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AN EXAMINATION OF SOME ASPECTS OF GROWTH AND NUTRITION OF JUVENILE GREY MULLET (MUGILIDAE) IN RELATION TO POTENTIAL MULLET FARMING IN BRITAIN

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University of Plymouth

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AN EXAMINATION OF SOME ASPECTS OF GROWTH AND NUTRITION OF JUVENILE GREY MULLET (MUGILIDAE) IN RELATION TO POTENTIAL MULLET FARMING IN BRITAIN

by

Alison J. Graham B.Sc., M.Sc.

A thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Plymouth Polytechnic

May, 1981
I declare that while registered as a candidate for the degree of Doctor of Philosophy I have not been registered for another award of C.N.N.A. or of a University. The work contained in this thesis is entirely my own.

Alison J. Graham
I am much indebted to Dr. P. Reay both for his supervision of the work and for his critical reading of the manuscript; and to Plymouth Polytechnic for the provision of a research assistantship. I am particularly grateful to Tony Maskell for so generously allowing me to use the facilities at his oyster hatchery at Steer Point.

A very special thanks is due to my husband for his encouragement and patience throughout. My thanks also to Michele Hennessy for her care with the typing, and to Bernie Phelan and Denise O'Reilly for their assistance at the proof reading stage.
AN EXAMINATION OF SOME ASPECTS OF GROWTH AND NUTRITION OF JUVENILE GREY MULLET (MUGILIDAE) IN RELATION TO POTENTIAL MULLET FARMING IN BRITAIN.

ALISON J. GRAHAM.

The growth of juvenile (0 - III group) C. labrosus, L. aurata and L. ramada was examined in the Tamar, Lynher and Yealm estuaries in South-west England. Two growth models were used to facilitate comparisons among species, age groups and sites of capture. The growth of C. labrosus and L. ramada was similar and faster than that of L. aurata. The growth and relative condition of 0 group fish tended to be greater in St. John's Lake than at other sites, but amongst older fish the reverse appeared to be true. Annual and daily cycles in feeding intensity were examined. A high daily food intake and fast passage of food through the gut appeared to be characteristic of both C. labrosus and L. aurata. In the spring 100% of fish examined were infected with cysts of Myxobolus exiguus but this did not appear to have any significant effect on growth or condition.

Three 12-15 week feeding experiments were undertaken to examine aspects of the nutrition of 1 group C. labrosus using experimental diets of semi-purified rations. In the first and second experiments the effect of dietary protein level and ration size on growth was investigated. There was a significant interaction between these factors with optimum dietary protein level decreasing with increase in ration size, and optimum ration decreasing with increase in dietary protein level. In the third experiment the ability of juvenile mullet to utilise dietary energy supplied as either lipid + carbohydrate or mainly carbohydrate in diets containing 20% and 40% protein was examined. Increase in dietary energy resulted in improved growth, but lipid energy had a greater protein sparing effect than carbohydrate energy. The effects of dietary protein and energy level, energy source and ration size on conversion efficiency, assimilation of the diet, histology and size of certain organs, and body composition were also investigated.
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## APPENDIX I.

REFERENCES.
INTRODUCTION

Grey mullet belong to the family Mugilidae. Fish of this family are widely distributed and are common in the shallow waters of all tropical and temperate seas. Thomson (1968) has reviewed the biology of the family, and more recently the biology of juvenile grey mullet has been reviewed by De Silva (1980). Further literature on specific aspects of mullet biology is referred to in the relevant chapters of this thesis.

Fish of the family Mugilidae are fished throughout their range, with figures reported from some 55 countries bordering the Pacific, Indian and Atlantic oceans, and the Mediterranean and Black Seas. The total catch in 1978 was 156,935 metric tons but the size and importance of the fishery varies enormously, from 24 metric tons in Romania to over 28,000 metric tons in Brazil, which in 1978 recorded 18.26% of the world mullet catch. After Brazil, the countries with the largest mullet fisheries are Indonesia (12.12%), U.S.A. (9.19%) and Nigeria (8.50%). By region, the largest mullet fishery is in the West Central Atlantic, with the second largest in the Mediterranean and Black Seas. In recent years there has been a trend for the world catch of mullet to increase. However, this reflects a relatively constant catch in most areas of the world, with a marked increase in the exploitation of mullet in certain regions, notably the Indian Ocean (India, Pakistan) and Southwest Pacific (Indonesia, Malaysia, Philippines, Thailand) where
catches in the period 1973-1978 have increased from 3,900 to 10,297 and 12,600 to 33,160 metric tons respectively (F..O. Fisheries Series: Yearbooks of Fishery Statistics).

In the North-east Atlantic region the total mullet catch in 1978 was 2,172 metric tons which included a British catch of only 122 metric tons (c. 0.08% of total world mullet catch). The annual grey mullet catch in Britain varies considerably but over the last 50 years there has been no continuing trend of either increase or decrease (from Sea Fisheries Statistical Tables, Ministry of Agriculture, Fisheries & Food).

Three species of grey mullet are found in British waters - Chelon labrosus (thick lipped mullet), Liza aurata (golden mullet) and Liza ramada (thin lipped mullet) (Wheeler, 1969). In the Mediterranean these three species not only make a significant contribution to the total annual mullet catch but also play an important role in an expanding fish farming industry. However, data on growth and other parameters collected in the Mediterranean does not provide a sound basis for assessment of aquacultural potential in the different climatic and biological environment of British estuaries.

A study of the British grey mullet was published by Hickling (1970b) but this was mainly concerned with the diet, feeding behaviour and maturation of adult fish. Data on growth of younger fish was mainly derived from studies of scales and opercular bones but these yielded no information about growth from 0-2 years, which was deduced from the lengths of some
samples captured in Plymouth estuaries.

The aim of this thesis was to make a fuller investigation of juvenile grey mullet in South-west England concentrating particularly on aspects relevant to a future assessment of their aquacultural potential.

The culture of mullet is known to have been practised from ancient times by the Romans and Egyptians, and also in parts of Asia and China. Today mullet are cultured in Europe, the Mediterranean, the Middle East, and throughout the Indo-Pacific region. On a smaller scale, experimental culture has occurred in N. America and certain regions of S. America. *M. cephalus* is by far the most commonly cultivated species (Ling, 1970) but other species are also involved e.g. *M. tade*, *M. dussumieri*, *M. macrolepis*, and *M. parsia* (Ling, 1970; Jhingran & Natarajan, 1970; Cervignon & Padron, 1974). In Europe and the Middle East mullet species cultured include *L. ramada*, *L. aurata* & *C. labrosus* in addition to *M. cephalus* (Perlmutter et al., 1957; El Zarka and Kamel, 1967; Zismann and Ben-Tuvia, 1975; Chervinski, 1975; Yashouv, 1969, 1972).

Despite its long history, techniques of mullet culture tend to be primitive - it has not developed along the same lines as the highly intensive production of trout and carp. Traditionally mullet are reared in brackish water ponds (called 'bheris' in West Bengal, India, 'valli' in Italy, 'limans' on the Russian coast of the Black Sea and 'barrachois' in Mauritius). However, these brackish water ponds range in size from 5-10 acres (12-25 ha) or less in India to more than 10,000 ha in ponds
bordering the Black Sea. The management of all these ponds is somewhat similar. In Spring, or whenever mullet fry are migrating shorewards, the sluice gates are opened. The young mullet enter the ponds with the high tide or are attracted into the ponds by the outflow of freshwater. During the growing season a grill is placed across the sluice to prevent the escape of the fish but permitting exchange of water. The main harvesting occurs at the end of the growing season, although there is some subsistence fishing in India and at Arcachon intermittent harvesting occurs to take advantage of particularly high fish prices caused by, for example, bad weather keeping fishing fleets in port. The fish are caught in traps at the sluice gates to which they are attracted by an inflow of sea water or the ponds may be partially drained on falling tides and fish caught using gill and seine nets.

The total annual yields from these ponds is very variable, ranging from 90-200 kg/ha in the Italian 'valli' and from 12 to several hundred kg/ha in Black Sea 'limans' (Hickling, 1971). In India annual yields tend to be higher, ranging from 150 to 1500 kg/ha depending on stocking rate, fertility etc. From this low intensity culture mullet yields have been improved by

(i) **Artificial stocking:**

Selective stocking allows exclusion of predators and permits some control over factors such as stocking density. Capture of wild fry, by means of small meshed seines, drag nets or dip nets, for stocking ponds is an essential part of mullet cul-
ture in most regions including Israel, Taiwan and Hong Kong where mullet culture is most highly developed (Jhingran & Natarajan 1970; Perlmutter et al., 1957). On a larger scale mullet fry have been stocked in large brackish water lakes such as Lake Mariut (Egypt) where transplantation resulted in a mullet catch of 67 tons in 1963 (El Zarka & Kamel, 1967).

(ii) Fertilisation:

In Taiwan, Bengal and much of the Indo-Pacific region, increased fertility is achieved by periodic drying out of the ponds and also tilling the soil, and working in fertiliser such as various oil cakes, rice bran and pig and chicken manures. The presence of diluted effluents from the Calcutta sewage works and the manuring of fish ponds is reported to increase the growth of mullet in the 'bheris' of W. Bengal (Kurian, 1974). In Taiwan and Hong Kong, pig, cow and poultry manure are added to mullet ponds (Lin, 1940), but in Israel & Egypt both inorganic and organic fertilisers are used (Bishara, 1978, 1979). In Taiwan production of mullet has been shown to increase linearly with the application of phosphate up to 180 kg/ha (Bardach et al., 1972) and in an experimental farm in Egypt treatment of ponds with 30 kg/ha of phosphate increased mullet yield by 166.7% compared with unfertilised ponds (Chan, 1970).

(iii) Feeding:

It is with both fertilisation of ponds and feeding that the highest yields have been attained - 2500 kg/ha in a 300 day growing season in Hong Kong ponds where mullet were reared with carp, milkfish and tilapia have been verified, but yields of up
to 3500 kg/ha have been claimed in the most intensively managed ponds. The total amount of food given over the 300 day growing period was 2500 kg/ha of rice bran and 3000 kg/ha of peanut cake, sometimes supplemented with soybean cake. In addition, the pig manure, rice bran and peanut cake added to the ponds for fertilisation may be used directly by mullet as food (Bardach et al., 1972; Lin, 1940).

Much controversy surrounds the diet of mullet and the effectiveness of various foods and methods of feeding in increasing mullet production. Feeding undoubtedly increases production but food added in excess of requirements is wasteful and may dangerously deplete oxygen content of the water. Feeding a fixed percentage of the total weight of the fish in a pond, as is common practice in Israel, reduces feed waste. However, the feeding levels are usually fairly arbitrarily chosen and do not take into account the food requirements of different species and age groups, the effect of environmental factors such as temperature and salinity on food requirements, and the varying contribution to the diet of natural food in the pond.

Although mullet are generally regarded as brackish water/marine fish, in parts of India they are successfully cultivated in freshwater (Hickling, 1971, Pillay, 1949). Experimental culture of mullet in freshwater is in progress in Israel (Chervinski, 1975) and in Yugoslavia (Morovic & Sabiocello, 1965). The potential of mullet production in freshwater is indicated by the results obtained from stocking mullet fry in
L. Kinnereth, Israel (Paperna, 1975). Over 11 years the stocking of 19 million fry yielded 1800 tons of mullet 1-4 kg in size. The cost of stocking was less than 10% of the total revenue of the yield. Recently De Silva & Perera (1976) and Vallet et al. (1970) have conducted some research into the effect of salinity on food consumption and food conversion. Boisseau et al. (1975), Nordlie & Leffler (1975) and Lasserre & Gallis (1975) have investigated osmoregulation and its energetic cost in several mullet species. However, much more information on the effect of salinity on food conversion and growth is required before the full potential of mullet culture in freshwater can be realised.

In most regions mullet are reared with other fish as part of a polycultural system resulting in fuller utilisation of the natural food in the pond and an increase in total fish yield although, due to competition, the yield of each species may be reduced. In W. Bengal (Pillay, 1949; Sarojini, 1951) and in Taiwan and Hong Kong there has been a long association between the culture of mullet and various species of carp. A more complex stocking procedure is followed in freshwater ponds in Taiwan which, in addition to mullet at 3-4000 per hectare, includes silver carp, bighead carp, grass carp, common carp, tilapia and sea perch. Well managed ponds under this system may yield 5-7000 kg/ha/yr which includes 1000 kg mullet. In Taiwan and Japan mullet and carp are included in ponds where eels are the principal crop (Bardach et al., 1972). In Israel mullet (M. cephalus & M. capito) and Tilapia sp. are reared in polyculture with common carp as the primary crop (Pruginin et al., 1975).
Yashouv (1966, 1972) examined growth of mullet with varying densities of carp and/or tilapia and concluded that mullet have some special property which makes their growth less than ordinarily dependent on density and the effect of other fish, a finding which has been supported by the results of Branch & Strawn (1978).

Following the success of polyculture in Israel, experiments are now in progress on the culture of *L. falcipinnis* with carp and tilapia on the Nigerian coast (Sivalingam, 1974), *M. cephalus* and *L. aurata* with carp in Yugoslavia (Morovic & Sabiocello, 1965) and *M. incilis* and freshwater prawns in Colombia (Martinez Silva & Pedini, 1977). In America mullet and tilapia have been experimentally stocked in ponds containing caged catfish. The total production was increased from 1020 lbs/acre (catfish alone) to 1430 lbs/acre for catfish with mullet (Perry & Avault, 1972).

Mullet play an important role in fish culture in the Mediterranean, and are of increasing importance in culture in the Indo-Pacific region (Ling, 1970). They have several characteristics which make them particularly suitable for pond culture including tolerance of salinity variation (Gopalakrishnan, 1970; Cervignon & Padron, 1974), temperature variation (Holt & Strawn, 1977) and high stocking densities (Yashouv, 1966, 1972). They also appear to be relatively resistant to pollution (Ezzat, 1964) and disease (Paperna, 1975; Minchew & Yarborough, 1977). In addition mullet have the energetic advantages of being near the base of the food chain and, in contrast to most herbivorous
fish, they can also utilise detritus which, particularly if finely divided, may have a rich bacterial flora and thus be an important protein source (Odum, 1968a, 1970). Mullet are opportunistic feeders and so, although able to thrive on natural foods, will also readily accept supplemental foods such as rich bran and peanut meal whenever these are available. Thus mullet farmers may take advantage of temporary gluts to boost fish growth without necessarily having to provide food regularly.

The acceptability of mullet as a food fish varies throughout the world. The low market price in America has been one of the main factors in discouraging investigation of mullet culture. This contrasts markedly with the situation in Hawaii where mullet are sold at 8-10 times the price obtained in other parts of America. In Israel mullet are considered to have a high quality flesh and sell at a higher price than carp or tilapia, and in Taiwan and India mullet roe is considered to be a gourmet food (Liao, 1974; Sarojini, 1951). In the Indo-Pacific region the increase in mullet farming is partly due to the fact that mullet is more acceptable as a food fish than milkfish in many Asian countries (Ling, 1970). In India mullet finds a ready market as fresh fish but is also extensively salted and dried and the roes are considered a delicacy (Sarojini, 1951). Experimental canning has been carried out with limited success in both India and Florida. The freezing of mullet reduces its quality and is not very satisfactory but in America and Australia a sausage type product is being developed (Daley et al., 1978).
In Australia attempts have been made to improve the image of mullet by marketing in smoked or fresh form under the name "lisa" but in Britain mullet are not highly valued, and because they are often found scavenging in harbours and estuaries they have the reputation of being 'unclean' and having a 'muddy' flavour. Despite this grey mullet are in demand in certain areas, especially London & Birmingham, and from 1960 to 1973 they increased in value from 63% to 110% of the price of plaice (Sea Fisheries Statistical Tables, Ministry of Agriculture, Fisheries & Food).

Thus although biologically grey mullet have many characteristics which would recommend them as a species for culture, in some regions this development will undoubtedly be hampered by their traditional unacceptability as a food fish, although the steeply rising costs of catching or rearing other fish may stimulate a broadening of tastes.

Even where mullet is highly valued as food one of the main obstacles to the development of mullet farming has been the erratic unreliable supply of fry from the wild. Neither Italian 'valli' nor Russian 'limans' can catch enough grey mullet fry and owners seek to buy fry from elsewhere (Hickling, 1971). In Israel pollution is blamed for the shortage of mullet fry which is affecting stocking programmes in L. Kinnereth (Paperna, 1975) and results in poor mullet yields from fish ponds in some years (Bardach et al., 1972). Interest in induced breeding of mullet was therefore initially stimulated by the need for a reliable source of large numbers of fry, although in the longer term there are other advantages, such as the scope for selective
breeding.

Artificial spawning of _M. cephalus_ was first achieved in Italy in 1930, but since then developments involving the use of purified salmon gonadotropin and human chorionic gonadotropin instead of mullet or carp pituitary extracts (Shehadeh et al, 1973), the cryogenic preservation of mullet sperm (Chao et al, 1975) and the induction of ovarian maturation out of season by manipulation of photoperiod and temperature cycle suggests that it will soon be possible to spawn mullet at any time of the year. Initial attempts at rearing fry from the eggs of artificially spawned mullet were characterised by very high mortalities. The best results have been obtained using natural foods such as oyster larvae, brackish water rotifers, copepods and unicellular algae (Ling, 1970; Liao, 1974; Nash et al, 1974). In addition, the light and temperature control, size of rearing tank, aeration etc. have also been shown to be critical to larval survival (Nash et al., 1974, Nash & Kuô, 1975). Research into techniques of mass propagation is currently in progress in several parts of the world (Sebastian & Nair, 1975, Liao, 1974) and it seems likely that large scale breeding and rearing of grey mullet will soon be a commercial reality.

