956).

KELSO, J.R.M. (1972). Conversion, maintenance and assimilation for walleye, Stizostedion vitreum vitreum as affected by size, diet and temperature. J. Fish. Res. Bd. Canada, 29, 1181-1192.

KEYS, A.B. (1931). A study of the selective action of decreased salinity and of asphyxiation on the Pacific killifish Fundulus parvipinnis. Scripps Inst. Ocean. Bull., Tech. Ser., 2, 417-490.

KINNE, O. (1960). Growth, food intake and food conversion in a euryplastic fish exposed to different temperatures and salinities. Physiol. Zoöl., 33, 288-317.

KINNE, O. (1962). Irreversible non-genetic adaptation. Comp. Biochem. Physiol., 5, 265-282.

KLEIBER, M. (1961). The Fire of Life. An Introduction to Animal Energetics. New York, John Wiley & Sons, Inc., 454pp.

KRAKOWER, C.A. and M. GOETTSCH (1945). Effect of excessive ingestion of sodium chloride on the chick, with particular reference to renal changes. Arch. Path., 40, 209.

KROGH, A. (1937). Osmotic regulation in fresh water fishes by active absorption of Cl^- ions. Z. vergl. Physiol., 24, 656-666.

LEPKOVSKY, S. (1948). The physiological basis of voluntary food intake. Advan. Food Res., 1, 105-148.

LISON, L. (1954). Alcian blue 8G with chlorantine fast red 5B. A technic for selective staining of mucopolysaccharides. Stain Technol., 29, 131-138.

LOVE, R.M. (1958). Studies on the North Sea cod. III. Effects of starvation. J. Sci. Fd. Agric., 9, 617-620.

LOVE, R.M. (1960). Water content of cod (Gadus callarias L.) muscle. Nature, London, 185, 692.

LOVE, R.M. (1970). The Chemical Biology of Fishes. London, Academic Press. 547pp.

MacCRIMMON, H.R. (1971). World distribution of rainbow trout. J. Fish. Res. Bd. Canada, 28, 663-704.

MADAN MOHAN RAO, G. (1968). Oxygen consumption of rainbow trout (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool., 46, 781-787.

MADAN MOHAN RAO, G. (1969). Effect of activity, salinity and temperature on plasma concentrations of rainbow trout. Can. J. Zool., 47, 131-134.

MADAN MOHAN RAO, G. (1971). Influence of activity and salinity on the weight-dependent oxygen consumption of the rainbow trout (Salmo gairdneri). Marine Biology, 8, 205-212.

MALOEUF, N.S.R. (1937). [Studies on the respiration of animals. II. Water animals having in their internal environment an oxygen carrier.] Ztsch. vergl. Physiol., 25, 29-42. (cited by WINBERG, 1956).

MARKOVA, L.I. (1949). [The effect of various salinities and temperatures on respiration of bream, Baltic herring and eels.] Izvestia VNIORKh, 29, 106-117. (cited by WINBERG, 1956).

MAYER, J. (1955). Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. In The regulation of hunger and appetite., (Ed. R.W. MINER) Ann. N.Y. Acad. Sci., 63, 15-43.

MAYNARD, A.L. and K.L. LOOSLI (1962). Animal Nutrition. 5th Ed. New York, McGraw-Hill.

MENEELY, G.R., R.G. TUCKER, S.H. AUERBACH and W.J. DARBY (1952). Renal damage in rats fed large quantities of sodium chloride. J. Clin. Invest., 31, 650.

MENZEL, D.W. (1959). Utilization of algae for growth by the angel fish. J. Cons. perm. int. Explor. Mer., 24, 308-313.

MENZEL, D.W. (1960). Utilization of food by a Bermuda reef-fish Epinephelus guttatus. J. Cons. perm. int. Explor. Mer., 25, 216-222.

MINER, R.W.(ed.) (1955). The regulation of hunger and appetite. Ann. N.Y. Acad. Sci., 63, 1-144.

MOTAIS, R., F. GARCIA ROMEU and J. MAETZ (1966). Exchange diffusion effect and euryhalinity in teleosts. J. Gen. Physiol., 50, 391-422.

MUIR, B.S. and A.J. NIIMI (1972). Oxygen consumption of the euryhaline fish aholehole (Kuhlia sandvicensis) with reference to salinity, swimming and food consumption. J. Fish. Res. Bd. Canada, 29, 67-77.

NELSON, J.S. (1968). Salinity tolerance of brook sticklebacks, Culaea inconstans, freshwater nine-spine sticklebacks, Pungitius pungitius and freshwater four-spine sticklebacks, Apeltes quadracus. Can. J. Zool., 46, 663.

NEUHAUS, O. and J.E. HALVER (eds.) (1969). Fish in Research. Academic Press Inc., New York.

NIEDERL, J.B. and V. NIEDERL (1942). Micro-methods of quantitative organic analysis. 2nd Ed. Wiley, New York.