Webber & Riordan (1976) identified a reliable source of seed as a major impediment to mullet culture in areas such as S. America and Africa where it has potential for alleviating serious protein shortages. Even in the Indo-Pacific region where the area already developed for coastal aquaculture exceeds 400,000 ha, at least a further 1.5 million ha is considered suit-
able for future development (Ling, 1970). Once large scale spawning and rearing are possible, mullet may well become the most important human food product of the estuarine environment (Bardach et al., 1972).

In Western Europe and North America economic factors, such as the high cost of labour, land etc., have concentrated fish farming effort on high priced luxury fish such as salmon, trout and turbot. However, increasing difficulties being encountered by the fishing industry, the high and increasing prices of traditional sources of protein and the growing awareness of the need to utilise resources to the full has reawakened some interest in culture of herbivorous fish in the United Kingdom and Western Europe. The high cost of land renders extensive culture as in the Indo-Pacific region uneconomic, but mullet may well have a place as a secondary crop in conjunction with trout, pig or poultry farming, or have a role to play in sewage treatment or the utilisation of industrial organic wastes (Chan, 1972). Growth of salmon was stimulated by exposure to papermill effluent (McLeay & Brown, 1974) and some success has been achieved in the introduction of brewers by-products, activated sludge, single celled proteins, waste from bleaching edible oils and hide fleshings into the diet of trout (Tacon & Ferris, 1976; Cowey et al., 1979; Austreng, 1978; Atack & Matty, 1979), although certain problems such as the high level of heavy metals in activated sewage sludge have yet to be overcome (Singh & Ferris, 1978). However, the natural feeding habits of a fish such as the grey mullet make it better suited to utilising waste products — both
directly and via the increased algal growth which their introduction into the pond is likely to stimulate. There is also some evidence to suggest that mullet may be able to synthesise amino acids from urea (Albertini-Berhaut & Vallet, 1971).

To date most research has concentrated on tropical and sub-tropical mullet species, especially M. cephalus. Much more information is required on the growth, food requirements and conversion of the species found on the United Kingdom coasts before their potential for culture in this country can be assessed.
SECTION 1. FIELD STUDIES

The growth, condition and feeding of juvenile
C. labrosus, L. aurata and L. ramada was examined in the estuaries
1.1. **Collection of fish**

A number of sites in the Tamar and Lynher estuaries were fished over a period of 18 months. A 10m sandeel seine net with a central minimum mesh size of 5mm bar was used. It was operated by two people. One stood on the shore holding a rope attached to one end of the net while the other rowed into the river in a rubber dinghy, laying the net in a line parallel to the shore before beaching the dinghy some distance from the other operator. The net was then pulled in, slowly and gently at first avoiding unnecessary movement or noise. When the fish were completely encircled by the net and the shore, the net was pulled in faster, particularly if mullet started to escape by jumping over it. The numbers of grey mullet captured at each site in each month is shown on Table 1.1. The area over which the net was dragged varied widely as a result of the irregular shoreline and numerous obstacles, e.g. posts, wrecks, buoys etc. For this reason, and because success of fishing depended on being able to encircle the fish without alarming them and on the fish not jumping the net, no attempt was made to use the numbers caught to estimate population density. The depth of the intertidal mud at most sites made operation of the seine net difficult, if not treacherous, except for a few hours at and preceding high water. Even so seine hauls were frequently abortive due to the net snagging on underwater obstacles, collecting so much weed and mud that it was too heavy to pull in, or becoming torn, twisted or rolled up so that all the fish could escape.
The sites fished are marked in Fig. 1.1. They were grouped according to estuary and, with the exception of St. John's Lake (Site 1) were all very similar - wide expanses of mud exposed at low water and shallowly covered to a depth of 0·2m at high water. St. John's Lake differed because the ford retained a certain depth of water even at low water. This site was fished within 2 hours of low water.

The captured mullet were placed in plastic bags labelled with date, location, and time of capture. On return to the laboratory the fish were rinsed to remove excess mud and placed in clean labelled plastic bags which were sealed and stored in a deep freeze. The fish were thawed in a refrigerator for 24 hours before being examined. To assess the effect of freezing, one batch of fish was weighed and measured before and after being stored in a deep freeze as above for a period of two weeks. Statistical comparison using t-tests indicated that the period of storage had no significant effect on either the weight or the length.

1.2. Examination of fish

For each fish the following were recorded.

(i) Species (see Chapter 2).
(ii) Length: A flat metre rule with a block attached to the end so that the snout of each fish, when just touching it, was on the 0mm mark, was used to measure length to the nearest mm. A group of 20 fish were measured twice.
An analysis of variance was carried out to resolve the total variance in the length measurement into variances due to differences between fish and variance due to the difference in measurement of the same fish. Repeatability was then calculated as:

\[
\text{Repeatability} = \frac{\text{variance due to differences between fish}}{\text{variance due to difference between fish} + \text{variance due to difference between 1st and 2nd measurement on the same fish}}
\]

The repeatability of the length measurement was calculated as .992.

Three measurements of length were used:-

a) Total length: the greatest length from the most anterior extremity (mouth retracted) to the end of the caudal fin with the fin closed so that length was obtained from the longest lobe.

b) Fork length: the greatest length from the most anterior extremity (mouth retracted) to the fork of the caudal fin with the caudal fin spread.

c) Standard length: the greatest length from the most anterior extremity (mouth retracted) to the base of the median tail fin rays where these meet the median hypural plate. This base, masked by overlying skin and musculature, was identified by a crease when the tail was bent sharply from side to side.

Standard length was used as the basic length measurement although the relationship between standard length and total length was calculated to enable comparison with data of authors using total length.
Standard length was chosen because, as was also reported by Sarojini (1957), some fish had damaged caudal fins which made measurement of total or fork length impossible whereas standard length could be measured on all fish.

(iii) Weight: Each fish was blotted dry with paper towels and weighed on a top pan balance to the nearest $10^{-2}$ g. A group of twenty fish were weighed twice. Repeatability was calculated as for length measurement and found to be .987.

(iv) Gutted weight and gut weight: The fish was slit along the ventral side and the gut and liver removed. The gutted fish, and the gut after separation from the liver, were weighed on a top pan balance to the nearest $10^{-2}$ g.

(v) Number of pyloric caeca, fullness of cardiac stomach and intestine: The gut was examined and the number of pyloric caeca was recorded. The fullness of the cardiac stomach and the intestine were recorded separately on an arbitrary basis - empty, $\frac{1}{2}$ full, $\frac{3}{4}$ full, full.

(vi) Fat index: The amount of fat surrounding the gut was recorded on an arbitrary scale as there was often too little, and the fish too small, for removal and weighing to be very accurate. The arbitrary scale was defined as:-

0 - None

1 - Fat forms a continuous line between the loops of the gut

2 - Fat forms a wide line between the loops of the gut and is also present between the pyloric caeca.

3 - Large amounts of fat around the pyloric caeca and stomach. Very wide line of fat between gut loops.
(vii) Parasites: The number of nematode worms found around the gut was recorded. The cysts of a myxosporidian parasite, identified as *Myxobolus exigus* (Matthews pers. comm.) were common on the gut and pyloric caeca. The degree of infestation was recorded on an arbitrary scale defined as:

0 - None
1 - A few cysts found on close inspection (Plate 1.1a).
2 - Cysts common (Plate 1.1b).
3 - Cysts covering most of gut (Plate 1.2a and b).

(viii) Scales: A few scales were removed from between the first dorsal fin and the middle flank. They were cleaned and mounted in glass slides for examination by projection onto a screen.

1.3. Year Group and Age Determination

Year group was determined using a combination of length frequency distribution and scale reading. For purposes of age calculation, November was chosen as the birth month for *L. ramada* and *L. aurata* and March for *C. labrosus*. However, for calendar and comparison convenience 1st January was chosen as the arbitrary birth date in determining year group after the first year. Thus, *L. ramada* and *L. aurata* become I group at 14 months of age, whereas *C. labrosus* become I group at 10 months, but in all species 0 group describes fish in their first full summer of growth. Fish commonly fell into fairly distinct length and weight groups, but as a check, and where there was any doubt, scales were examined. Although some difficulty was encountered as regards overgrowth in the centre of the scale, as
also described by Kennedy & Fitzmaurice (1969) and Hickling (1970b) up to the III group age determination from the scales could be made with some confidence.

1.4. Statistical Methods

Statistical techniques were used to test the normality of length-frequency distributions. Although the mesh size of the net would be expected to dictate the smallest size of fish captured, in practice weed etc. caught in the net meant that the effective mesh size was considerably smaller than 5mm. As regards the larger fish, the success of the fishing method depended on being able to surround the fish within the net and the shore. If they were alarmed too early it was common for the whole catch to be lost. Once surrounded the only method of escape was over the top of the net. *Liza aurata* (also called the jumping mullet) may be more likely to jump the net than other species (Le Dantec, 1955). There was no indication from observations during this study that any one size group was more likely to escape than another, although faster swimming speeds of larger fish might be expected to give them some advantage in evading the net.

As far as possible, data for statistical analysis were drawn from individual fish; thus condition, for example, was calculated for each fish individually and the means obtained for each group as required. A Dec-20 computer and the University of Pittsburgh SPSS-H (Statistical Package for the Social Sciences) version 6.02C was used for some of the statistical analysis.
CHAPTER 2 NOMENCLATURE AND IDENTIFICATION

The nomenclature of the grey mullets has become very confused due in part to the limited variation in anatomical characters which caused considerable difficulties in arranging the species into genera. In this work the classification suggested by Trewavas & Ingham (1972) has been adopted. According to this scheme, the three British species of grey mullet fall into two genera:

1) Liza (Jordan & Swain (1884) type Liza ramada (Risso, 1826))
   Liza ramada
   Liza aurata (in Walbaum, 1793)

2) Chelon (Rose(1793), type Chelon labrosus (Risso, 1826)) A specialised offshoot of Liza.
   Chelon labrosus.

Ingham, in an unpublished revision of Mugilidae in 1952, rejected Chelon as a genus and included C. labrosus in the genus Crenimugil. It was from this manuscript that Wheeler (1969), Hickling (1970) and Kennedy & Fitzmaurice (1969) took their usage of Crenimugil for labrosus. Schultz (1946) considered C. labrosus to be closely related to Liza and so included both in the genus Chelon. Trewavas & Ingham (1972) considered Chelon to be an offshoot from Liza in which the lips have become specialised.

The close relationship between Liza ramada and Liza aurata has been confirmed by Lee & Juge (1965) who identified a common antigen for these species. However, there was also some evidence from their work that Liza ramada had particular associations with Mugil cephalus. This is supported by a report case of hybridisation between a female M. cephalus and a male L. ramada (Yashouv et al., 1969). More recently
a system of mullet identification by disc electrophoresis of myogen has been developed by Herzberg & Pasteur (1975) who noted certain similarities between _L. aurata_ and _M. cephalus._

Confusion has been increased by the fact that several different names have been used for what are widely considered to be the same species. Some name equivalents are listed below.

<table>
<thead>
<tr>
<th>Author</th>
<th>Name</th>
<th>Name</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risso (1810)</td>
<td><em>M. cephalus</em></td>
<td><em>M. cephalus</em> var.A, &quot;ramada&quot;</td>
<td><em>M. auratus</em></td>
</tr>
<tr>
<td>Risso (1826)</td>
<td><em>M. labrosus</em></td>
<td><em>M. ramada</em></td>
<td><em>M. auratus</em></td>
</tr>
<tr>
<td>Cuvier &amp; Valenciennes (1836)</td>
<td><em>M. chelo</em></td>
<td><em>M. capito</em></td>
<td><em>M. auratus</em></td>
</tr>
<tr>
<td>Arné (1938)</td>
<td>Mugil chelo/ labrosus</td>
<td>Mugil ramada/ capito</td>
<td>Mugil auratus</td>
</tr>
<tr>
<td>Le Dantec (1955)</td>
<td>Mugil chelo</td>
<td>Mugil ramada</td>
<td></td>
</tr>
<tr>
<td>Perlmutter et al. (1957)</td>
<td>Mugil chelo</td>
<td>Mugil capito</td>
<td>Mugil auratus</td>
</tr>
<tr>
<td>El Zarka &amp; Kamel (1967)</td>
<td>Mugil chelo</td>
<td>Mugil capito</td>
<td></td>
</tr>
<tr>
<td>Erman (1961)</td>
<td>Mugil chelo</td>
<td>Mugil capito</td>
<td>Mugil auratus</td>
</tr>
<tr>
<td>Thong (1969)</td>
<td>Mugil labrosus</td>
<td>Mugil ramada</td>
<td>Mugil auratus</td>
</tr>
<tr>
<td>Wheeler (1969)</td>
<td>Crenimugil labrosus</td>
<td>Liza ramada</td>
<td>Liza aurata</td>
</tr>
<tr>
<td>Kennedy &amp; Fitzmaurice (1969)</td>
<td>Crenimugil labrosus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hickling (1970b)</td>
<td>Crenimugil labrosus</td>
<td>Liza ramada</td>
<td>Liza aurata</td>
</tr>
<tr>
<td>Trewavas &amp; Ingham (1972)</td>
<td>Chelon labrosus</td>
<td>Liza ramada</td>
<td>Liza aurata</td>
</tr>
</tbody>
</table>

In this thesis the names of Trewavas and Ingham are used irrespective of those chosen by original authors.
C. labrosus is the least modified of the Chelon species and several authors have reported difficulty in distinguishing the young C. labrosus from young Liza sp. (Trewavas & Ingham, 1972; Thong, 1969).

A number of characteristics have been used in identification:

1) Number and length of pyloric caecae:

As in Perlmutter et al. (1957), Thong (1969) and El Zarka et al. (1970) this was found to be the most reliable characteristic for identification of species, particularly for fish $\leq$ 50mm. This characteristic also appears to be fairly uniform for each species throughout the Mediterranean (Perlmutter et al., 1957; El Zarka & Kamel, 1967; Heldt, 1948), French (Arné, 1938; Thong, 1969) and British coasts (this work).

2) Thickness of the upper lip:

As in Wheeler (1969) and Arné (1938) this character was found to be useful in separating C. labrosus from the Liza sp. Perlmutter et al. (1957) working with juvenile fish do not record or illustrate a notable difference in lip thickness and Thong (1969) refers to the upper lip of both C. labrosus and Liza ramada as being thick although his illustrations show that of C. labrosus to be thicker.

3) Teeth/papillae on upper lip:

None of the C. labrosus examined in this study bore the stout horny papillae referred to by several authors (e.g. Wheeler, 1969; Thong, 1969; Trewavas & Ingham, 1972) although the upper lip did sometimes appear slightly 'lumpy' especially in larger specimens. Trewavas & Ingham (1972) have found that lip papillae are absent from individuals $\leq$ 90mm standard length, develop earlier in Madeira than in the
Mediterranean and earlier in the Mediterranean than in northern populations. Evidence from this study seems to confirm this since in South-west England papillae were not observed in fish younger than year group III. Some relationship with the onset of sexual maturity has been suggested, which is consistent with the slow development noted for this species in British waters (Hickling, 1970b)

Adult *C. labrosus* do not seem to possess teeth on the upper jaw and this has been used to distinguish them from *Liza ramada* which has small fine teeth (Wheeler, 1969). In the juvenile *C. labrosus* examined in this study very fine teeth were clearly visible under X25 magnification in some specimens. Short fine teeth were also observed in juvenile *C. labrosus* by Perlmutter et al (1957) and Arné (1938) and it seems probable that these are present in the juvenile but absent in the adult. The teeth of juvenile *L. ramada* and *L. aurata* were found to be as described for the adults.

4) Width of jugular space:

This was found to be useful in identification except in small fish (≤c.50mm) and in fish where the jugular space had been deformed by muscular distension of the mouth. It was also used by Thong (1969), Boer (1975) and Arné (1938).

5) Protrusion of maxillary lobe from beneath preorbital bone:

This was particularly useful in separating *Liza ramada* from *Liza aurata*. It was also used by Thong (1969), Boer (1975) and Arné (1938).

6) Shape of preorbital bone:

Variation in shape and serration of this bone was used in identification by Perlmutter et al. (1957), Wheeler (1969) and Trewavas &
Ingham (1972). In this work the usefulness of the characters was limited by considerable individual variation and the gradation of one type into another.

7) Body shape and pigmentation:

Although body shape was dismissed by Thong (1969) as being subjective, the longer slimmer body form was used to identify L. aurata in initial sorting, although reliance was never placed on this character alone. The amount of head pigmentation was not found to be a useful characteristic - possibly because the fish used in this study were larger than those of Perlmutter et al. (1957) for which pigmentation differences were described.

8) Golden patches on head and operculum:

Wheeler (1969) characterised L. aurata by the possession of these patches and Arnê (1938) and Trewavas & Ingham (1972) described these as being conspicuous and well defined in L. aurata compared with the diffuse gold spots on L. ramada. In this work, as reported by Thong (1969), gold patches were sometimes found on all three species especially on the gill cover.

9) Spleen:

Differences in spleen shape between C. labrosus and L. ramada were described by El Zarka & Kamel (1967). In this work these differences did not appear to be distinctive and spleen shape seemed to vary with size of the fish.

10) Black patch at base of pectoral fin:

This character, although possibly more distinct in L. ramada, was found on specimens of all species. This was also noted by Thong (1969).

11) Length of pectoral fin:

As described by Wheeler (1969) the pectoral fin of L. aurata tended
to be larger than in the other species but variation was considerable. The differences were not so great, particularly in the smaller specimens, for this to be very useful distinguishing characteristic.

More recently, other characteristics for the identification of juvenile grey mullet have been suggested. Herzberg and Pasteur (1975) produced a tentative key for species determination based on the myogen electrophoretic pattern of five species - *L. ramada*, *L. aurata*, *L. provensalis*, *L. saliens*, and *M. cephalus* and van de Elst and Wallace (1976) devised a key based on lateral scale counts and dentition of juvenile mullet from the East coast of South Africa. However, in the work described in this thesis it was found that even small juvenile grey mullet could be identified using a combination of characters 1 - 11 above. The key factors used for identification are shown in Fig. 2.1.
CHAPTER 3  GROWTH

3.1.  RESULTS

3.1.1.  Length-frequency distributions

Length-frequency distributions for C. labrosus, L. aurata and L. ramada are shown in figs. 3.1 & 3.2. Estimates of skewness and kurtosis were calculated for groups of fish (n ≥12) of the same species captured at the same site and date, and the significance of the difference of these values from zero was tested using t-tests. The results are shown on Table 3.1. Negative estimates of skewness were more commonly recorded amongst 0 group fish of all species suggesting that some of the smallest fish might be escaping the net. However, skewness was only significant in 3 (p < .05) and kurtosis in 2 (p < .05) out of 22 distributions examined. In most cases neither skewness or kurtosis was significant and it was decided that it was justified to assume normal distributions for the purposes of statistical analyses.

3.1.2.  Relationship of standard length and total length

Regressions of standard length on total length were calculated for each species and each age group of each species (Table 3.2). All regression coefficients were found to be highly significant (p < .001) and analysis of variance showed that in each case the variance due to the regression was significantly greater than the variance due to deviations from regression (p < .001) (Table 3.2.). The best fit regression lines obtained by the method of least squares are plotted in Fig. 3.3.
Covariance analyses were carried out to compare the regression of total length on standard length among age groups of the same species and among species (Table 3.3). There was no significant difference in slope or elevation of the regression lines for age groups 0 and I of L. ramada. Among age groups of L. aurata there was no significant difference in slope but a highly significant difference in elevation \( p < .001 \) and among age groups of C. labrosus there was a marginally significant difference in slope \( p < .05 \).

There was a slight tendency for the total length relative to standard length to increase with increasing age of the fish, i.e. for the caudal fin to become proportionately longer, in both C. labrosus and L. aurata.

3.1.3. Growth curves

Growth curves of standard length and weight were plotted for each of the three species and are shown in Figs. 3.4 to 3.9. Seasonal variation in growth was a marked feature of all the growth curves. Growth was rapid from May to August but there was little or no growth during the winter months.

3.1.4. Methods for the description and analysis of growth

It is difficult to visually compare growth curves due to seasonal variation in growth among species and to difference in size among age groups. The description of growth by a single growth equation has many advantages. Several attempts have been made to define such an equation, but as stated by Paloheimo & Dickie (1965) growth is a very complex process affected by a range of factors which may or may not be altering.
throughout the life history of the fish. They considered that it was unlikely that the growth of many species of fish could be described over their entire life span by a single simple growth model. Thus, in this work, the growth equations were not intended to be a perfect mathematical description of growth, but rather a simple approximation to the description of growth which would allow statistical comparisons to be made between species and age groups, between growth of fish at different sites, and between growth of fish in the natural environment and in laboratory experiments where they were fed on artificial rations.

The growth models of Paloheimo & Dickie (1965) take as a starting point the basic energy equation defined by Winberg (1956).

\[ \frac{\Delta W}{\Delta t} = R - T \]

\[ R = \text{rations} \]

\[ T = \text{total metabolic expenditure} \]

However, both \( T \) and \( R \) vary with weight of the fish, \( W \), and they are also dependent to a certain extent on one another. In the relationship between total metabolic expenditure and weight, \( T = \alpha W^\gamma \), \( \alpha \) has been shown to depend on the level of \( R \). The relationship between \( R \) and \( W \) is not well defined and is complicated by the fact that the greater the level of \( R \), the more energy must be expended in acquiring it, and that the efficiency of use of food for growth tends to decline as rations increase. All these relationships may also be influenced by the type of food consumed and ambient temperature. This type of growth model was used by Elliott (1976c) for trout but it requires more detailed knowledge of the physiology of the fish than is yet available for juvenile grey mullet.

The growth model of Von Bertalanffy (1938) has its basis in the
difference between the rates of catabolism and anabolism. One form of the Von Bertalanffy growth equation is:-

\[ l_t = l_\infty - (l_\infty - l_0) \cdot e^{-kt} \]

where \( l_t \) = length at time \( t \)

\( l_\infty \) = asymptotic length

\( l_0 \) = length at time \( 0 \)

\( k \) = constant

It has since been shown that this cannot be interpreted in simple physiological terms (Richards, 1959) and the existence of an asymptotic length is rather rare (Paloheimo & Dickie, 1965). The Von Bertalanffy growth model has been used extensively for many species of fish including mullet (Thong, 1969; Albertini-Berhaut, 1975) and has been reported to be an adequate description of fish growth, particularly during the exploited phase of their life history.

For most fish growth curves are of the type:

Below the inflection point, the rate of increase of \( W \) increases with time, and above the inflection point the rate of increase of \( W \) decreases with time. Walford (1946) described a graphical method of approximately representing the growth curve by a straight line which is utilised by both Parker & Larkin (1959) and Von Bertalanffy (1938). Length at time \( (t+1) \) is plotted on the \( y \) axis against length at time \( t \) on the \( x \) axis as in Fig. 3.14. Below the inflection point this
straight line will have a slope $\geq 1$ and above the inflection point, a slope $< 1$. In estimating the parameters of the Von Bertalanffy growth equation, the fact that the Walford plot has a slope $< 1$ is utilised to estimate the asymptotic length, or length at which $l_{t+1} = l_t$. It is thus only applicable to growth of fish after the inflection point, when growth conforms to an inverse exponential pattern i.e. additions to linear dimensions decrease in geometric progression with increase in time. In most fish the part of the growth curve below the inflection point is completed early in life and is relatively unimportant in terms of the whole life history. However, it can be seen from Fig. 3.14. that for the species and age groups of this study the slope of the plot of $l_{t+1}$ against $l_t$ was $> 1$, indicating that the portion of the growth curve involved was below the inflection point. Table 3.17. shows growth increments collected from the literature and is discussed more fully in section 3.2.4. The occurrence of a maximum growth increment at ages 2-4 reported by several authors, particularly those working on grey mullet in British and Irish waters, does suggest that the inflection point of the curve occurs relatively late in these species. The relative importance of the phase of growth below the inflection point may be related to the growth rate. Thus, in Irish and British waters, where grey mullet are long-lived, slow-growing fish, not reaching maturity until 9-11 years old (Hickling, 1970b; Kennedy & Fitzmaurice, 1969), the inflection point occurs at 2-4 years, whereas on the Brittany coast, where the grey mullet become mature at 3-4 years (Thong, 1969) or in the Dardanelles Straits where first maturity occurs at 4-5 years (Erman, 1961), the inflection point of the growth curve seems to occur when
fish are less than one year old. Albertini-Berhaut (1975) found that the plot of $l_{t+1}$ against $l_t$ for *L. ramada* in their first year of growth had a slope less than 1 suggesting that, in the Gulf of Marseille, the first phase of the growth curve is completed early, before the fish reach 100mm. The evidence suggested that the inverse exponential growth model of Von Bertalanffy was not suitable for the juvenile fish of the study described in this thesis and that exponential models would be more appropriate to this phase of growth.

Other growth models tend to be more empirically derived from observed growth curves. One of the simplest is an exponential expression in which the rate of growth is proportional to the weight/length attained i.e. \[
\frac{dw}{dt} = kw
\]

Parker & Larkin (1959) developed this model further based on the equation $dW/dt = kW^x$. Paloheimo & Dickie (1965) suggest that the exponent 'x' in this equation is equivalent to their exponent 'y' ($T = \alpha W^y$) when $\alpha$ is constant and growth efficiency does not alter with ration size. The fact that Parker & Larkin found that $x$ was often less than 0.8, which is the commonly found value of $y$, is due to the fact that growth efficiency does change with ration size, and $\alpha$ varies with food and temperature. This may explain why Parker & Larkin found that it was necessary to divide the life history into stanzas, and calculate a separate equation with a different $x$ value for each stanza.

In addition to those described above numerous other models for fish growth have been put forward including the Gompertz growth curve, Putter growth curve, Johnson's growth curve and Richard's function (Ricker, 1979). Most have been proposed with mathematico-physiological
theories relating them to growth processes but according to Ricker (1979) the only criteria for choosing a growth curve that have proved valid are goodness of fit and convenience. On this basis the models chosen to describe the growth of juvenile grey mullet in this work were (a) the simple exponential growth curve and (b) its modified form as described by Parker & Larkin (1959).

(a) The exponential growth model:

The equation commonly associated with such growth is of the type:

\[ W = A \cdot e^{bx} \]

where \( W \) = weight, \( x \) = age, \( A, b \) are constants. Applying logarithms gives \( \log_e W = \log_e A + b \cdot x \). If growth is exponential a plot of \( \log_e W \) against age is linear and the regression coefficient, \( b \), is the relative increase of weight, i.e. rate of increase per unit time per unit weight. The calculation of the instantaneous growth coefficient, \( G \), is based on this model.

\[ G = \frac{\log_e W_2 - \log_e W_1}{\Delta t} \]

These equations can also be applied to standard length such that \( \log_e SL = \log_e A' + b'x \) and \( b' \) is the relative rate of increase of standard length, i.e. the rate of increase per unit time per unit standard length.

(b) The Parker & Larkin growth model.

The basic relationship for describing growth in weight was taken as

\[ \frac{dw}{dt} = k \cdot w \cdot x \]

Where \( w \) = weight
\( t \) = time
\( k, x \) = constant

(1)
Integrating

\[ \int_{w_0}^{w_t} w^{-x} \, dw = k \int_0^t \, dt \]

\[ w_t^{(1-x)} = (1-x)kt + w_0^{(1-x)} \]

If \( t \) is set \( t=1 \), i.e. growth is only considered from time \( t \) to \( t+1 \), the relationship becomes a regression of \( w_t^{(1-x)} \) on \( w_t^{(1-x)} \) with intercept \( k(1-x) \) and slope always equal to unity: thus

\[ w_{t+1}^{(1-x)} = k(1-x) + w_t^{(1-x)} \quad (2) \]

This holds for all values of \( w_t \) without regard to absolute age.

Assuming the weight-length relationship to be adequately described by the expression

\[ w = q_l y \]

where \( l = \) length \( \quad (3) \)

\( q, y = \) constants

and substituting for \( w \)

\[ l_{t+1}^{(1-x)} = \frac{k(1-x)}{q^{(1-x)}} + l_t^{(1-x)} \quad (4) \]

or

\[ l_{t+1}^x = \alpha + l_t^x \quad (5) \]

where

\[ \alpha = \frac{k(1-x)}{q^{(1-x)}} \quad (6) \]

and

\[ z = y(1-x) \quad (7) \]

In this work the unit of time was taken to be one year. Where growth varies seasonally, as in grey mullet, this was necessary so that difference between length at time 't' and 't+1' always included the slow growing and fast growing periods of the year. If units of time less than one year were considered, the variation in the coefficients of the growth equation with time of year would have to be taken into account as in Cloern & Nichols (1978).
To estimate \( z \) (eqn. 5) from a set of measurements of \( l_t \) and \( l_{t+1} \), \( z \) was set at values 0.5, 1.0, and 1.5 and three corresponding sets of data of \( l_t^z \) and \( l_{t+1}^z \) were estimated. For each set a series of \( \alpha \) values and their mean and standard deviation were calculated. The best estimate of \( z \) corresponds with the minimum variance of \( \alpha (s_{\alpha}^2) \).

\[
s_r = a + bz + cz^2
\]

where \( s_r = \text{relative standard deviation} = \sqrt{\frac{\sigma^2}{\mu^2}} \) and \( a, b, c \) are constants.

The best estimate of \( z \) corresponds with minimum variance of \( \alpha (s_{\alpha}^2) \) i.e. when the derivative of equation 8 is zero and

\[
2cz + b = 0 \text{ or } z = - \frac{b}{2c}.
\]

From the three sets of values of \( s_r \) and \( z \) equation 8 was solved using simultaneous equations and optimum \( z \) calculated. Using the optimum \( z \) the length data was converted to \( l_t^z \) and \( l_{t+1}^z \).

If \( l_{t+1}^z \) is plotted against \( l_t^z \) the points will lie \( \alpha \) distance above a 45° diagonal originating at the origin (equn. 5). Thus \( \alpha \) expresses length increment in a manner which is comparable regardless of size or age and thus has the same utility as instantaneous relative growth coefficient would have in the case of an animal growing exponentially.

The relationship between length and weight for each species is described in Chapter 4, so that the \( q \) & \( y \) value of each species was known (eqn. 3). Substituting in equation (7) \( x \) was estimated. Substituting in equation (6) \( k \) was estimated.

A growth equation of the type \( \frac{dw}{dt} = k \cdot w^x \) was thus obtained.
It is most unlikely that growth is exponential for any long period of time, but the highly significant correlation between both $\log_e W$ and $\log_e SL$ and age and the significance of the variance due to the regressions, suggested that growth of juvenile grey mullet was approximately exponential, at least within each age group over the first three years. This model was, therefore, of particular use in the comparison of growth rates between age groups and between sites within age groups, i.e. to compare growth over relatively short periods. Although the exponential growth model was used as a first approximation to compare growth of the three species, this was not entirely satisfactory as the increase of $\log_e W$ and $\log_e SL$ varied with age group. Thus to compare growth over a period of several years, the Parker & Larkin growth model was used.

3.1.5. Growth curves: comparison among age groups and species

For each fish $\log_e$ weight ($W$) and $\log_e$ standard length ($SL$) were determined and the regressions of $\log_e W$ and $\log_e SL$ on age in months were calculated for each age group of each species (except $L.$ ramada age groups 0, I, II and $L.$ aurata age group 0 where insufficient data were available) and for each species over all age groups. Correlation coefficients were calculated and variance ratio tests carried out to compare variance due to the regression and variance due to deviations from the regression. The data are summarised in Tables 3.4 and 3.5. All regressions and all correlations were highly significant ($p < .001$). Best fit straight lines were calculated for the regressions of $\log_e W$ and $\log_e SL$ on age for age groups 0, I, II, III of $C.$ labrosus and age groups I and II of $L.$ aurata (Figs. 3.10, 3.11). Best straight lines were also
calculated for the regressions of \( \log_e W \) and \( \log_e SL \) on age for each species, all age groups combined (Figs. 3.12, 3.13). The relative rates of increase of weight and standard length are shown in Table 3.6.

Covariance analysis was used to compare the regressions of \( \log_e W \) on age among age groups within species. The results are summarised in Table 3.7. There was a significant difference in the slope of the regression lines of the different age groups of *C. labrosus* \( (p < .001) \) and *L. aurata* \( (p < .01) \). Similar covariance analyses were carried out on the regressions of \( \log_e SL \) on age. The results are summarised in Table 3.8. There was a significant difference in the slope of the regression lines of different age groups of *C. labrosus* \( (p < .001) \) and *L. aurata* \( (p < .05) \). The relative rates of increase of weight and standard length are shown in Table 3.6. They tended to decrease with increasing age with the exception of *C. labrosus* group III. The high relative increase of weight and standard length of this age group was possibly due to the erroneous inclusion of group IV fish in group III or, more likely, due to the fact that data for group III fish was only available from mid-April to mid-July which included the period of fastest growth. In contrast, data on other age groups included fish caught in the fast and slow growing seasons of the year.

Although it is highly unlikely that the exponential growth model satisfactorily describes growth over several age groups, both \( \log_e SL \) and \( \log_e W \) were highly significantly correlated with age \( (p < .001) \) in each species. It was therefore considered justified to use this model for a first approximation of the comparison of
growth among species. Covariance analysis showed highly significant differences (p < .001) in the slopes of the regressions for the three species (Tables 3.7, 3.8). In Figs. 3.12 and 3.13 the regression lines of $\log_e SL$ vs $\log_e W$ on age for C. labrosus and L. ramada were approximately parallel indicating a similar relative rate of growth, but with L. ramada being heavier and longer at any given age. The regression lines of $\log_e W$ vs $\log_e SL$ on age for L. aurata were of similar elevation but less steep than those for L. ramada, suggesting a slower rate of growth. C. labrosus and L. ramada had similar overall relative rates of increase of standard length and weight which were higher than those of L. aurata (Table 3.6).

However, growth cannot realistically be described as exponential except for short periods. Therefore, a modified exponential model as described by Parker & Larkin (1959) was also used to compare growth among species.