OGINO, C., J. KAKINO and M.S. CHEN (1973). Protein nutrition in fish. II. Determination of metabolic faecal nitrogen and endogenous nitrogen excretions of carp. Bull. Jap. Soc. Sci. Fish., 39, 519-523.

OTTO, R.G. (1971). Effects of salinity on the survival and growth of pre-smolt coho salmon (Oncorhynchus kisutch). J. Fish. Res. Bd. Canada, 28, 343-349.

PAINTAL, A.S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation and thirst. J. Physiol., 126, 271-285.

PALOHEIMO, J.E. and L.M. DICKIE (1965). Food and growth of fishes. I. A growth curve derived from experimental data. J. Fish. Res. Bd. Canada, 22, 521-542.

PALOHEIMO, J.E. and L.M. DICKIE (1966a). Food and growth of fishes. II. Effects of food and temperature on the relation between metabolism and weight. J. Fish. Res. Bd. Canada, 23, 869-908.

PALOHEIMO, J.E. and L.M. DICKIE (1966b). Food and growth of fishes. III. Relations among food, body size and growth efficiency. J. Fish. Res. Bd. Canada, 23, 1209-1248.

PANDIAN, T.J. (1967a). Intake, digestion, absorption and conversion of food in the fishes Megalops cyprinoides and Ophiocephalus striatus. Marine Biology, 1, 16-32.

PANDIAN, T.J. (1967b). Transformation of food in the fish Megalops cyprinoides. I. Influence of quality of food. Marine Biology, 1, 60-64.

PANDIAN, T.J. (1967c). Transformation of food in the fish Megalops cyprinoides. II. Influence of quantity of food. Marine Biology, 1, 107-110.

PANDIAN, T.J. and R. RAGHUMURAMAN (1972). Effects of feeding rate on conversion efficiency and chemical composition of the fish Tilapia mossambica. Marine Biology, 12, 129-136.

PARRY, G. (1958). Size and osmoregulation in fishes. Nature, London, 181, 1218.

PARRY, G. (1960). The development of salinity tolerance in the salmon, Salmo salar and some related species. J. Exp. Biol., 37, 425-434.

PARRY, G. (1964). Organ systems in adaptation: the osmotic regulating system. In Handbook of Physiology. Section 4: Adaptation to the environment. American Physiological Society, Washington, D.C.

PARVATHESWARARAO, V. (1965). Influence of different temperature-salinity combinations on the oxygen consumption in the freshwater fish Etroplus maculatus. Helgoländer wiss. meeresunters, 12, 301-314.

PAVLOVSKII, E.N. (1962). (ed.) Techniques for the investigation of fish physiology. Israel Program for Scientific Translations. Jerusalem 1964.

PENTELOW, F.T.K. (1939). The relationship between growth and food consumption in brown trout. J. Exp. Biol., 16, 446-473.

PETERS, D.S. and M.T. BOYD (1972). The effect of temperature, salinity and food availability on the feeding and growth of the hogchoker Trinectes maculatus (Bloch & Schneider). J. exp. mar. Biol. Ecol., 7, 201-207.

PHILLIPS, A.M. Jr. (1954). Effect of starvation upon brook and rainbow trout. Progr. Fish-Cult. 16, 124.

PHILLIPS, A.M. Jr. (1959). The known and possible role of minerals in trout nutrition and physiology. Trans. Am. Fish. Soc., 88, 133-135.

PHILLIPS, A.M. Jr., D.L. LIVINGSTON and R.F. DUMAS (1960). Effect of starvation and feeding on the chemical composition of brook trout. Progr. Fish-Cult., 22, 147-154.

PHILLIPS, A.M., A.V. TUNISON, A.H. FENN, C.R. MITCHELL and C.M. McCAY (1940). The nutrition of trout. Cortland Hatchery Report for 1940, No. 9, 32pp.

PHILLIPSON, J. (1964). A miniature bomb calorimeter for small biological samples. Oikos, 15, 130-139.

RAFFY, A. (1932). Recherches physiologiques sur le mécanisme de la mort des poissons stenohaline soumis à des variations de salinité. Bull. Inst. océanogr. Monaco, 602, 257-291.

RAFFY, A. (1933). Recherches sur le metabolisme respiratoire des poikilotherms aquatiques. Annls. Inst. oceanogr. Monaco, 13, 259-293.

RAFFY, A. (1955). L'euryhalinité des jeunes plies Pleuronectes platessa. C.r. Séanc. Soc. Biol., 149, 2122-2123.

ROBERTSON, O.H., M.A. KRUPP, S.F. THOMAS, C.B. FAVOUR, S. HANE and C.B. WEXLER (1961). Hyperadrenocorticism in spawning migratory and non-migratory rainbow trout; comparison with Pacific salmon (Oncorhynchus). Gen. Comp. Endocrinol., 1, 473-484.

ROBERTSON, O.H., S. HANE, B.C. WEXLER and A.P. RINFRET (1963). The effect of hydrocortisone on immature rainbow trout. Gen. Comp. Endocrinol., 3, 422-436.