The mean standard lengths of different age groups of each species in the different months of the year were calculated (Table 3.9). From this a table of $l_t$ and $l_{t+1}$ was drawn up. $l_t$ is plotted against $l_{t+1}$ in Fig. 3.14. $z$ was calculated as described in Section 3.1.3. and in Fig. 3.15 $l^Z_t$ is plotted against $l^Z_{t+1}$. The points on this graph lie at a distance $\propto$ above the 45° diagonal passing through the origin and $\propto$ represents the growth increment irrespective of size or age. From Fig. 3.15 it is evident that the points for all species lie at approximately the same height above the diagonal i.e. the difference in growth rate among the species was not very great. However, when a line is drawn at a distance $\propto$
above the diagonal it can be seen that values of \( L. \) aurata tend to be below \( \alpha \). The mean values of the three species were compared using t-tests. The results are summarised in Table 3.10. The mean \( \alpha \) of \( C. \) labrosus and \( L. \) ramada were similar indicating a similar growth rate. The mean \( \alpha \) of \( L. \) aurata was significantly lower than that of \( C. \) labrosus \((p < .05)\) indicating smaller yearly length increments at least over the age groups examined. These results agree with the conclusions reached using an exponential growth model.

As the growth rates of \( C. \) labrosus and \( L. \) aurata were apparently different it was necessary to calculate separate growth equations for each species.

Growth equations for \( C. \) labrosus and \( L. \) aurata were calculated as described in section 3.1.3.b.

These calculations are summarised in Table 3.11. The equations are:

\[
C. \text{labrosus} \quad \frac{dw}{dt} = 2.82w^{.84}
\]

\[
L. \text{aurata} \quad \frac{dw}{dt} = 3.76w^{.70}
\]

There were too few data to enable an equation to be obtained for \( L. \) ramada.

3.1.6. Growth curves: comparison among sites

In Figs. 3.4. to 3.9. site of capture is indicated on the growth curve of each species. At no site was the rate of growth obviously faster or slower. In order to compare the growth rate of fish at different sites statistically, regressions of \( \log_{e} W \) and \( \log_{e} SL \) on age were calculated for each age group of each species where fish had been captured in two or more months at two or more

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39
sites. The regression equations are shown in Tables 3.12 and 3.13 and the best fit regression lines are plotted in Figs. 3.16 and 3.17. With the exception of two _L. aurata_ groups which contained relatively few fish, log$_e$W and log$_e$SL were highly correlated with age (p < .001), and the variance due to the regression was significantly greater than the variance due to the deviations from regression (p < .001). The slopes of each regression line were compared with that of all fish of the same species and age group using t-tests. (Figs. 3.16 and 3.17).

All age groups were then combined, and for each site the regressions of log$_e$W and log$_e$SL on age were calculated for fish of each species. The regression equations are shown in Tables 3.12 and 3.13 and the best fit regression lines are plotted on Figs. 3.18 and 3.19. In all cases the correlation coefficient and the variance due to regression were highly significant (p < .001). The regressions for the fish caught at different sites were compared using covariance analysis. The results are summarised in Tables 3.14 and 3.15.

Comparisons of growth in log$_e$W and log$_e$SL gave rise to very similar conclusions. The growth in log$_e$SL and log$_e$W of _C. labrosus_ age group II and III at St. John's Lake was significantly slower than average, whilst in the R. Lynher and R. Tamar it appeared that growth was rather faster than average (Figs. 3.16 and 3.17). The reverse seemed to be true in the case of _L. aurata_ year group I where growth in St. John's Lake was significantly faster than average for the age group. This suggested that St. John's Lake was an area which favoured the growth of very young fish, whilst older fish tended to grow faster.
in the main rivers. Growth of _L. aurata_ of both age groups in the R. Lynher was not significantly different from the mean for all fish of the same species and age group.

When all age groups of _L. aurata_ were combined, the regressions of log<sub>e</sub>SL or log<sub>e</sub>W on age for fish from different sites were not significantly different in slope, i.e. log<sub>e</sub>W and log<sub>e</sub>SL were increasing at a similar rate at all sites. However, the regression lines were significantly different in elevation (Figs. 3.18, 3.19). This difference was only barely significant for the regressions of log<sub>e</sub>W on age (p < .05) but highly significant (p < .001) for the regressions of log<sub>e</sub>SL on age. At the same age _L. aurata_ from the R. Lynher were slightly longer than those from the R. Yealm and fish from the R. Yealm were slightly longer than those from St. John's Lake.

When all age groups of _C. labrosus_ were combined, covariance analysis indicated that the regressions of log<sub>e</sub>SL and log<sub>e</sub>W on age for fish from different sites were highly significantly different in slope (p < .001). The regression coefficients indicated that there was little difference in the growth rate of fish from the R. Tamar and the R. Lynher although fish in the R. Tamar appeared to be both longer and heavier than those of the same age in the R. Lynher. As age was not defined in units less than one month, such a difference might arise if the R. Tamar was fished later in the month than the R. Lynher. However, examination of the original data indicated that this had not been the case. The rate of increase of log<sub>e</sub>SL and log<sub>e</sub>W of fish from St. John's Lake was markedly slower than at the other two localities.
In summary, from the data available, it appeared that growth of young fish was faster in St. John's Lake than at other sites, although the reverse was true for older age groups.

3.1.7. Seasonal variation in growth

Examination of growth curves (Figs. 3.4 to 3.9) indicated that there was a very marked seasonal variation in growth with little or no growth occurring in the winter months. L. ramada were not captured regularly enough throughout the year for a seasonal pattern to be identified although it is probably similar to that of the other species. The growth of C. labrosus recommences in May/June. There was some indication that the growth of L. aurata began slightly earlier in April/May. Growth in all age groups appeared to recommence at approximately the same time. Maximum growth occurred during the summer in July and August. O group C. labrosus had ceased growing in October. This was the only age group sampled during the later months of the year.

3.2. DISCUSSION

3.2.1. Difficulties in drawing comparisons with other authors

Several authors have studied the growth of C. labrosus, L. ramada and L. aurata and a summary of their estimates of growth is given in Table 3.16. However, difficulties arise when comparing these both with each other, and with the results of the present study.

Apparent differences in growth rate may arise from:
(i) Sampling technique:-

Differences in the mesh size of the net, its mode of operation, and the topography and water depth of the area fished affect the part of the population which is sampled.

(ii) Use of different length measurements:-

Although most authors have used total length, some, for example Thong (1969) and Kennedy & Fitzmaurice (1969), have used fork length. However, their conversion factors are similar - 1.08 (Thong, 1969) and 1.10 (Kennedy & Fitzmaurice, 1969) - and have been used to convert fork lengths to total lengths for easier comparison with other work.

(iii) Procedures of scale reading:-

Most authors have used scales of older fish for back calculation of the growth of young fish. Although this method has some advantages, e.g. in estimating length at the end of the growing season, certain errors can arise, such as Lees phenomenon whereby back calculations of length show a tendency to be smaller the older the fish from which they were computed. Back calculation of growth assumes annual ring formation but Kesteven (1953) found that scales of M. cephalus were eroded in the winter - which might be expected to affect the scale length - body length relationship. However, most authors find that in mullet, scale length does seem to be proportional to body length (Thong, 1969). Nearly all authors report difficulties in ageing mullet from scales. (Ezzat, 1964; Le Dantec, 1955; Thong, 1969; Hickling, 1970b; Kennedy & Fitzmaurice 1969). Although the scales of young fish are thin and the structure fairly clear, with increasing age the scale becomes thicker. Growth of
spines on the posterior of the scale and spine bosses over the
central part, may obscure the first and even the second annual ring. Some authors (Kennedy & Fitzmaurice, 1969; Hickling, 1970b; have taken this into account by assuming the first visible ring to be the second or third. Using this method Kennedy & Fitzmaurice (1969) report good agreement between the observed length of juvenile C. labrosus and that calculated from the scales of older fish. Others appear to have ignored the overgrowth of early rings raising some doubt about the accuracy of the ageing of fish, particularly in view of the fact that their lengths given at 1 year correspond more closely to those given by other authors at 2 years. Thong (1969) gives the length of L. aurata at one year estimated from scales as 9.1cm. but reports L. aurata of 4.1cm. in October from the Gulf of Morbihan. However, Le Dantec (1955) considered the first visible scale ring as the second and still calculated a growth to 13.1cm. in the first year. Other authors such as Erman (1961) have used otoliths and cross sections of fin rays as well as scales to age fish, and also report very fast growth in the first year.

(iv) Spawning time:

Differences in size at year 1 might also be partly accounted for by differences in the time and duration of spawning. Observations on the spawning of grey mullet are very few and estimates of spawning time are made from the degree of maturation of adult fish, e.g. Hickling (1970b) and the size and time of arrival of young mullet on the coast, e.g. Demir (1971). There are numerous reports of ripe mullet migrating towards the sea (Thomson, 1954a; Kilby, 1948; Hickling, 1970b; Sarojini, 1958b; Le Dantec, 1955) and it is suggested that
spawning occurs just offshore where ripe and spent fish are
cought together (Erman, 1961). Reports are sometimes conflicting.
For example it has been suggested that *L. ramada* can spawn in
fresh water (Wimpenny & Faouzi, 1935; Arné, 1938) and Demir (1971)
suggested a spawning time for *C. labrosus* in South-west England
from the end of May to the end of August, which contrasts markedly
with the January to April spawning time suggested by Hickling (1970b)
for the same species off the Scilly Isles. A summary of reported
spawning times is given in Figs. 3.20 to 3.22. Although there is
some general agreement, for example most authors give the spawning
time of *L. aurata* in the autumn and that of *C. labrosus* in the spring,
within each species reported spawning times vary widely. It is
difficult to assess how much variation is due to error in the estim­
ation of spawning time and how much is due to genuine differences in
spawning behaviour in different areas.

Yashouv (1966) presented some evidence to suggest that
the spawning time was partially related to temperature. At temper­
atures of less than 18°C the eggs of *L. ramada* perish and so a drop
in water temperature in January marks the end of the spawning season.
He found that the eggs of *M. cephalus* had a different temperature
tolerance and suggested that this might be an explanation of the
successive spawning times of different species. If spawning is tem­
perature governed in this way it might be expected to vary in diff­
erent parts of the world. However, different spawning times reported
in the literature do not seem to be directly attributable to water
temperature. Particularly for *L. ramada*, the period of arrival of
fry in the estuaries seems to be of longer duration in the Nile Delta
region than in South-west England or in western France. This may be due to a temperature effect on the duration of spawning or it may be due to other factors such as the large area of the Mediterranean influenced by the discharge of the Nile in comparison to the smaller areas influenced by discharges of the rivers of South-west England.

Perlmutter et al. (1957) found that fry of *C. labrosus* arrived on the Israeli coast in May 1956 and September 1955. This would suggest that behaviour of the fry or the spawning period may vary considerably from year to year. This may partly explain different spawning times reported from the same area in different years, and similarly, different rates of growth in the first year.

(v) Duration of spawning:

Estimates of the duration of spawning time vary greatly, from one month (Ezzat, 1964; Serbetis, 1939) to five months (Demir, 1971). Many authors give spawning periods of approximately three months. This may partly account for apparent differences in growth rate. For example, Kennedy & Fitzmaurice (1969) attribute the wide size variation of 0 group fry in October to a combination of individual variation in growth rate and a difference in age of weeks or even months. Similarly, Roule (1917) attributed the wide variation in growth from the same spawning in the Mediterranean to varying age due to the long spawning period.

Ezzat (1964) found that the scales of young *M. cephalus* hatched soon after the start of the spawning period showed a winter check, but those hatched at the end of the spawning period (October) showed no check in scale growth until the following winter. Thus,
young of the same year group may show a different number of winter rings resulting in erroneous ageing and growth estimates.

(vi) Distance of spawning grounds from the coast:

Even when reported spawning times coincide, the time of arrival of fry at the coast may differ markedly. Thus, Hickling (1970b) and Thong (1969) both report spring spawning for C. labrosus but the fry arrive in April in Brittany but not until July in South-west England and Ireland. One explanation is that the spawning grounds of the Brittany mullet are closer inshore than those of the mullet of South-west England or Ireland. The earlier arrival of fry in the rich feeding grounds of the estuaries may partly account for the faster growth observed in the first year in Brittany.

(vii) Ageing strategy:

Differences, real or apparent, in the time of spawning have contributed to the confusion over the ageing of the mullet. Thong (1969) illustrates his ageing scheme as:

\[
\text{hatching} \quad \longrightarrow \quad \text{1st winter} \quad \longrightarrow \quad \text{2nd winter} \\
\quad \text{0 group} \quad \longrightarrow \quad \text{1 group} \quad \longrightarrow \quad \text{I group}
\]

\[
\text{L}_1 \quad \longrightarrow \quad \text{L}_2
\]

On a similar scheme the strategy of ageing used in this study would appear:

\[
\begin{align*}
\text{winter:} & \quad C. \text{labrosus} & \quad L. \text{aurata} / L. \text{ramada} \\
\text{hatching} & \quad \longrightarrow \quad \text{1st} \quad \longrightarrow \quad \text{2nd} & \quad \longrightarrow \quad \text{1st} \quad \longrightarrow \quad \text{2nd} \\
\text{0} & \quad \longrightarrow \quad \text{I} & \quad \longrightarrow \quad \text{0} \quad \longrightarrow \quad \text{I} \\
\text{L}_1 & \quad \longrightarrow \quad \text{L}_2 & \quad \longrightarrow \quad \text{L}_1 \quad \longrightarrow \quad \text{L}_2
\end{align*}
\]
Thus, for *L. aurata* and *L. ramada* what is denoted L1 in this study should correspond to the L2 of Thong (1969). However, it does not appear to be the case, the L1 of Thong in both cases exceeding the L1 of this study. Thong measured *L. aurata* fry in the Gulf of Morbihan. He found two groups of fry with mean total lengths of 21mm and 82mm respectively in July, and 44mm and 114mm in October. In spite of the fact that mullet of the larger group were captured at 56mm in May before his suggested spawning time of June, he appears to consider both groups as 0 group mullet. Thus, the L1 of Thong appears to be a mixture of L1 and L2 of this study. Serbetis (1939) gives a spawning time of Feb-March for *L. ramada* with the appearance of fry of 95-114mm in April, reaching 145mm by the first winter. Where the growth of the fry of these species has been directly observed, most workers report a growth rate of about 10mm per month (Thong, 1969; Kennedy & Fitzmaurice, 1969). Even the fast growing *M. cephalus* in the warm coastal waters of Georgia, where water temperatures are greater than 20°C, only achieves a maximum growth of 17mm per month. It therefore seems unlikely that fry spawned in February could attain a length of 95-114mm by April. It seems more likely that they were spawned in the previous year, probably in the autumn which is the more favoured spawning time for *L. ramada*. The extremely fast growth of *L. ramada* in the first year observed by Wimpenny (1955) must also be viewed with some doubt in view of the observations of El Zarka & Kamel (1967) from the same region. The difference in the recorded lengths of fish of the same quoted age group in January (160mm and 19.5mm respectively) seems to be attributable to the ageing strategy.

Thus, the ageing procedure adopted must also be taken into account when comparing age-length data from different sources.
3.2.2. Comparison of the growth of the three species in the same region

Statistical comparisons of the growth in weight and length using the exponential growth model indicated that there were significant differences in the growth of the three species over the first three years in the area of this study. Comparison of relative rates of increase of \( \log_e W \) and \( \log_e SL \) indicated that the growth of *C. labrosus* and *L. ramada* was very similar and rather faster than that of *L. aurata*. When the Parker & Larkin model was used to compare the growth of the three species similar results were obtained; the growth of *C. labrosus* and *L. ramada* being very similar, and the growth of *C. labrosus* being significantly greater than that of *L. aurata*.

Apparent differences in growth rate may occur for several reasons including:

(i) Differences in the length of growing season:

There was no evidence to indicate that the growing season of *C. labrosus* and *L. ramada* was longer than that of *L. aurata*.

(ii) Months of sampling:

*C. labrosus* tended to be more regularly sampled during the winter when growth was slowest and so differences in sampling months cannot account for the observed differences in growth rate.

(iii) Migration:

Larger, faster growing *L. aurata* individuals may migrate sooner to the deeper waters of the estuary, particularly since *L. aurata* is known to be the most stenohaline of the three mullet species (Perlmutter et al, 1957; Morovic & Sabicello, 1965). However, al-
though this might account for the apparently slow growth rate of group II, it cannot explain the relatively slow growth rate also observed for group I fish.

(iv) Age group representation:

As relative rate of growth tends to decrease with age, the overall value for each species will depend to some extent on the age groups represented, e.g. relatively more fish of age group 0 are included in the _C. labrosus_ calculations. However, when group II or group III _C. labrosus_ were compared with group I _L. aurata_ their relative growth rates were higher in spite of the fact that they were larger and older. Thus differences in growth rate cannot be attributed to different age group representation alone.

Observed differences in the growth rates of grey mullet species in the estuaries examined in this study cannot be attributed satisfactorily to any of the above factors.

Several authors have examined the growth of two or more of _C. labrosus, L. ramada_ and _L. aurata_ where they occur at the same locality. For the reasons stated in Section 3.2.1. this is preferable to comparisons of growth at different localities and in different years. Some results are illustrated in Fig. 3.23. Difficulties in ageing mean that it is not necessarily significant that Thong (1969) shows _C. labrosus_ larger than _L. aurata_ where Hickling (1970b) shows the reverse.

Both Hickling (1970b) & Thong (1969) report only small differences in growth rate among the three species. Thong (1969) found that _L. ramada_ and _C. labrosus_ from the Brittany coast grew slightly faster than _L. aurata_ which agrees with the finding of this study and
also Hickling (1970b), although in the Mediterranean L. aurata and L. ramada appear to grow at approximately the same rate in the first year (Albertini-Berhaut, 1978). Arné (1938) found that L. ramada grew less quickly than the other two species but this was probably related to the special salinity and temperature conditions of the Bassin d'Arcachon. Serbetis (1939) found that on the Italian coast L. ramada and L. aurata grew at a similar rate and at an equivalent age were smaller than M. cephalus. Morovic (1960) working on mullet from lakes on the Adriatic coast of Italy found that the growth of C. labrosus and M. cephalus was approximately equivalent over the first five years. In the Black Sea the growth rates of C. labrosus and L. aurata were approximately the same (Nikolskii, 1954). Perlmutter et al. (1957) examined the growth in nearly freshwater ponds in Israel and found that over a period of six months L. ramada grew faster than C. labrosus which grew faster than L. aurata.

This would seem to indicate that the relative growth rates of C. labrosus, L. aurata and L. ramada in one locality may be affected by their salinity preferences. Vallet et al. (1970) found that the growth of L. aurata was a maximum at 20‰. C. labrosus and particularly L. ramada are more tolerant of low salinity than L. aurata (Serbetis, 1939; Boisseau et al., 1975).

In L. ramada & C. labrosus the prolactin cells show increased activity in freshwater. In L. aurata they have a relatively high activity in sea water and seem unable to increase it further to adapt successfully to freshwater (Chambolle et al., 1979). The results of Perlmutter et al. (1957) suggest that maximum growth of L. ramada occurs
at a lower salinity than the 20% observed for *L. aurata*.

The sites sampled in this study tended to be in the upper reaches of estuaries where the salinity varied widely, particularly in St. John's Lake where it approached 0°/oo at low tide. Thus the salinity regimes in these areas may have hindered the growth of *L. aurata*.

Thong (1969) reported that the relative growth of the three species was affected by 'unspecified local conditions'. In the mouth of La Rance *L. ramada* and *L. aurata* grew faster than *C. labrosus* but in the Bay of Morlaix, *C. labrosus* attained a length clearly superior to *L. aurata*. However, these differences could not satisfactorily be attributed to salinity variations.

In general it appears that all three species have a similar capacity for growth and under certain conditions they can apparently grow almost as fast as *M. cephalus*, the species most favoured in mullet culture. There is some indication that local conditions of salinity, temperature, exposure etc. may have a differential effect on the growth rate of the three species.

In an experiment weight increase of juvenile *L. ramada* varied from 56.8% to 153.6% when they were fed on diets ranging from sorghum to copepods (Yashouv & Ben Shachar, 1967). Thus, differences in the availability of their preferred diet may partially account for differences in growth rate at the same locality.

Some work in the Mediterranean (Albertini-Berhaut, 1973) showed that although harpacticoid copepods were approximately equally common in the diet of *L. aurata* and *L. ramada* fry, other crustaceans were twice as common in *L. aurata* as in *L. ramada*. 
L. *ramada* fry showed a particular preference for very small diatoms of the genus *Navicula*. Ezzat (1964) found that *L. ramada* preferred the blue-green algae in the same area as *M. cephalus* were feeding mainly on green algae. In a laboratory food preference experiment (Yashouv & Ben-Shachar, 1967) *M. cephalus* fry ingested algae especially diatoms, sand and mud as well as animal matter including cladocerans, copepods and ostracods. In the same experiment *M. capito* fry, presumably of an equivalent size although this is not stated, fed mainly on chironomid larvae and rotifers ingesting no sand at all. Although no obvious dietary differences were observed it is possible that the type of food available at the sites sampled may have been more appropriate for, and therefore promoted faster growth in, juvenile *C. labrosus* and *L. ramada* than in *L. aurata*.

3.2.3. **Comparison of growth among age groups**

The relative rate of increase of standard length (SL) or weight (W) differed significantly with age group for *C. labrosus* and *L. aurata*, tending to decline as age increased. The increase observed in group III *C. labrosus* is probably anomalous due to the relatively small numbers of fish, the limited sampling period, and the possible erroneous inclusion of group IV fish. In *C. labrosus* the decline in relative growth rate from 0 to I group was greater than the decline from I to II group.

As has already been noted, when annual length increments were compared it was found that in *C. labrosus* there was a progressive increase in the annual increment from the first to the fourth
year of growth. For *L. aurata* and *L. ramada* the length increment in the third year was greater than in the second. The growth increment of the first year was also greater than that of the second, but this may have been partly due to the ageing strategy employed, which meant that the first 'year' of growth was 14 months.

Annual growth increments reported by several authors for these and other species of mullet are summarised in Table 3.17. In some cases, e.g. Thong (1969), growth increment declines with age and growth conforms to an inverse exponential pattern. However, others working on the same species have found that growth does not conform to this pattern. Nearly all authors report particularly rapid growth in the first year, but in some cases this may be exaggerated due to ageing problems. Morovic (1960) found that in *L. Pantan* the length increment of *C. labrosus* increased from the second year to a maximum in the third and fourth years, declining thereafter. Similarly Rossignol (1951), Hickling (1970b) Arné (1938), Le Dantec (1955) and possibly this work suggest a growth maximum for *C. labrosus* in the third and fourth years. In Ireland a growth maximum in the third year is reported (Kennedy & Fitzmaurice, 1969), while in the Black Sea growth in the sixth year is reported as being four times that in the fifth and more than twice that in the fourth year (Nikolskii, 1954). For *L. ramada* a maximum growth in the third year is suggested by the results of Hickling (1970b) and this study. Maximum growth of *L. aurata* apparently occurs in the second year (Serbetis, 1939), third year (this study; Hickling, 1970b) or fourth year (Ezzat, 1964).
There are some cases where the growth maximum is quite small and its real existence must be doubted, as it could be attributed to sampling errors or scale reading anomalies, e.g. Serbetis (1939) for L. aurata. However, in other cases the increase in increment is too large to be easily discounted e.g. the annual increment of C. labrosus in the fourth year has been reported as double that in the third (Arné, 1938; Le Dantec, 1955). Kesteven (1953) found that erosion of the scales of M. cephalus occurred during the period of growth cessation. Differential erosion might lead to an apparent growth maximum but this seems an unlikely explanation, as the increase in annual increment was also observed when otoliths were used for ageing (Ezzat, 1964; Kennedy & Fitzmaurice, 1969) and where growth was observed by the sampling of juveniles (Kennedy & Fitzmaurice, 1969; this study).

One explanation may derive from the shape of the growth curve. In most species of fish the phase of the growth curve below the inflection point, where growth rate is increasing with time, is completed very early in life and is relatively unimportant. However, in British and Irish waters the grey mullet is known to be a very slow growing fish, which may result in the point of inflection of the growth curve not occurring until the second to fourth years. In contrast, in Brittany and the Mediterranean the grey mullet grow faster and mature much earlier - at 3-4 years rather than 9-11 years - (Thong, 1969; Erman, 1961) and so the point of inflection of the growth curve occurs during the first year, with no later growth increment maximum (Thong, 1969; Erman, 1961; Albertini-Berhaut, 1975). This may also explain the comparative
rarity of a maximum growth increment after the first year in the faster growing *M. cephalus*. However, this is not consistent with the high growth increments observed in the first year (although a possible explanation has been suggested) or the growth maxima in the second to fourth years observed by Morovic (1960), Rossignol (1951), Le Dantec (1955) and Arné (1938) in the Mediterranean and the Gulf of Arcachon where the mullet are relatively fast growing and early maturing.

Another possible explanation for the change in the growth rate is a change in diet. The gradual change from a predominantly carnivorous planktonic to a predominantly herbivorous benthic mode of feeding at 20-50mm is well documented (Albertini-Berhaut, 1973; De Silva & Perera, 1976; Sarojini, 1954; Suzuki, 1965; Zambriborsch, 1964) but the diet may also change at later ages. Ezzat (1964) examined the food of *L. aurata* and found that the % occurrence of diatoms varied from 60-90% in fish of 100-150 mm to less than 5% in fish of 300-350mm. The ingestion of blue-green algae and green algae increased with fish length, but the nutritional value of these is uncertain as both have been found in an undigested state in the rectum (Yashouv & Ben-Shachar 1967; Payne, pers. comm.). Investigations on the chemical composition of the alimentary canal of mullet from South Africa (Marais & Erasmus, 1977) and *M. cephalus* from India (Das, 1978) suggested that smaller fish consumed the most nutritious food material. In this study all mullet except very small specimens were found to be feeding on mud rich in diatoms although a detailed study of species or particle size was not undertaken. The reason for the maximum growth increment observed in the second to fourth years re-
3.2.4. Seasonal variation in growth

A marked seasonal variation in growth was evident in *C. labrosus* and *L. aurata* with maximum growth in July and August and little or no growth during the winter. The growing season of juvenile *C. labrosus* was from the end of May until the end of October. There was some indication that the growth of *L. aurata* might have recommenced earlier in the year in April/May.

Almost all authors report a winter check in growth. The length of the period of growth cessation varies. In Western Australia the growth of *M. cephalus* ceases for about two and a half months (June to September) which is a considerably shorter period than the 6-7 months observed for *C. labrosus* in this study and by Kennedy & Fitzmaurice (1969) in S.W. Ireland. In other areas growth continues throughout the year. In N.W. Florida the growth of *M. cephalus* in spring and summer is approximately twice that in autumn and winter but even during these seasons growth proceeds at .08mm/day (Broadhead, 1953). Similarly the growth of *M. cephalus* in the Bay of Bengal proceeds at the comparatively slow rate of 8.5mm per month from November to April with a much higher growth rate (c. 30mm/month) during the rest of the year (Jhingran & Mishra, 1962).

The causes of the growth cessation are not clearly understood. There is some evidence for seasonal variation in feeding intensity (Kennedy & Fitzmaurice, 1969; Albertini-Berhaut, 1973; Le
Dantec, 1955; De Silva, & Wijeyaratne, 1976; Kuthalingham, 1966) which is discussed in Chapter 5. Observations by Sarojini (1954) on the Indian grey mullets suggested that feeding ceased in the rainy season because the bottom flora was disturbed rendering their normal food unavailable to the mullet. There is some evidence that the type of food as well as the quantity may vary seasonally. Albertini-Berhaut (1973) examined the food of juvenile L. ramada, L. aurata and M. saliens in the Mediterranean and found that the most important element of the diet varied from harpacticoid and planktonic copepods in the spring to amphipods in May and insects falling into the water in October. Epiphytic diatoms were important in the diet in the spring, whereas diatoms free in the sediment were most commonly ingested in the Autumn. De Silva & Wijeyaratne (1976) found M. cephalus (20-55m) fed predominantly on diatoms for most of the year, but in August and September, Chlorophycae and Xanthophycae became more important. Kuthalingham (1966) reported that the seasonal variation in diet of M. cephalus depended on the environment. Thus, in inshore waters, the proportion of algae in the diet tended to be lower from May to August, whereas mullet feeding in offshore waters consumed more algae during these months. Similarly in India M. parsia from the sea consumed very little decayed organic matter during the rainy season and relatively large amounts during the cold season, but in estuaries and brackish water the reverse was true - less mud was consumed in the winter season, correlated with a greater intake of algae (Sarojini, 1954).

Although in other species, e.g. trout (Swift 1955) and
coregonids (Hogman, 1968) temperature did not have a direct effect on the beginning and ending of the growth period, observations on juvenile C. labrosus show that feeding and activity decline below 10°C, and feeding ceases at less than 8°C (Kennedy & Fitzmaurice, 1969). Thomson (1951) reported that the growth period of M. cephalus in Western Australia was related to water temperature. If this is generally the case, one might expect the length of the growth period, and thus the annual rate of growth, to vary from region to region and from year to year.

3.2.5. Variation in growth rate from year to year

In this study group I C. labrosus captured in St. John's Lake in January 1976 were significantly longer than the equivalent age group captured in February 1975. Similarly, Albertini-Berhaut (1978) found that the annual linear growth increment of juvenile L. ramada and L. aurata varied from year to year. Possible reasons for this are numerous. Temperature fluctuations and other physical factors vary from year to year. Rainfall affects nutrient concentrations and hence primary productivity. In January 1976 the area beyond the ford at St. John's Lake was more silted up than it had been in the previous year and so larger fish were not captured with the group I fish. Possibly a reduction in competition in 1976 stimulated growth rate amongst the smaller fish. Zambriborsch (1964) reported that growth rate of L. aurata in the estuaries of the Black Sea was at least partly density dependent and so a year class weak in numbers may show particularly fast growth. It is also possible that the 1975 I group belonged to a younger sub-brood than
the fish captured in 1976 - such sub-broods may remain distinct for 1-2 years (Thomson, 1951). Time of spawning and conditions affecting growth and migration of the larvae may also vary from year to year. Perlmutter et al. (1957) found that C. labrosus fry arrived at the coast at a length of 25-35mm in September 1955 and in May in 1956. El Zarka & Kamel (1967) found that the number of fry moving into brackish water lakes was highly correlated with the amount of discharge of nearly fresh water. Thus the amount of rainfall, which varies from year to year, may influence the success of the coastal migration of the young mullet.

Several authors have noted differences in growth rate between different year classes of grey mullet. Thomson (1951) found that the 1931 M. cephalus year class was particularly fast growing. Wmmpenny (1955) reported that 0 group L. ramada reached 16.3cm in 1928 and 21.3cm in 1933. However, standard deviations are not given. Similarly Morovic (1954b) found that the growth of M. cephalus in first year was greater in 1949 than 1952.

Growth rate of any particular age group seems to vary from year to year. Growth studies should, therefore, span several years and ideally be coupled with comprehensive data on temperature, rainfall, primary productivity etc.

3.2.6. Comparison of growth at different localities and in different parts of the world.

There was some evidence to suggest that growth of fish older than 0 group tended to be slower in St. John's Lake than in the R. Lynher or the R. Tamar. However, several factors may result in
this being an apparent difference in growth rate. Where all age groups are combined it might be expected that a particularly fast relative growth rate would be shown at sites where a high proportion of young fish were caught, since younger age groups tend to have higher relative growth rates. However, St. John's Lake, where growth was relatively slow, was the site of capture of most of the younger fish which would seem to indicate that this was not a significant factor. If growth within age groups does not adhere precisely to the exponential pattern, differences in sampling months may also give rise to apparent differences in growth rate. The particularly fast rate of growth of *C. labrosus* age group III in the R. Lynher may be partly due to the fact that samples were only captured in June and July, in the season of fastest growth. Similarly *C. labrosus* age group I from the R. Tamar were only sampled from June to August. Apparently slow growth rates may also arise from migration of larger fish from St. John's Lake to the main river. However, this would be expected to affect estimates of growth rate of the oldest age group, not successive age groups, as was observed in *C. labrosus* group II and III. Although estimates of skewness of length distributions of age groups II and III tended to be positive, the majority were not significantly different from zero.

When the relative condition of fish of equivalent species and age captured in the same month at different sites were compared (4.1.5.) fish from St. John's Lake had a lower relative condition than fish captured in the R. Tamar and R. Lynher. This suggested that the St. John's Lake site was in some way less favourable than
other sites, and that the observed differences in growth were not due to differences in sampling months, age groups or migration alone.

All sites had many similarities being characterised by wide expanses of mud, exposed at low water and shallowly covered at high water. However, St. John's Lake differed from the other sites in that the ford retained the fish in the upper estuary at low water, and there was a sewage outlet from a nearby village. Both the retention of mullet in the feeding grounds and the input of organic material might be expected to cause increased growth in juvenile mullet. There was some indication that the growth of 0 group mullet was faster in St. John's Lake but the growth of older C. labrosus was rather slower than in the R. Tamar or the R. Lynher. Factors such as the depletion of dissolved oxygen caused by the high oxygen demand of the sewage may have adversely affected growth. The wide salinity fluctuations in the pool behind the ford, from almost freshwater at low tide to full seawater at high tide may also have impaired growth since Boisseau et al. (1975) have shown that C. labrosus expends considerable energy in maintaining osmotic balance in freshwater.