ROZIN, P. and J. MAYER (1961). Regulation of food intake in the goldfish. Am. J. Physiol., 201, 968-974.

SAUNDERS, R.L. and E.B. HENDERSON (1969a). Survival and growth of Atlantic salmon fry in relation to salinity and diet. Fish. Res. Bd. Canada., Tech. Rep. 148, 9pp.

SAUNDERS, R.L. and E.B. HENDERSON (1969b). Survival and growth of Atlantic salmon parr in relation to salinity and diet. Fish. Res. Bd. Canada., Tech. Rep. 147, 5pp.

SAUNDERS, R.L. and E.B. HENDERSON (1969c). Survival and growth of Atlantic salmon smolts and post-smolts in relation to salinity and diet. Fish. Res. Bd. Canada, Tech. Rep. 149, 24pp.

SCHLIEPER, C. (1936). [Regulation of the relation between basal metabolism and temperature in animals with variable body temperature.] Biol. Ztbl., 56, 87-94. (cited by WINBERG, 1956).

SEDGWICK, S.D. (1970). Rainbow trout farming in Scotland. Farming trout in salt water. Scottish Agriculture 1970, No. 3, 180-185.

SHEHADEH, Z.H. and M.S. GORDON (1969). The role of the intestine in salinity adaptation of the rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 30, 397-418.

SMITH, F.E. (1963). Population dynamics in Daphnia magna and a new model for population growth. Ecology, 44, 651-663.

SMITH, H.W. (1930). The absorption and excretion of water and salts by marine teleosts. Am. J. Physiol., 93, 480-505.

SOLOMON, D.J. and A.E. BRAFIELD (1972). The energetics of feeding, metabolism and growth of perch (Perca fluviatilis). J. Anim. Ecol., 41, 699-718.

STEWART, N.E. (1962). The influence of oxygen concentration on the growth of juvenile largemouth bass. M.S. Thesis, Oregon State University, 46pp.

SURBER, E.W. (1935). Trout feeding experiments with natural food (Gammarus fasciatus). Trans. Am. Fish. Soc., 65, 300-304.

SWIFT, D.R. (1955). Seasonal variation in the growth rate, thyroid gland activity and food reserves of brown trout. J. Exp. Biol., 32, 751-764.

SWIFT, D.R. (1960). An improved feed for experimental fish. Nature, London, 187, 1133.

SWIFT, D.R. (1961). The annual growth rate cycle in brown trout (Salmo trutta L.) and its cause. J. Exp. Biol., 38, 595-604.

TUBA, J. and N. DICKIE (1955). The role of alkaline phosphatase in intestinal absorption. 4. The effects of various proteins on levels of the enzyme in intestinal mucosa. Can. J. Biochem. Physiol., 33, 89-92.

TUNISON, A.V., A.M. PHILLIPS, C.M. McCAY, C.R. MITCHELL and E.O. RODGERS (1939). The nutrition of trout. Cortland Hatchery Report for 1939, No. 8. N.Y. Conservation Department, Albany, N.Y.

USSING, H.H. (1960). The frog skin potential. J. Gen. Physiol., 43, 135-147.

UTIDA, S. (1967). Effect of sodium chloride on alkaline phosphatase activity in intestinal mucosa of the rainbow trout. Proc. Jap. Acad., 43, 783-788.

UTIDA, S. and N. ISONO (1967). Alkaline phosphatase activity in intestinal mucosa of the eel adapted to fresh water or sea water. Proc. Jap. Acad., 43, 789-792.

VALLET, F., J. BERHAUT, C. LERAY, B. BONNET and P. PIC (1970). Preliminary experiments on the artificial feeding of Mugilidae. Helgoländer wiss. Meeresunters, 20, 610-619.

VIRABHADRACHARI, V. (1961). Structural changes in the gills, intestine and kidney of Etroplus maculatus (Teleostei) adapted to different salinities. Quart. J. Microscop. Sci., 102, 361-369.

WALLACE, J.C. (1973). Observations on the relationship between food consumption and metabolic rate of Blennius pholis L. Comp. Biochem. Physiol., 45A, 293-306.

WARREN, C.E. and G.E. DAVIS (1967). Laboratory studies on the feeding, bioenergetics and growth of fish. In The Biological Basis of Freshwater Fish Production (Ed. S.D. GERKING), 175-214, Oxford, Blackwell Scientific Publications.

WEINREB, E.L. and N.M. BILSTAD (1955). Histology of the digestive tract and adjacent structures of the rainbow trout, Salmo gairdneri irideus. Copeia, (3), 194-204.

WESTERN, J.R.H. (1969). Studies on the diet, feeding mechanism and alimentary tract in two closely-related teleosts, the freshwater Cottus gobio L. and the marine Parenophrys bubalis Euphrasen. Acta Zool. Stockh., 50, 185-205.