When all age groups were combined, growth of L. aurata was similar at all sites and growth of C. labrosus was similar in the R. Lynher and R. Tamar. It may be that juvenile mullet stock of the R. Tamar, R. Lynher and R. Yealm intermingle and migrate from one river to another, so that mullet feeding in R. Tamar at one time may be captured in the R. Yealm or the R. Lynher at another. Thomson (1954a) found that 0 group mullet repopulated creeks which
had been netted out with fine netting in the course of 2-3 weeks. He used opercular tags to examine the movements of *M. cephalus* over 10cm long. In addition to the spawning migration of adults, group I and II fish sometimes migrated along the coast from one river to another. As the fish had empty guts he suggested this was a trophic migration, possibly stimulated by floods which caused a food scarcity. *L. aurata* from estuaries around the Black Sea winter in deeper waters off the coast (Zambriborsch, 1964) and the seasonal variation in the abundance of grey mullet in estuaries and on the coast, noted by several authors (e.g. Thong, 1969; Hickling, 1970b; Erman, 1961) suggests that some mullet move away from the estuaries in winter. Such migrations may permit mixing of stocks from different rivers. Thomson (1951) tagged immature *M. dobula* in Western Australia. Although one was recaptured after 2-3 years only three miles from where it was tagged, two others were recaptured in river systems 50 and 350 miles away after 310 and 455 days respectively. Two tagging experiments involving immature *M. cephalus* were carried out in Chilka Lake off the Bay of Bengal (Jhingran & Patro, 1969; Jhingran & Mishra, 1962). The tagged fish spread out in different directions over the 400 sq. ml. lake, and the population of the whole lake was probably homogeneous. Thus, what evidence there is from tagging experiments would seem to suggest that in addition to the intermingling of mature fish from various rivers during spawning (Kesteven, 1953) there is also some fairly wide ranging migration of immature fish from one river system to another. In addition, if, as Hickling (1970b) suggests, mullet spawn off the Scilly Isles, it is likely that the juvenile mullet
captured at all 4 sites of this study are part of the same population.

Several authors have compared the growth of grey mullet at different localities. The growth of *M. cephalus* at widely separated parts of the coast of Florida was similar (Anderson, 1958; Kilby, 1948). Kennedy & Fitzmaurice (1969) found no significant differences in growth of *C. labrosus* on the south coast of Ireland and the cooler waters of the Irish Sea. Thomson (1951) found that the growth rate of *M. cephalus* on the east and west seaboard of Australia was similar and Thakur (1967) reported that growth of the same species was similar in India, Australia, western and north-western Florida. Morovic (1954) found no significant differences in the growth of *M. cephalus* in four lakes along the Adriatic coast of Italy.

However, other authors do report differences in growth of the same species at different localities. *C. labrosus* grows faster in the reservoirs of Certes than in the Gulf of Gascony or on the Moroccan coast (Le Dantec, 1955). The work was carried out by different authors in different years, and scales were used for ageing which Le Dantec acknowledged could lead to errors because the first ring was not always visible. Morovic (1960) reported 'notable differences' in the growth of *C. labrosus* in L. Pantan on the Adriatic coast of Italy compared with that given by Rossignol (1951) for the Moroccan coast. Thomson (1951) found some variation in the growth of *M. cephalus* according to locality, but there was no continuing trend of growth at a slow or fast rate at any one locality. Although Thong (1969) found that *C. labrosus*
and *L. ramada* generally showed comparable growth at all localities, there were several exceptions - the growth of *C. labrosus* was more rapid in the Bay of Morlaix and *L. ramada* grew particularly fast in the mouth of La Rance. In contrast, *L. aurata*, particularly in the Gulf of Morbihan region, apparently showed a different growth rate at most localities. El Zarka et al. (1970) examined the growth of *M. saliens* in two lakes on the Nile delta. Growth was considerably faster in *L. Qarun* than in *L. Edku* possibly due to reduced competition because the mullet of *L. Edku* were not exploited.

The growth of *C. labrosus*, *L. aurata*, *L. ramada* and *M. cephalus* has been examined by various authors in different parts of the world. Some of their results are shown in Figs. 3.24 - 3.27 and Table 3.16. Results of different authors can only be compared with great caution. Apparent differences in growth rate at different localities may arise from differences in various factors which have been discussed in section 3.2.1.

Real differences in growth rate at different localities may arise from differences in:

1) Race of genotype
2) Food and feeding behaviour
3) Salinity regime
4) Temperature regime.
5) Environmental effects, e.g. pollution, gas regime and their diurnal and annual fluctuations.
(1) Racial or genetic differences between the stocks of different areas:

The growth of individual mullet is noted by most authors to be very variable. After the third year, and sometimes even earlier, length is not a good indicator of age (Morovic, 1954b; Sarojini, 1957). Wide variation in growth of individuals was also noted in tagging experiments (Thomson, 1951) and growth experiments (De Silva & Perera, 1976). However, it was not known how much of the variation was due to genetic variation and how much due to the other factors, e.g. age, food supply etc. or in the case of the feeding experiment, crowding and hierarchy effects. Thomson (1951) found no evidence of racial variation between the stocks of East and West Australia. There was no evidence of morphological variation between the C. labrosus, L. ramada and L. aurata stocks of Brittany and the Mediterranean (Thong, 1969). Detailed studies have not been undertaken and the extent and scale of genotypic variation is unknown.

(2) Food supply and feeding behaviour:

Albertini-Berhaut (1973) found that the relative importance of different diatom species in the food of mullet fry varied with locality in the Mediterranean. At Pointe-Rouge epiphytic diatoms accounted for 44% and mobile diatoms found in the sediment for 10.7% of the diet of juvenile mullet, whereas at Brusc diatoms from the sediment formed 69.4% and epiphytic diatoms only 8.2% of the diet. Le Dantec (1955) reported that the diet of C. labrosus in the Reservoirs de Certes (Bay of Arcachon) in March consisted mainly
of yellow algae - *Ruppia maritima* and *Ruppia rostellata*. These species were not found in any of the *C. labrosus* examined by Hickling (1970b) in South-west England. *M. cephalus* fry in the coastal lagoons of Sri Lanka feed only rarely on animal matter but its proportion in the diet increased nearer to the sea mouth of the lagoon (De Silva & Wijeyaratne, 1976). Kuthalingham (1966) investigated the diet of adult *M. cephalus* in India at locations off-shore, inshore and in brackish water. The consumption of algae was slightly higher in brackish water and that of decayed organic matter was higher inshore but the differences were not very great. The feeding intensity was greatest off-shore and least in brackish water. Similarly *M. parsia* fry were found to be feeding more intensively in the foreshore areas of Jaunput (Bengal) than in the poorer feeding grounds of the estuaries (Sarojini, 1957). The diet of *M. tade* was compared in the sea, estuary, a brackish water farm and freshwater tanks. Decayed organic matter was most important in the diet of fish from the estuary, whereas diatoms were most important as a dietary constituent in the sea. In both the brackish water farm and the freshwater tanks the diet was predominantly algae (Pillay, 1953). Similarly *M. dobula* in Australia consumed mainly detritus and diatoms in estuaries but in freshwater was more dependent on algae. In the Albert River mullet were found to be feeding almost exclusively on filamentous green algae (Thomson, 1954b). In analysis of the food of mullet from South African estuaries, all mullet were feeding on sand and detritus but the chemical composition of the
food varied from estuary to estuary (Blaber, 1977). In none of these examples was a difference in diet shown to be directly related to growth.

Evidence from comparison of the occurrence of prey items in the environment and mullet stomachs (Pillay, 1953), seasonal variation in diet (previous section) and various observations of mullet feeding on floating bread (Kennedy & Fitzmaurice, 1969) suggest that mullet are opportunistic feeders and variation in stomach contents is due to availability of food items. However, such variation in diet with locality would only be expected to have an effect on growth if mullet habitually feed in one area. It has been shown that immature mullet may move from one river system to another, and larger scale feeding migrations have been observed, probably in response to special food shortages caused by factors such as floods (Thomson, 1954a). Kennedy & Fitzmaurice (1969) tagged mullet feeding on bread and reported seeing them at the same locality feeding in the same manner on several occasions in subsequent weeks. In spite of the fact that mullet spread out to feed, a school may persist for a period of 6 months or more and while some schools appear to move more or less continuously, others remain in one locality within a river system for a considerable period (Thomson, 1954a). In a tagging experiment off Florida (Idyll & Sutton, 1952) 87% of M. curema were captured within 20 miles of the place of release. The evidence would seem to indicate that some mullet may stay in one locality long enough for local differences in food availability to have some effect on growth.
Salinity regime

Salinity has been found to have a direct effect on the growth and survival of several euryhaline fish (e.g. flounder (Dewbler, 1960); coho salmon (Canagarathnam, 1959)) independent of its effect via food supply. Boisseau et al. (1975) reported that Na- and K- ATPase activity in *C. labrosus* increased at low temperatures and low salinities suggesting an increased energy requirement. Nordlie & Leffler (1975) found that the metabolic rate of *M. cephalus* varied with salinity. In environments of 100-300 mOs/l it was a minimum increasing at lower and higher salinities. Transfer from one salinity to another caused an increase in oxygen consumption. The thyroid activity of *L. aurata* varied according to ambient salinity, and in a feeding experiment, growth and growth efficiency were higher at 20°/oo than at 5, 12 or 37.5°/oo. (Vallet et al., 1970). Similarly De Silva & Perera (1976) found that in young *M. cephalus* both food intake and growth was a maximum at 20°/oo decreasing at lower and higher salinities. Thus one might expect that mullet in brackish water ponds would grow faster than those exposed to extremes of salinity in freshwater ponds or hypersaline lagoons (e.g. Curacao, Kristensen, 1964) or highly variable salinities as in St. John's Lake. This may partly explain why Le Dantec (1955) found that the growth of *C. labrosus* was faster in the stable salinity of the reservoirs than on the coast where salinity varied from 6 to 9 g NaCl/l. Salinity may also effect growth indirectly. For example, the intestinal microflora, which has been implicated in digestive efficiency, was different in mullet of the same species captured in
fresh and sea water (Hamid et al., 1977).

(4) **Temperature regime**

The effect of temperature on the growth rate of fish is complex and its mediation is not clearly understood. Bromhall (1954) found that sea temperature does not affect growth rate of *M. cephalus* fry but the slow rates of growth of *C. labrosus* observed in South-west England or Ireland compared to regions further south may be at least partially due to temperature. Besides the effect of temperature on the environment (oxygen concentration, food supply etc.) it may also exert an indirect effect on growth via hormone release from the pituitary gland (Swift & Pickford, 1965) and the thyroid gland (Swift, 1955, 1959). Leray & Febvre (1968) investigated the thyroid activity of *L. aurata* held at 18°C and 10°C and found marked reduction in the rate of fixation of $^{131}$I at 10°C. The metabolic cost of osmotic regulation of *M. cephalus* was found to be higher at lower temperatures (Nordlie, 1976) which may explain why salinity tolerance is at least partly governed by temperature. Mires (1970) found that *L. ramada* fry suffered far greater mortality from sudden changes in temperature than salinity.

Temperature may also affect growth by influencing the length of the growing season. Thomson (1951) found that the growth of *M. cephalus* ceased when the temperature fell below 16-18°C, recommencing when the temperature rose to that level again, although in other fish species seasonal changes in growth rate have been found to be more closely correlated with day length than temperature (Hogman, 1968). There are several reports of seasonal variation in
feeding intensity and diet of mullet in which it is suggested that temperature plays a part (Albertini-Berhaut, 1973; Hickling, 1970b; De Silva & Perera, 1976). Kennedy & Fitzmaurice (1969) observed juveniles of C. labrosus and found that feeding and activity decreased markedly at < 10°C.

(5) **Other environmental effects**

Mullet appear to be generally very tolerant of adverse environmental conditions. The mullet fishery of L'Etang de Bérre survived in spite of oil pollution which severely damaged other fisheries (Ezzat, 1964). Savchuk (1967) reported the presence of young L. aurata in waters with gas regimes which would be lethal to other fish. The presence of young mullet in shallow waters where the temperature may rise above 30°C (Savchuk, 1967) and areas with a high input of organic waste (Kristensen, 1964; this study) suggest that mullet are also tolerant of extremes of oxygen concentration. Nordlie & Leffler (1975) found that young M. cephalus were good oxygen regulators maintaining the same oxygen uptake irrespective of environmental oxygen concentration between 1.5 and 5.5 ml O₂/l. Kutty & Mohammed (1975) reported that the activity of Rhinomugil corsula increased with a decrease in oxygen concentration from 3mg O₂/l to 1.6mg O₂/l. In air saturated water the fish had normal aerobic metabolism, but below air saturation there was an increase in anaerobic metabolism and protein utilisation. This would permit prolonged tolerance to low oxygen levels without incurring an oxygen debt. However, the amount of energy derived from anaerobic metabolism is less than from aerobic metabolism of the same substrate and so low oxygen concentrations in the environ-
ment are disadvantageous for growth and may be a cause of differential growth in different areas.

In view of these problems few conclusions can be drawn from a comparison of mullet growth in different parts of the world. In general it can be said that mullet do seem to be larger at a given age in coastal lagoons or reservoirs than elsewhere, larger in the Mediterranean than in Brittany, and larger in Brittany than in South-west England and Ireland. There seems to be good agreement between the results of this study and those of Hickling (1970b) for *L. ramada* and *L. aurata* and Hickling (1970b) and Kennedy & Fitzmaurice (1969) for *C. labrosus*. Some data on the growth of *M. cephalus* has been included for comparison. In general growth of *M. cephalus* does appear to be faster than for the other species and more variable at different localities. This might be expected in view of its wider geographic range, but this cannot account for all the observed differences since Thakur (1967) found that the growth of *M. cephalus* in the Mahanadi Estuary, Bay of Bengal, was similar to that reported by Thomson (1951) in Australia, but very different from the growth of the same species in Chilka Lake which is only about 50 miles from the Mahanadi Estuary.
Analysis of the length-weight relationship has two purposes: (a) the mathematical description of the length-weight relation so that weight can be predicted from length. This is particularly relevant when length is estimated from scales. (b) the measurement of variation from expected weight of fish or groups of fish as an indication of fatness or condition.

The length-weight relationship of most fish has the form:

\[ W = aL^n \quad \text{Where } W = \text{weight} \]
\[ L = \text{length} \]
\[ n = 2.5-4.0 \quad (I) \]

The value of \( n \) varies with locality, sex and state of maturity but is usually constant for fish similar in these respects. In the log form:

\[ \log W = \log a + n \log L \quad (II) \]

Thus, if log \( W \) is plotted against log \( L \) and the regression method of least squares used to fit a best straight line to the data, log \( a \) and \( n \) can be estimated.

Various condition factors have been defined, their usefulness varying according to the purpose of the analysis. Condition can be defined as the ratio of the observed weight to the expected weight as calculated from the observed length. Expected weight is calculated as that of an 'ideal' fish with length-weight relation \( W = aL^3 \). Then condition = \( W / L^3 \). However, as condition is then often an awkward decimal number, alternative condition factors may be defined as:

\[ K = W / CL^3 \quad \text{or} \quad K = C.W / L^3 \quad \text{Where } K = \text{a condition factor} \]
\[ C = \text{'constant', chosen so that } K < 1 \]
However, when calculated in this way, $K$ varies with length, because in the majority of fish the length-weight relationship does not obey the cube law. To eliminate the effect of length on condition factor, a relative condition factor is used. The length-weight relation is calculated using the log formula given above. From the estimated value of $a$ and $n$, the smoothed mean weight, $W$, can be calculated for any given length. This weight is then compared with the observed weight according to the formula $K_n = W/aL^n = W/W$ Where $K_n =$ relative condition.

Analysis of condition can also be made by using the length-weight formula itself. Provided the slope of the regression does not alter, differences in the elevation of the regressions calculated for different groups of fish reflect differences in the weight of the fish relative to its length i.e. differences in condition. In this study both direct comparisons of the length-weight relationships and the relative condition factor $K_n$ were used.

4.1. RESULTS

4.1.1. Comparison among species

The length-weight relationship was calculated separately for each species. The regression equations are given in Table 4.1. In each case $\log_e$ standard length (SL) and $\log_e$ weight (W) were highly correlated ($p < .001$) and an F ratio test showed that the variance due to the regression was highly significant ($p < .001$). The best straight lines relating $\log_e W$ and $\log_e SL$ were plotted in
Fig. 4.1. The lines appeared similar in slope but although those for *C. labrosus* and *L. ramada* are approximately coincident, that of *L. aurata* is slightly lower. This is as expected as *C. labrosus* and *L. ramada* are similar in body shape whereas *L. aurata* is markedly slimmer and more elongated. Covariance analysis was used to compare the regressions of log$_e$W on log$_e$SL (Table 4.2). The slopes of the lines for different species were significantly different (*p* < .001). The regression coefficients were tested for the significance of their deviation from 3, the expected value for the 'ideal' fish where weight increases proportionally with SL$^3$. All regression coefficients were significantly different from 3 (*C. labrosus* and *L. ramada*, *p* < .001; *L. aurata*, *p* < .05).

To compare the proportion of total weight which was gut and liver, regressions of gutted weight on weight were calculated for all fish of each species (Table 4.3). All correlation coefficients and regression coefficients were highly significant (*p* < .001). The regression lines are plotted in Fig. 4.2. Covariance analysis indicated that the small differences in slope were highly significant (*p* < .001; see Table 4.3). The slope of the regression for *L. aurata* was greater than that for either *C. labrosus* or *L. ramada*. Thus, as the fish grew, gutted weight increased faster with increase in total weight in *L. aurata*, i.e. the gut and liver tended to form a lower and increasingly lower proportion of the total weight in this species compared with *C. labrosus* and *L. ramada*.
4.1.2. **Comparison among age groups**

Separate length-weight relationships were calculated for each age group of each species. The regression equations are shown in Table 4.1. In all cases the correlation between \( \log_{e}W \) and \( \log_{e}SL \) and the variance due to the regression were highly significant (\( p < .001 \)). The best fit regression lines are plotted in Fig. 4.3. and compared using covariance analysis (Table 4.2). In all three species, the slopes of the regression lines calculated for fish of different age groups were significantly different (C. labrosus, L. aurata \( p < .001 \); L. ramada \( p < .05 \)).

Examination of the regression equations and Fig. 4.3 indicated that, with the exception of L. aurata year group II, there was a tendency for the regression lines to increase in steepness (increase in \( n \)) and decrease in elevation (decrease in \( a \)) with increase in age group. Thus, with increasing age there was a tendency for weight to increase proportional to a higher power of standard length.

4.1.3. **Seasonal variation in condition**

Separate length-weight relations were calculated for all fish of the same species and age group captured in the same month. The regression equations are shown in Tables 4.4 and 4.5. In all cases the correlation between \( \log_{e}SL \) and \( \log_{e}W \) was significant and the variance due to the regression was significantly greater than the residual variance. Covariance analysis was used to compare the regressions. The results are summarised in Tables 4.6 and 4.7.

In spite of the fact that in most cases the differences in the slopes of the regressions of \( \log_{e}W \) on \( \log_{e}SL \) for each month
were not significant, they were sufficient to affect the intercepts of the lines of the y axis. Thus the elevation of the \( \log_e W - \log_e SL \) regressions did not, in all cases, provide a satisfactory measure of condition and it was necessary to calculate relative condition as well. Relative condition, \( K_n \), was calculated for each fish using the length-weight relationship appropriate to the species and age group. Means for each month were computed for each age group of each species (Table 4.8) and plotted in Figs. 4.4 and 4.5. Analyses of variance were carried out on the relative condition values of each age group within species to resolve the total variance into variance due to month of the year and residual variance (Table 4.9).

(a) *C. labrosus*

Age group 0:

The slopes of the regressions of \( \log W \) on \( \log_e SL \) were significantly different in different months of the year (\( p < .001 \)), with that of July being particularly low. Thus the 'a' values (equation II) could not be used as a measure of condition. The relative condition of the fish rose markedly between July and August and the variance due to month of the year was highly significant (\( p < .001 \)).

Age group 1:

The regression lines calculated for fish captured in different months were not significantly different in slope but highly significantly different in elevation (\( p < .001 \)). Elevation increased from January to June indicating an increase in condition but declined in July. Relative condition showed the same trend and the
same decrease in July. Earlier in the year results were confused by the fact that the condition of fish caught in January 1976 was significantly higher than that of those caught at the same time of the year in 1975 ($p < .001$).

Age group II:

The regression lines calculated for fish captured in different months were significantly different in slope. Examination of the regression equations showed that the regression calculated for fish caught in February had a slope very different from that of other months. The reason for this is unclear. When this month was excluded from the covariance analysis, the regression lines were not significantly different in slope but were highly significantly different in elevation ($p < .001$). The elevation tended to increase from a minimum in January to a maximum in July. However, examination of the slopes and the 'a' values (equation II) showed that although the differences in slope were not significant, slight differences in slope had a relatively large effect on the 'a' value masking differences in the condition of the fish. Thus the relative condition factor was more suitable for comparing condition in different months. Relative condition showed a fairly steady increase from January to June, a slight decline in July, and an increase in August. The variance of relative condition due to the month of the year was highly significant ($p < .001$).

Age group III

There was no significant difference in the slope or elevation of the regression lines for different months suggesting
that no significant alteration in shape occurred from April to July. Relative condition appeared to increase from April to June, and decrease in July. The variance of relative condition due to month of capture was marginally significant ($p \approx .05$).

(b) *L. aurata*

Age group I:

The $\log_e W - \log_e SL$ regression lines for fish captured in different months were not significantly different in slope but highly significantly different in elevation ($p \ll .001$). The elevation of the $\log_e W - \log_e SL$ regression line increased from February to June indicating that the fish were becoming heavier for a given length over this period. Relative condition was similar in February and March but increased from April to June. The variance of relative condition due to month of the year was significant ($p \approx .01$).

Age group II:

The regression lines for fish captured in different months were not significantly different in slope but highly significantly different in elevation ($p \ll .001$). However, the elevation does not increase with month of the year as might be expected and it is evident that here, as in the case of *C. labrosus* year group II, the differences in the slopes of the regression lines from month to month, though not significant, were sufficient to affect the intercept. Thus the elevation of the $\log_e$ weight on $\log_e$ standard length regressions did not provide a satisfactory measure of condition. Relative condition increased progressively from
March to June with a slight decline in July. The variance of relative condition due to month of the year was highly significant ($p \ll .001$).

**L. ramada**

Although there was some evidence to suggest an increase in condition from January to June, and a decrease from June to July in year group I, too few fish were captured in too few months of the year for a seasonal pattern to be identified.

In almost all age groups there was an increase in condition of the fish from January-February to June. In *C. labrosus* age group I, II, and III and in *L. aurata* age group II there was a slight reduction in condition in July.

### 4.1.4. The effect of seasonal variation in gut weight

In almost every age group examined, for a given length fish became heavier from January-February to June. However, over this period feeding intensity might be expected to increase, increasing the weight of food in the gut, and hence increasing the estimate of condition. The relationship between the gutted weight and the total weight of the fish in different months of the year was, therefore, examined for each age group of *C. labrosus* and *L. aurata*.

The regression of gutted weight on weight was calculated for each age group and species caught in each month. The regression equations are shown in Table 4.10 and 4.11. In all months gutted weight was highly correlated with total weight ($p \ll .001$).
and the variance due to the regression was significantly greater than the variance due to deviations from the regression ($p < .001$). Covariance analysis was used to compare the regressions for each age group in different months of the year. In all age groups the regressions of the different months were found to have significantly different slopes, i.e. gutted weight was increasing as a different proportion of total weight (Tables 4.12, 4.13). However, closer examination of the regression equations indicated that the regression coefficient did not vary in any consistent way with month of the year. In *C. labrosus*, the elevation of the line appeared to increase with age group, i.e. gutted weight was proportionally greater in older age groups. However, with the exception of *C. labrosus* age group III and *L. aurata* age group II, there was no indication that the elevation of the regression lines varied consistently with month of the year. In these two age groups elevation of the lines increased from April to June-July, i.e. gutted weight increased relative to total weight. Any increase in condition was, therefore, due to a genuine increase in weight of the fish rather than an increase in the weight of the gut caused by higher feeding intensity in the summer months.

4.1.5. **Comparison of condition at different localities**

$\log_{e}W - \log_{e}SL$ regressions were calculated for the *C. labrosus* and *L. aurata* captured at each site (Table 4.14). Best fit regression lines are plotted in Fig. 4.6. The results of covariance analysis to compare regressions is summarised in Tables
4.17 and 4.18. The regressions calculated for *C. labrosus* captured at St. John's Lake, R. Lynher and R. Tamar were marginally significantly different (*p* < .05) in slope. The regressions calculated for *L. aurata* caught in the R. Yealm, R. Lynher and St. John's Lake were not significantly different in slope but were significantly different in elevation. Examination of the lines showed that the slight differences in their slope caused them to cross over, and so differences in the elevation could not be used to compare condition of fish at the different sites. In general, the regression of loge*W* on loge*SL* calculated for fish of the same species captured at different sites was very similar. However, the elevation of this regression had already been shown to vary significantly with month of the year & age group, and so by combining all fish of the same species from one site, irrespective of the month of capture, real differences in the condition of fish captured at different sites may be masked.

Therefore, loge*W* - loge*SL* regressions were calculated for fish from different sites in months where fish of the same species and age group had been captured at more than one site. The regression equations are given in Tables 4.15 and 4.16. With the exception of *L. aurata* group II, R. Tamar, all regression coefficients were significant and the regressions are plotted in Fig. 4.7. The results of comparisons of regressions by covariance analysis are summarised in Tables 4.17, and 4.18. Except in the case of *O* group *C. labrosus* captured in August, regressions calculated for fish from different sites did not differ significantly in slope. For *L. aurata* group II in June and *C. labrosus* group II in June
there was also no significant difference in elevation suggesting that the condition of these fish from different sites was similar. However, in other groups differences in elevation were significant. For _L. aurata_ group I the difference in the elevation of the regression lines calculated for fish from different sites was only marginally significant and the difference in slope meant that the elevation was not a reliable index of condition. For _C. labrosus_ group III in June, and _C. labrosus_ group II and III in July, the differences in elevation were highly significant (p < .01) and in each case, over the range of length and weight appropriate to the age group, fish from St. John's Lake were slightly lighter at a given standard length than fish from either the R. Lynher or R. Tamar. However, this was not necessarily apparent from a comparison of the 'a' values due to slight, although non significant, differences in 'n' values (equation II). Relative condition was, therefore, calculated for groups of fish of the same species and age group captured at different sites in the same month. Mean and standard deviation of relative condition and weight of the fish were calculated. Analysis of variance was used to assess the significance of the variance of both weight and relative condition attributable to the site of capture (Table 4.19.).

For _L. aurata_ age group I captured in June, and _L. aurata_ age group II captured in June, fish from different sites were not significantly different in weight or relative condition. However, the weight and relative condition of _C. labrosus_ varied significantly with the site of capture, with the exception of _C. labrosus_ group II caught in July when the fish from different sites were not significantly different in weight. In general, fish with high relative
condition tended to be from sites where the fish were also heaviest. With the exception of the 0 group, fish from St. John's Lake had a lower mean relative condition than fish from either the R. Lynher or R. Tamar, where the relative condition of the fish appeared to be approximately equal. Among the 0 group fish captured in August, both weight and condition were markedly higher in St. John's Lake than in either the R. Lynher or R. Tamar. The R. Yealm was only sampled in June but on that occasion it was notable that for *L. aurata* groups I and II and *C. labrosus* group II, while the weight of the fish was similar, smaller or larger, their relative condition was consistently lower than that of fish of the same species and age group captured in the same month at other sites.

4.1.6. Fat deposition

The quantity of fat in the body cavity was judged visually according to the scale described in section 1.2. For each group of fish (fish of the same species and age group caught at the same location on the same day), the number of fish on each point of the arbitrary fat scale was expressed as a percentage of the total number of fish in the group. This data is given in Tables 4.20 and 4.21.

The number of *C. labrosus* containing fat, and the amount of fat in the fish as judged on the fat scale, increased from April to July-August in age groups I, II and III. Age group 0 fish contained no visible fat when they arrived in the estuary in July. In August they still contained no visible fat in the body cavity.
but in October, December and January a fairly high level of fat was maintained in the body. This declined rapidly from February to April.

The limited data available for *L. ramada* suggested that the pattern of fat deposition in this species was similar to that in *C. labrosus*, but *L. aurata* appeared to be less fatty than the other two species. The amount of fat in *L. aurata* examined never exceeded scale 1.

4.2. **DISCUSSION**

4.2.1. **Comparison of condition among species**

Overall length-weight relationships for the three species were (Table 4.1):

- *C. labrosus*: $W = SL^{3.1067} \times 0.0154$
- *L. aurata*: $W = SL^{3.0244} \times 0.0147$
- *L. ramada*: $W = SL^{3.2493} \times 0.0106$

The exponents were significantly different from each other and significantly different from 3.

Thong (1969) reported no significant differences in slope or elevation of the log$_e$ weight - log$_e$ length plots of the same three species from the coast of Brittany. The exponent varied from 2.85 to 3.20, and the constant or 'a' value varied from $3.52 \times 10^{-6}$ to $25.09 \times 10^{-6}$. Taking into account that Thong (1969) used mm rather than cm, the 'a' values were still rather lower than those obtained in this study because total length rather than standard length was used, and possibly because Thong's calculations included fish up to 40 cm in length. In the Lac de Tunis *C. labrosus* was heavier than
L. ramada of the same length (Farrugio and Quignard, 1974) which contrasts with this study where the length-weight relationships of juvenile C. labrosus and L. ramada were approximately similar. Ezzat (1964) calculated the length-weight relationship of L. aurata varying in length from 160 to 350mm, and found that the exponent was lower than that of M. cephalus in the same region. Similarly in this work it was found that the exponent for L. aurata was lower than that for the other species. The relative increase of weight with length would be expected to be lower in a slimmer fish but Hickling (1970b) found that the exponent of the length-weight relationship for L. aurata was greater than that for either L. ramada or C. labrosus. This may be due to the difference in the size range of fish (23 - 60cm) or to the fact that gutted weight rather than total weight was used, implying that increase in gut and liver weight forms a greater proportion of the increase in weight with length in L. ramada and C. labrosus than in L. aurata.

In view of this, the relationship between total weight and gutted weight was investigated further. Gutted weight rather than gut weight was used as this could be measured more accurately, particularly in very small fish. The gut frequently broke as it was removed and spillage of gut contents occurred. Also, the liver was always removed as part of it frequently came away with the gut anyway. Although the difference in the slope of the regressions of gutted weight on weight were not large they were statistically significant. The gutted weight of L. aurata increased faster with increase in total weight than in the other species, suggesting that the gut and liver of L. aurata formed an increasingly lower
proportion of the total weight in this species. This may explain why Hickling (1970b), in contrast to this and other studies, found that the exponent of the length-weight relationship was greater for *L. aurata* than for other species.

Arné (1938) reported differences in the shape of *C. labrosus, L. ramada & L. aurata* by expressing total length in terms of the height of the body. This was 4.6 - 5.0 for *C. labrosus*, 5.0 - 5.3 for *L. ramada* and 5.2 - 5.6 for *L. aurata*, suggesting that *L. aurata* was relatively longer for a given depth of body than *L. ramada* which was relatively longer than *C. labrosus*.

4.2.2. Comparison of condition among age groups

Ezzat (1964) suggested that not separating the fish according to age was the reason why the observed weights of fish of a given length often deviated considerably from those calculated from the log$_e$W - log$_e$SL regression. Le Cren (1951), in a detailed study of the length-weight relationship of perch, found that no single regression would adequately describe the relationship for all age groups. Therefore, in this study, separate log$_e$W - log$_e$SL regressions were calculated for each age group of each species. Within species the difference in the slopes of regressions for different age groups was highly significant. With one exception (*L. aurata*, group II) the slope tended to increase with age group indicating that in older age groups weight increased faster relative to standard length. This is consistent with the observations of Kennedy & Fitzmaurice (1969) that juvenile grey mullet were slimmer than the adults. Thong (1969) also found a tendency for condition to
increase with size, i.e. for fish to become relatively heavier for their length, in grey mullet from the Brittany coast. However, this does not appear to be the case for all species of grey mullet. Sarojini (1957) found that there was no significant difference between the regressions of log \( W \) on log \( SL \) for adult female, adult male, and 0 group \( M. \) parsi, and Thomson (1951) reported that when two year classes of \( M. \) cephalus were sampled simultaneously at the same site in Western Australia, the older group of fish had a lower mean condition factor.

4.2.3. Seasonal variation in condition

The standard deviations of mean relative condition were high. Several authors have commented on the variability of the individual condition factor in mullet (Thomson, 1951; Thong, 1969). Kennedy & Fitzmaurice (1969) state that few fish vary so much in build as mullet. In spite of this the variance of relative condition due to month of capture was significantly greater than the residual variance.

In both \( C. \) labrosus and \( L. \) aurata the increase in condition from a low value in January to a high value in June was as expected. The amount of fat surrounding the gut increased markedly in June suggesting that some of the increase in condition was due to the laying down of fat reserves (Tables 4.20, 4.21.). In 0 group fish fat deposition appeared to be delayed until later in the year. In \( L. \) aurata the increase in fat around the gut was not as marked as in other species. This may reflect a real difference in the amount of fat stored, or it may be due to a species difference
in the location of fat storage.

Amongst all age groups of both species condition tended to reach a maximum value in June, with the exception of 0 group C. labrosus where condition and slope of the regression line relating $\log_e W$ and $\log_e SL$ increased considerably from July, when the fish had just arrived, to August when they had been in the estuaries for a few weeks. This was possibly associated with the change in environment and diet which occurred at the home of migration from coastal waters to the estuary. Both Yashouv & Ben Shachar (1967) and Suzuki (1965) found that weight increased more rapidly with length on a mixed diet than on a carnivorous diet, although Suzuki’s results were not statistically significant.

In all other age groups there was a decline in condition in July. This may be attributable to an out of phase growth of weight and length-growth in weight occurring more rapidly in June followed by a proportional increase in length in July. Brown (1946 b) reported cycles in the growth of weight and length of trout consisting of approximately 2 weeks fast and 2 weeks slow growth. Fluctuations in length and weight increase alternated, resulting in fluctuations of the condition factor. Such cycles would be best investigated by frequent measurement of the growth of individual fish but in the study described in this thesis mean growth curves of C. labrosus, L. ramada & L. aurata indicated that growth in length and weight occurred approximately simultaneously. The July decline in relative condition may be a reflection of an early and late summer blooming of diatoms which Hickling (1970b), this study and others suggest form an important part of the diet.
However, one might expect such an interruption of food supply to be reflected in the growth curves, which does not appear to be the case. Le Cren (1951) in an analysis of the seasonal variation in condition of the perch also reported a decline in relative condition of the immature fish in July. This he attributed to a temporary food shortage caused by a decline in zooplankton in this month. The subsequent increase in relative condition of juvenile mullet in August is probably due to the deposition of fat.

Most authors have examined seasonal variation in the condition of adult mullet correlated with gonad development. Thomson (1951) found that condition of immature \textit{M. cephalus} from Western Australia fell slightly in the winter, possibly due to scarcity of food.