WESTERN, J.R.H. (1971). Feeding and digestion in two cottid fishes, the freshwater Cottus gobio L. and the marine Enophrys bubalis (Euphrasen). J. Fish. Biol., 3, 225-246.

WIKGREN, B. (1953). Osmotic regulation in some aquatic animals with special reference to the influence of temperature. Acta Zool. Fennica No. 71, 1-102.

WILKINS, N.P. (1967). Starvation of the herring Clupea harengus L. Survival and some gross biochemical changes. Comp. Biochem. Physiol., 23, 506-518.

WINBERG, G.G. 1956 (transl. 1960). [Rate of metabolism and food requirements of fishes.] Fish. Res. Bd. Canada. Transl. Ser. No. 194, 202pp.

YAMAMOTO, T. (1966). An electron microscope study of the columnar epithelial cell in the intestine of fresh water teleosts: goldfish (Carassius auratus) and rainbow trout (Salmo irideus). Zeitschrift für Zellforschung, 72, 66-87.

ZAUGG, W.S. and L.R. McLAIN (1970). ATP-ase activity in gills of salmonids: seasonal variations and salt water influence in coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol., 35, 587-596.

ZAUGG, W.S. and L.R. McLAIN (1969). Inorganic salt effects on growth, salt water adaptation and gill ATP-ase of Pacific salmon in Fish in Research (ed. NEUHAUS, O. & J.E. HALVER), 293-306. Academic Press Inc., New York.

APPENDIX 1.

Effects of salinity on food intake.

Salinity (p.p.t.)	Mean weekly intake ± s.e. (% dry matter)		
Expt. No.	1	2	3
0.0	24.42±1.47	20.39±0.84	23.49±0.79
7.5	26.09±2.00	16.69±0.91	27.59±0.34
15.0	-	24.92±1.85	26.74±1.14
28.0	29.10±1.44	22.95±1.78	-
32.5	-	-	17.34±1.25

APPENDIX 2

Effects of salinity on absorption efficiency.

Salinity (p.p.t.)	0.0	7.5	15.0	32.5
Total food intake (g)	110.0	110.0	110.0	110.0
Total dry food intake (g)	101.66	101.66	101.66	101.66
Total dry faecal production (g)	11.90	13.53	15.96	23.02
Nitrogen content of feed (% dry wt.)	6.97±s.e.0.15			
Energy content of feed ($J.mg^{-1}$ dry wt.)	20.76±s.e.0.52			
Nitrogen content of faeces (% dry wt.)	2.46±s.e.0.07	2.92±0.11	3.11±0.14	3.18±0.09
Energy content of faeces ($J.mg^{-1}$ dry wt.)	15.61±s.e.1.77	15.73±0.03	14.62±1.02	15.97±0.68
Total N input (g)	7.09	7.09	7.09	7.09
Total N output (g)	0.29	0.40	0.50	0.73
Total **E** input (J)	$2.11x10^6$	$2.11x10^6$	$2.11x10^6$	$2.11x10^6$
Total E output (J)	$1.86x10^5$	$2.13x10^5$	$2.33x10^5$	$3.68x10^5$
Dry matter absorption efficiency	0.89	0.88	0.86	0.80
Energy absorption efficiency	0.91	0.90	0.89	0.83
Nitrogen absorption efficiency	0.96	0.94	0.93	0.90

APPENDIX 2

Effects of salinity on absorption efficiency.

Salinity (p.p.t.)	0.0	7.5	15.0	32.5
Total food intake (g)	110.0	110.0	110.0	110.0
Total dry food intake (g)	101.66	101.66	101.66	101.66
Total dry faecal production (g)	11.90	13.53	15.96	23.02
Nitrogen content of feed (% dry wt.)	6.97±s.e.0.15			
Energy content of feed ($J.mg^{-1}$ dry wt.)	20.76±s.e.0.52			
Nitrogen content of faeces (% dry wt.)	2.46±s.e.0.07	2.92±0.11	3.11±0.14	3.18±0.09
Energy content of faeces ($J.mg^{-1}$ dry wt.)	15.61±s.e.1.77	15.73±0.03	14.62±1.02	15.97±0.68
Total N input (g)	7.09	7.09	7.09	7.09
Total N output (g)	0.29	0.40	0.50	0.73
Total E input (J)	$2.11x10^6$	$2.11x10^6$	$2.11x10^6$	$2.11x10^6$
Total E output (J)	$1.86x10^5$	$2.13x10^5$	$2.33x10^5$	$3.68x10^5$
Dry matter absorption efficiency	0.89	0.88	0.86	0.80
Energy absorption efficiency	0.91	0.90	0.89	0.83
Nitrogen absorption efficiency	0.96	0.94	0.93	0.90

APPENDIX 3

(a) Effects of salinity on conversion efficiency

	Salinity	K_1			K_2			n	s.e.
		dry matter	total energy	total nitrogen	dry matter	total energy	total nitrogen	(No. of obsn. periods)	
Gp. 1 (0+,50-95 g)	0.0	0.24	0.26	0.35	0.27	0.29	0.36	5	0.01
	7.5	0.23	0.25	0.34	0.26	0.28	0.36	5	0.01
	15.0	0.19	0.21	0.28	0.22	0.24	0.30	5	0.01
	28.0	0.19	0.21	0.28	0.23	0.25	0.31	3	0.03
	32.5	0.14	0.15	0.20	0.17	0.18	0.22	2	0.01
Gp. 2 (0+,80-155 g)	0.0	0.20	0.22	0.29	0.23	0.24	0.30	6	0.03
	7.5	0.19	0.21	0.28	0.22	0.23	0.30	6	0.02
	15.0	0.20	0.22	0.29	0.23	0.25	0.31	6	0.02
	28.0	0.20	0.22	0.29	0.24	0.26	0.32	3	0.04
	32.5	0.12	0.13	0.18	0.15	0.16	0.20	3	0.01
Gp. 3 (1+,115-155g)	0.0	0.12						3	0.03
	7.5	0.15						3	0.03
	28.0	0.16						3	0.02

(b) Analyses of whole trout carcass and feed pellets

	Trout carcass (10)	Coopers' Floating Pellets (10)	Coopers' "Growers'" Pellets (10)
% dry matter	26.44±0.77(s.d.)	92.42±1.38	93.29±1.62
% water content	73.56±0.77	7.58±1.38	6.71±1.62
nitrogen content (% dry)	10.19±0.63	6.45±0.15	5.98±0.11
protein content (% dry:N x 6.25)	63.67±3.97	43.56±0.94	40.00±0.69
energy content ($J.mg^{-1}$ dry)	22.77±0.21	20.76±1.04	17.87±0.26

APPENDIX 4

Effects of fasting.

Experiment (1)

a) Weight loss.

Day No.	Salinity	Total mean wt.	s.d.	n	Sacrificed mean wt.	s.d.	n	Continuing mean wt.
0(2/5/72)	0.0	121.92	19.84	19	119.92	12.50	5	122.63
	7.5	141.35	33.15	20	123.77	30.18	5	147.20
	15.0	156.74	21.38	18	147.67	23.47	5	160.22
	32.5	101.90	19.90	18	99.08	22.50	5	102.97
7	0.0	118.45	21.71	14				118.45
	7.5	140.09	33.61	14				140.09
	15.0	155.85	19.78	13				155.85
	32.5	100.23	18.77	13				100.23
19	0.0	115.14	21.29	14	106.11	14.92	5	120.16
	7.5	136.33	33.36	14	145.20	26.65	5	131.40
	15.0	153.86	20.54	12	146.30	14.72	5	159.25
	32.5	97.72	16.87	13	98.38	24.45	5	97.31
37	32.5	77.22	8.77	7	77.22	8.77	7	
48	0.0	113.09	24.07	9	113.09	24.07	9	
	7.5	125.99	37.91	8	125.99	37.91	8	
	15.0	147.66	25.54	6	147.66	25.54	6	

b) Water and lipid content of epaxial white muscle.

Day No.	Salinity	Water % wet wt.	s.d.	Lipid % wet wt.	s.d.	n
0(2/5/72)	0.0	77.64	0.49	0.67	0.24	5
	7.5	77.99	0.92	0.51	0.14	5
	15.0	77.17	0.86	0.63	0.17	5
	32.5	78.00	0.50	0.60	0.17	5
19	0.0	78.00	0.45	0.60	0.10	5
	7.5	77.97	0.39	0.66	0.10	5
	15.0	78.14	0.22	0.61	0.14	5
	32.5	78.26	1.58	0.57	0.14	5
37	32.5	74.06	2.28	0.51	0.30	6
48	0.0	78.46	0.56	0.48	0.24	5
	7.5	79.39	2.03	0.43	0.10	5
	15.0	78.46	0.73	0.50	0.14	5

APPENDIX 4 (CONTD.)

c) Water and lipid content of liver.

Day No.	Salinity	Mean wt. as % fish live wt.	s.d.	Water % wet wt.	s.d.	Lipid % wet wt.	s.d.	n
0 (2/5/72)	0.0	0.88	0.10	75.18	1.85	1.68	0.17	5
	7.5	1.06	0.24	74.68	0.63	1.88	0.48	5
	15.0	1.26	0.33	75.17	0.45	1.80	0.35	5
	32.5	0.86	0.10	74.64	0.64	1.97	0.20	5
19	0.0	0.91	0.14	75.79	1.09	1.23	0.14	5
	7.5	1.17	0.34	75.59	0.91	1.20	0.44	5
	15.0	1.05	0.20	75.74	0.56	1.29	0.30	5
	32.5	0.95	0.20	75.04	1.48	0.99	0.37	5
37	32.5	1.32	0.26	73.95	2.08	1.24	0.44	6
48	0.0	0.84	0.17	75.23	0.26	1.38	0.30	5
	7.5	1.02	0.26	75.94	0.69	1.39	0.30	5
	15.0	0.79	0.17	75.40	0.26	1.29	0.14	5

Experiment (2)

Weight loss and condition factor.

Day No.	Salinity	Total mean wt.	s.d.	n	Sacrificed mean wt.	s.d.	n	Continuing mean wt.	Total mean length	Continuing mean length	C.F.
0 (28/5/73)	0.0	95.42	26.33	16	104.23	20.88	5	91.41	20.09	19.73	1.15
	7.5	79.66	22.15	16	84.24	10.55	5	77.57	18.99	18.72	1.13
	15.0	80.83	18.70	16	71.08	19.36	5	85.26	19.05	19.41	1.15
	32.5	84.41	20.97	13	83.64	30.64	5	84.89	19.12	18.98	1.19
23	0.0	85.94	27.54	11	86.12	30.14	5	85.78	19.97	19.88	1.05
	7.5	70.91	25.34	11	76.93	19.83	5	72.29	18.72	18.94	1.05
	15.0	78.62	17.39	11	84.73	16.39	5	73.55	19.22	19.02	1.06
	32.5	76.07	12.09	8	71.45	14.46	5	83.77	19.03	19.83	1.09
48	0.0	77.93	32.47	6	77.93	32.47	6		19.82		0.96
	7.5	67.34	20.69	5	67.34	20.69	5		18.96		0.96
	15.0	66.86	15.42	6	66.86	15.42	6		18.88		0.98
	32.5	72.20	16.75	3	72.20	16.75	3		19.63		0.95

APPENDIX 5

Effect of ration on conversion efficiency.

Initial total live weight	Final total live weight	Dry matter increase (g)	Median dry weight (g)	Total food intake (g dry)	K_1 (dry matter)	Total food intake (% dry)	Weekly food intake (% dry)	Weekly % dry matter increase
1056.58	1050.98	-1.48	278.62	21.25	-0.07	7.63	3.82	-0.53
1056.39	1054.41	-0.52	279.05	22.17	-0.02	7.94	3.97	-0.19
1026.89	1024.53	-0.62	271.20	23.19	-0.03	8.55	4.28	-0.12
1027.67	1056.58	7.64	275.54	42.51	0.18	15.43	7.72	1.41
1029.91	1056.39	7.00	275.81	44.34	0.16	16.08	8.04	1.28
1003.20	1026.89	6.26	268.38	46.39	0.14	17.29	8.65	1.18
970.48	1027.67	15.12	264.16	110.51	0.14	41.83	13.94	1.77
948.27	1029.91	21.59	261.52	115.32	0.19	44.10	14.70	2.87
859.12	925.34	17.51	235.91	72.26	0.24	30.63	15.32	1.59
925.59	1003.20	20.52	254.99	120.60	0.17	47.30	15.76	2.79
820.12	875.27	14.58	224.13	75.40	0.19	33.64	16.82	3.34
818.10	855.95	10.01	221.31	78.85	0.13	35.63	17.82	2.32
925.34	970.48	11.94	250.63	102.01	0.12	40.70	20.35	2.44
875.27	948.27	19.30	241.07	106.45	0.18	44.16	22.08	4.17
855.95	925.59	18.41	235.52	111.32	0.17	47.27	23.64	4.07
1050.98	1141.66	23.98	298.87	145.30	0.17	50.13	25.07	5.26
1054.41	1157.01	27.13	292.35	150.73	0.18	51.56	25.78	4.87
1024.53	1146.73	32.31	287.04	153.44	0.21	53.46	26.73	5.97
765.90	859.12	24.65	214.83	137.72	0.18	64.11	32.06	6.09
668.95	765.90	25.63	186.69	125.39	0.20	66.10	33.05	7.25
745.19	820.10	19.81	206.93	143.71	0.14	69.45	34.73	5.03
650.19	745.19	25.12	184.47	130.85	0.19	70.93	35.47	7.31
719.96	818.10	25.95	203.33	150.29	0.17	73.91	36.96	6.81
634.05	719.96	22.71	179.00	136.84	0.17	76.45	38.23	6.77

APPENDIX 6

Phillipson micro-bomb calibration.

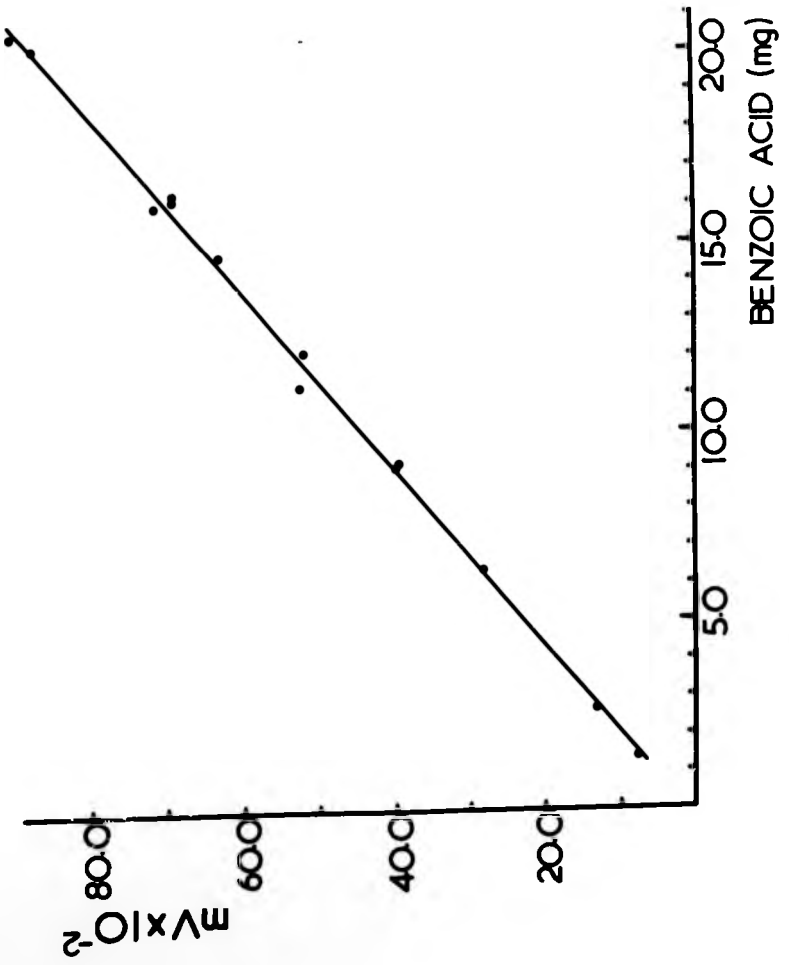
No.	Pellet wt. (mg)	Energy content of benzoic acid	Total energy content of pellet (J)	Potential rise (mV x 10^{-2})
1	14.6	26.455 $J.mg^{-1}$	386.24	63.0
2	20.0		529.10	87.0
3	11.2		296.30	52.5
4	2.7		71.43	13.0
5	6.4		169.31	28.5
6	12.1		320.11	52.0
7	16.2		428.57	69.0
8	16.1		425.93	69.0
9	1.4		37.04	8.0
10	9.1		240.74	40.0
11	9.2		243.39	39.5
12	20.3		537.04	90.0
13	15.9		420.63	71.5

FIGURE 22. Electrical output (mV x 10^{-2}) of the Phillipson micro-bomb calorimeter *versus* weight (mg) of benzoic acid combusted.
($y = 4.29x + 1.33$)

0^{-2}) of the

orimeter

zoic acid



x 10^{-2}) of the
calorimeter
benzoic acid

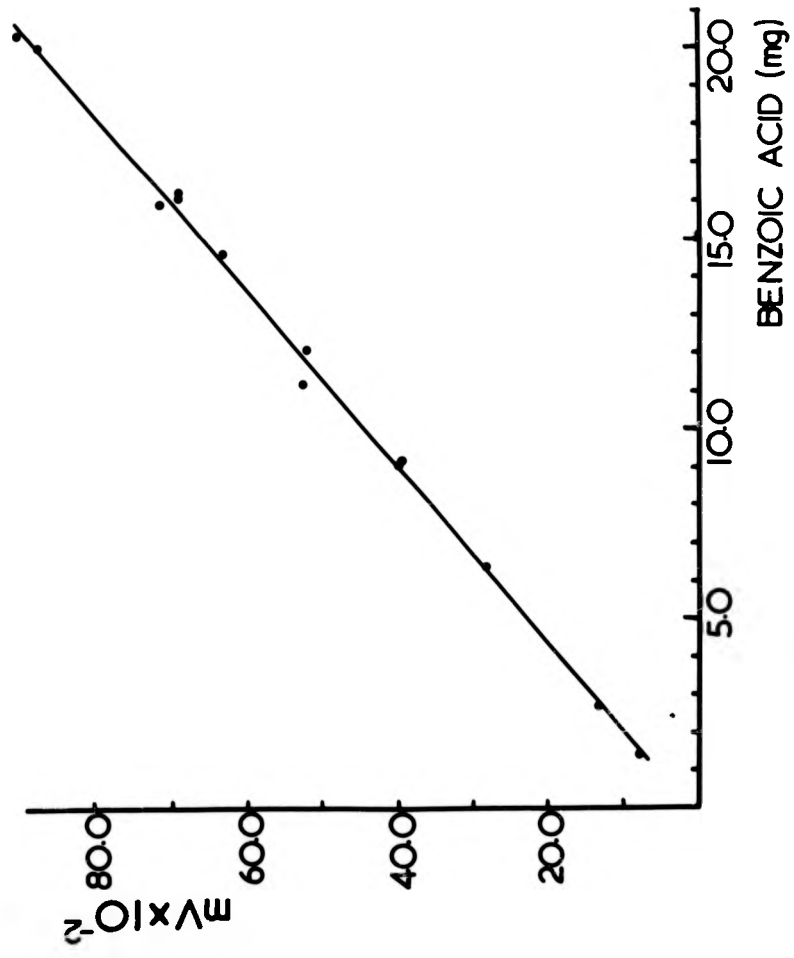
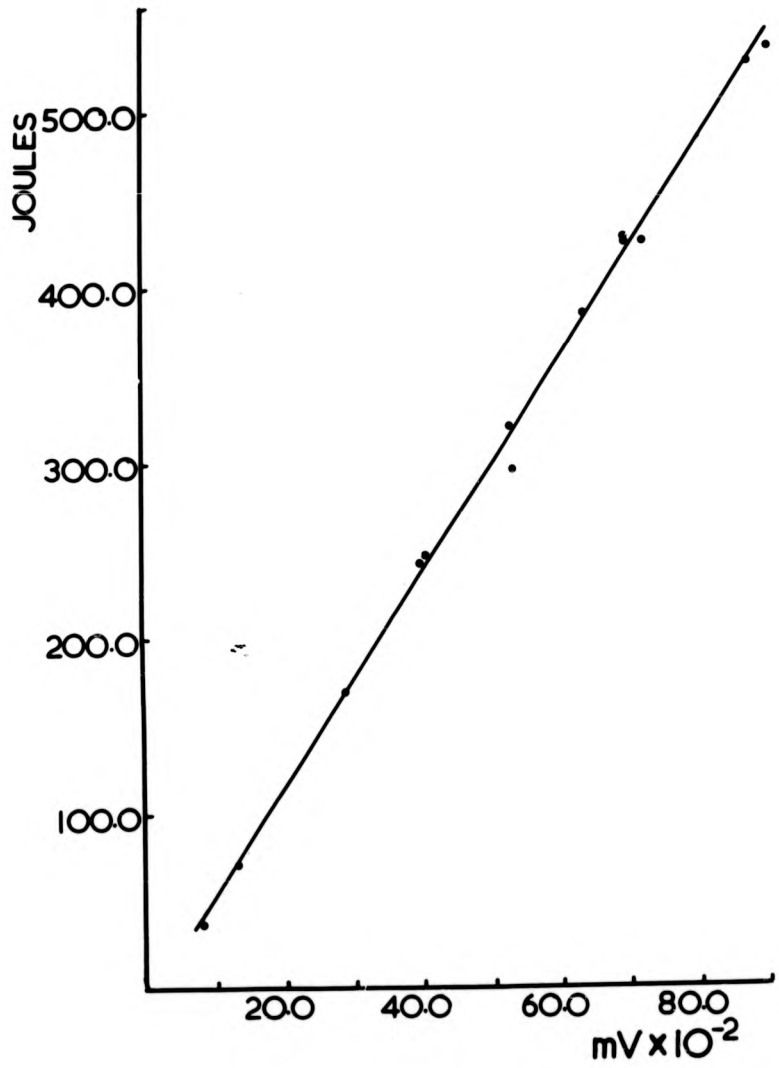


FIGURE 23. Energy (joules) versus electrical output (mV x 10^{-2}) in the Phillipson micro-bomb calorimeter.
($y = 6.15x - 7.16$)

electrical

the Phillipson

.



APPENDIX 7

Histological techniques

1) Dehydration	Time (h)
1 50% methylated spirit	1
2 80% methylated spirit	2
3 8% phenol meth.	3
4 8% phenol meth.	2
5 8% phenol meth.	2
6 absolute alcohol	2
7 absolute alcohol	1
8 chloroform	1
9 chloroform	1
10 wax 56°C	2
11 wax 56°C	2
12 wax 56°C	2

2) Dewaxing	Time (min)
1 xylol	5
2 absolute alcohol	2
3 methylated spirit	2
4 water	2

3) Haematoxylin & Eosin	Time	
1 Mayer's haemalum	5 min	
2 wash		
3 1% acid alcohol	differentiate	
4 wash		
5 Scott's tap-water substitute	3 min (blue)	
6 wash		
7 eosin	30 s	
8 wash		
9 methylated spirit	2 min	dehydration and clearing
10 absolute alcohol	2 min	
11 absolute alxohol	2 min	
12 xylol	1 min	
13 xylol	1 min	
14 mount		

4) Lison's Alcian Blue/Chlorantine Fast Red

		Time
1	Mayer's Haemalum	5 min
2	wash	
3	Scott's tap-water substitute	3 min
4	wash	
5	Alcian blue	10 min
6	wash	
7	1% phosphomolybdic acid	10 min
8	wash in distilled water	
9	chlorantine fast red	10 min
10	wash in distilled water	
11	dehydrate, clear and mount	

5) PAS/Light green

		Time
1	aqueous periodic acid	10 min
2	wash	
3	wash in distilled water	
4	Feulgen's reagent	20 min
5	wash	
6	methylated spirit	
7	20% alcoholic light green	30 s
8	wipe off excess	
9	dehydrate, clear and mount	