In South-west England Hickling (1970b) reported an increase in feeding intensity of mullet in the summer months. Thus, at least some of the seasonal variation in relative condition may have been due to the weight of food in the stomach and intestine, and so the regressions of gutted weight on total weight were calculated for each month for each age group and species, and the months were compared. There was no indication that the elevation of the regressions declined in the summer as might be expected if the gut was becoming progressively heavier relative to the body weight. In fact in two groups (\textit{C. labrosus} group III and \textit{L. aurata} group II) the reverse appeared to be true. The observed increase in condition could not, therefore, be attributed to an increase in the weight of the gut contents.
Le Cren (1951) examined the seasonal variation in the ratio of stomach contents : body weight in perch and concluded that seasonal changes in the weight of the stomach contents were not important. Sarojini (1957) reported that seasonal variation in weight of viscera did not exert a marked effect on condition in M. parsia. Hickling (1970b) used gutted weight in length-weight calculations in order to examine condition unaffected by gonads or gut contents. In the work described in this thesis total weight was preferred, firstly because it seemed logical that fat laid down around the gut, sometimes almost covering it, be taken into account, and secondly to enable comparison with live fish in experimental work.

The I group C. labrosus were sampled at St. John's Lake in January in successive years. In 1976 the fish were longer and heavier and had a higher mean relative condition than in 1975. This difference was highly significant ($p \leq .001$). Several authors have reported that the condition of mullet captured in one month varies from year to year (Thomson, 1951; Wimpenny, 1955). Le Cren (1951) also noted differences in the condition of perch from year to year in L. Windermere. This has generally been attributed to variations in weather, temperature, food supply etc., although in St. John's Lake other factors, such as competition and age of the fish may also have been important (Chapter 3).

4.2.4. Variation of condition with locality

Condition has already been shown to vary with month of the year and age group and so comparisons of all fish would be influenced
by the time of year that the sites were sampled. Comparisons were, therefore, limited to fish of the same species and age group caught at different sites in the same month. The elevation of the $\log_e W - \log_e SL$ regression line, and the mean relative condition of fish from St. John's Lake tended to be lower than those of fish from the R. Tamar and R. Lynher. A notable exception was $C. \text{l}ab\text{rosus}$ O group in August, when fish from the R. Lynher had a markedly lower relative condition and the slope of the $\log_e W - \log_e SL$ regression was steeper for fish from the R. Tamar and St. John's Lake than from R. Lynher. This may have been related to diet since Suzuki (1965) and Yashouv & Ben-Shachar (1967) found that the slope of the $\log W - \log SL$ regression for young mullet fed on a mixed diet was steeper than for those fed on an animal diet. A relatively large proportion of animal matter was observed in the stomachs of the O group $C. \text{l}ab\text{rosus}$ from the R. Lynher in August, whereas in the same month the stomachs of O group $C. \text{l}ab\text{rosus}$ from St. John's Lake contained mostly mud. Both weight and relative condition of O group $C. \text{l}ab\text{rosus}$ were higher in St. John's Lake than in the R. Lynher and R. Tamar. This may indicate that St. John's Lake provided a genuinely more favourable environment for O group fish, or it may be that due to the long drawn out spawning season, the fish captured in St. John's Lake were older, and had arrived in the estuary rather earlier, than the school of fish which were captured in the R. Lynher.

In the June sample from the R. Yealm mean relative condition of each group of fish was considerably lower than that of fish of equivalent species and age group captured at other sites in
the same month, although the difference was not statistically significant in all cases. Thomson (1951) suggested that the weight-length relationship of *M. cephalus* altered with salinity, with osmotic forces tending to cause an increase in condition at low salinities and a decrease at high salinities. The R. Yealm was fished at high water when salinity approached 33°/oo whereas St. John's Lake was sampled at low water when salinity was very low, approaching 0°/oo. However this explanation is not consistent with the lower condition of fish older than 0 group in St. John's Lake compared with R. Lynher and R. Tamar, both of which were fished approaching high water. If feeding is governed by tide, then the fact that St. John's Lake was fished at a different state of the tide from the R. Lynher & R. Tamar may have had some effect on the estimates of relative condition. Feeding cycles are discussed in more detail in Chapter 5 (Section 5.4.). It is possible that the older fish in St. John's Lake were, perhaps genetically, the 'runts' of their age group, and that the 'typical' older fish moved out into the rivers. However, although estimates of skewness tended to be positive, length distributions of older age groups were not significantly different from normal suggesting that migration was not an important factor.

Farrugio (1975) found that the condition of Tunisian grey mullet was dependant on environmental conditions, particularly primary productivity. Although Thong (1969) and Rangaswamy (1976) found no significant differences in grey mullet condition at different localities, other authors have reported to the contrary. Le Dantec (1955) found that the weight : length ratio for fish from
coastal reservoirs was greater than that of fish from the Gulf of Gascony. Ranganathan & Natarajan (1969) reported that the condition of *M. corsula* in one coastal lagoon in the Bay of Bengal was consistently higher than in another. They attributed this to its higher salinity, silicate content and primary productivity.

From this work it appears that while St. John's Lake may provide a favourable environment for very young mullet, older age groups have a higher relative condition in a given month in the R. Lynher and R. Tamar, which appears to be a genuine reflection of disadvantageous conditions and stress in St. John's Lake. The retention of a shallow pool behind the ford at St. John's Lake, its high temperatures and the sewage input from a local village, would be expected to stimulate primary productivity; but the extremes of temperature and salinity and the high oxygen demand of the sewage apparently combine to make the site less favourable for the condition and growth of older fish.
5.1. Feeding Behaviour

When the mud flats were exposed at low water the two-grooved scrape marks characteristic of mullet (Thong, 1969) were readily visible, particularly in the summer when a dark brown scum covered the mud surface. Some small mullet (3-5cm) were maintained in the laboratory in a perspex tank with a layer of mud at the bottom. They were regularly observed feeding at the mud surface and the same parallel grooved scrape marks could be seen on the sides of the tank as the young mullet scraped at the algae layer.

When stomachs were examined, most, irrespective of mullet size and species, were filled with unidentifiable organic matter and a large proportion of sand and mud particles. A few harpacticoid copepods and ostracods and some diatoms (genus Nitzschia) were more or less entire. The diet was consistent with that reported for these species (Hickling, 1970b; Thong, 1969) and other species (Thomson, 1954b; Sarojini, 1954; Pillay, 1953)

The exception to this was a sample of 0 group C. labrosus captured in R. Lynher in August, 1975. Their mean length was 2.0 cm, and about half the stomachs contained predominantly material of animal origin. Distortion and fragmentation made identification very difficult, but amphipod and insect larvae were among the prey. Nearly all the stomachs contained some mud, and some contained only mud, suggesting a mixed diet.
In larger fish the predominance of animal food was not noted, but in a food preference experiment individuals 3.7 - 4.7 cm in length, which had been feeding on diatoms and detritus in St. John's Lake, showed a marked preference for live Artemia nauplii over their normal diet (Section 5.2.). Yashouv & Ben-Shachar (1967) conducted food preference experiments with *M. cephalus* and *L. ramada* fry. After they had been feeding in the same tank for a fixed period stomachs of *M. cephalus* were found to contain cladocerans, ostracods (present in mud layer), copepods, diatoms, and relatively large amounts of sand particles and unidentifiable organic material. Whereas those of *L. ramada* contained only chironomid larvae and rotifers, and no sand particles at all. Yashouv & Ben-Shachar (1967) also reported *L. ramada* 8-12 cm long feeding on chironomid larvae, and in South Carolina adult *M. cephalus* were observed feeding on swarming polychaetes (Bishop and Miglarese, 1978). This suggests that feeding behaviour differs with species and that the change to a benthic herbivorous feeding habit may occur partly because animal food is not readily available.

The change from a predominantly carnivorous diet to a benthic herbivorous diet in juvenile mullet has been observed by most authors although the size given for the change varies from 16-20 mm for *Liza subviridis* in S. African estuaries (Chan & Chua, 1979), 23 mm for *M. cephalus* in Japan (Suzuki, 1965), and 25 mm for *M. parsia* and *M. spiegleri* in India (Sarojini, 1954) to 50 mm for *M. aurata*, *M. capito* and *M. saliens* in the Mediterranean (Albertini-Berhaut, 1973). It seems likely that the dietary change occurs gradually as De Silva & Perera (1976) reported an increase in the
proportion of sand in the stomach of *M. cephalus* with increase in length from 25-55mm. The change in diet of *C. labrosus* in South-west England appeared to occur over the smaller size range of 20-30mm.

5.2. **A FOOD PREFERENCE EXPERIMENT**

A food preference experiment was carried out in the laboratory on 13 group I *C. labrosus*, 3.7-4.7cm in standard length. They were placed in a tank of 50% sea water with a bottom layer of surface mud collected from St. John's Lake. 1-2 day old *Artemia* nauplii larvae and 20-30 white worms were added to the tank. After 1 hour 3 of the fish were removed and killed by overanaesthetisation. The remaining fish were transferred to a tank containing 50% sea water with no food added. After 6 and 12 hours, 3 fish, and after 14 and 72 hours, 2 fish, were killed in the same way. After 2, 4, 6, 8, 10, 12, 24, and 72 hours faeces were collected and preserved in formal saline. The fish were starved for 4 days prior to the experiment. The temperature of the water varied between 11 and 14°C during the course of the experiment.

After 1 hour all three stomachs were full. The stomachs of 2 fish contained *Artemia* nauplii only, whilst the third contained 3 white worms as well as numerous nauplii. In later samples the stomachs were empty except for a small amount of mucus. No mud was ingested in spite of the fact that stomachs of similar sized fish from St. John's Lake contained mud only. It appears that when animal food is available young *C. labrosus* prefer it to their more normal diet of mud and diatoms, although it is possible that
the mud layer had not been allowed sufficient time to develop its natural flora in this experiment.

The intestines were empty at 1 hour, contained 5-6 food pellets at 6 hours, 0, 1, or 2 pellets at 12 and 24 hours and were empty at 72 hours. The quantity of faeces declined rapidly after 6 hours, but some faeces could still be collected at 72 hours.

No recognizable remains were found in the faeces or the pellets in the intestine. The faeces collected at 2 hours contained some reddish pigment. The faeces collected at 24 and 72 hours, when stomachs and intestines were mostly empty, suggested that the fish were ingesting faeces or obtaining some food from the tank or the sea water.

When live Artemia nauplii were ingested at 10-14°C by C. labrosus of 3.7 - 4.7cm standard length, the stomach was evacuated within 6 hours and the entire gut was largely evacuated after 24 hours and totally evacuated after 72 hours. 1-2 day old Artemia nauplii appeared to be almost totally digested.

5.3. SEASONAL VARIATION IN FEEDING INTENSITY

For each fish captured the fullness of the stomach and intestine were assessed separately on a visual arbitrary scale of:
empty - ¼ full - ½ full - ¾ full - full

For each group (fish caught at the same site and time and which were of the same species and age group) the percentages of fish within each of the above categories were determined. The data are presented in histogram form in Fig. 5.1. The fullness of stomachs and intest-
ines was very variable as was also noted by Hickling (1970b) and feeding and non-feeding individuals were captured in most months from January to August. The fullness of the intestine did not always reflect the fullness of the stomach. For example, in June the proportion of empty stomachs was very high although the fullness of the intestines showed that the fish had been feeding. This may have been due to a particularly rapid rate of gastric evacuation, or to regurgitation during the trauma of capture, although this was never observed and is not recorded in mullet by other authors. There was a marked similarity between the fullness of stomachs and intestines of all groups of fish captured on the same occasion, irrespective of age group and species. This suggested that, if feeding cycles existed, they were similar for all species and age groups, and depended on some external factor such as tide or time of day.

In some samples, e.g. *L. aurata* group II March and *C. labrosus* group 0 July, there was an obvious preference for allocating stomachs to categories empty, \( \frac{1}{4} \) full and full, rather than \( \frac{1}{2} \) full or \( \frac{3}{4} \) full. This reflects the disadvantages of an arbitrary, subjective scale. A vacuity index was calculated (Albertini-Berhaut, 1973):

\[
\text{Stomach vacuity index} = \frac{\text{no. of stomachs empty}}{\text{no. of stomachs examined}} \times 100\%
\]

As there was some indication that regurgitation of stomach contents might have occurred, an intestine vacuity coefficient was also calculated.

Feeding intensity of mullet has been expressed by a variety
of indices. De Silva & Wijeyaratne (1976) defined the feeding intensity by a number of food organisms per feeding individual, but the majority of authors have used a subjective arbitrary scale of fullness of stomach similar to that used in this study (Sarojini, 1954; Hickling, 1970b; Pillay, 1953). Hickling (1970b) and Sarojini (1954) estimated feeding intensity from the number of stomachs 'full', and 'gorged, full and $\frac{1}{3}$ full' respectively. The vacuity coefficient, as used by Albertini-Berhaut (1973) was preferred because it was felt that less subjectivity was involved in allocating stomachs to the 'empty' category. In a later experiment when feeding was assessed by variations in the weight of the stomach and intestine and contents, gut weight as a % of total body weight was found to have a significant positive correlation with % stomachs containing food and % intestines full and $\frac{1}{3}$ full, and a significant negative correlation with the stomach and the intestine vacuity coefficient.

The stomach and intestine vacuity coefficients, calculated for each group of fish, are plotted in Fig. 5.2 and 5.3. A marked feature was the similarity of the vacuity coefficients for groups of fish of different species and age groups captured on the same occasion, and the great variation of vacuity coefficients of samples captured within the same month. For example, in June stomach vacuity coefficients for $C. \text{labrosus}$ of 5 and 100% were recorded.

Due to the wide variability, comparisons among species and age groups were difficult. All $C. \text{labrosus}$ samples captured in the same month were pooled, irrespective of age group, and a stomach vacuity coefficient for the month was calculated. As com-
paratively few *L. aurata* and *L. ramada* had been caught these were combined, and the pooled monthly stomach vacuity coefficient calculated. An overall monthly stomach vacuity coefficient for all species was also calculated. Similar calculations were made for the intestine vacuity coefficient (Fig. 5.4).

In all species a marked decline in stomach vacuity coefficient i.e. an increase in feeding intensity, was apparent from April to July - August, and from April to June - July for the intestine vacuity coefficient. Hickling (1970b) found that the proportion of *C. labrosus* with 'full' stomachs in estuaries of South-west England was highest in the late summer and autumn, and De Silva & Wijeyaratne (1976) found that feeding intensity of *M. cephalus* in Sri Lanka increased from February to a maximum in August. Albertini-Berhaut (1973) working on mullet fry and fingerlings in the Gulf of Marseille similarly found that the vacuity coefficient was a minimum in summer. A seasonal variation in feeding intensity seems to be a common feature of grey mullet, noted in *C. labrosus* in Ireland (Kennedy & Fitzmaurice, 1969); in various species in the Mediterranean (Albertini-Berhaut, 1973); *M. cephalus* in Sri Lanka (De Silva & Perera, 1976); *M. parisa* (Sarojini, 1954) and *M. cephalus* (Kuthalingham, 1966) in India. However, the degree to which feeding intensity varies seasonally may differ. Albertini-Berhaut (1973) found that the vacuity coefficient of juvenile grey mullet in the Mediterranean varied from 8.33% in summer to 44.16% in winter, whereas the vacuity coefficient of *M. tade* from Indian waters was zero all the year, with the percentage of stomachs gorged or full varying seasonally from 25%
to 100% (Pillay, 1953). Kennedy & Fitzmaurice (1969) reported a partial winter fast with no trace of food in juvenile or adult *C. labrosus* in March. In South-west England feeding individuals were found in all months and there was no evidence of a winter fast, at least amongst juveniles. De Silva & Wijeyaratne (1976) suggested that variation in feeding intensity was a reflection of food availability, which may partly explain differences in the seasonal variation of feeding intensity in different parts of the world.

The intestine vacuity coefficient tended to decline earlier in the year than the stomach vacuity coefficient. From June to August the stomach vacuity coefficient decreased whereas the intestine vacuity coefficient remained approximately constant, even rising slightly in August. This may have been due to a faster rate of digestion, and hence intestine evacuation, due to rise in temperature and an increase in the activity of digestive enzymes (Jobling & Spencer-Davies, 1979). A marked increase in the activity of pepsin, trypsin, and lipase activity to coincide with the period of maximum feeding intensity in turbot, pike perch, and bream was observed by Ananichev (1959) and enzyme activity in roach & rudd varies with season and diet. (Hofer, 1979 a,b, Niederholzer & Hofer, 1979). However, other factors besides temperature affect rate of gastric evacuation, such as meal size and meal type (Elliott, 1972; Fange & Grove, 1979). Thus, the rate of gastric evacuation of juvenile grey mullet might be expected to vary since fish feed to satisfy a calorie requirement (Rozin & Mayer (1961)), and the calorific content of estuarine mud.
is reported to vary widely (Sarojini, 1957; Anderson, 1939). All factors which affect gastric and entire gut evacuation influence estimates of feeding intensity when these are made from the fullness of stomachs and intestines.

In view of the wide variability described between samples captured in the same month, it was obviously unsatisfactory that, for example, in April all fish were caught on the same occasion at one site. More extensive sampling was necessary. However, the low stomach vacuity coefficient of group \( \text{I } \) \( \text{C. labrosus} \) in January, February & March was found in three separate samples from the same locality and the March sample of group \( \text{II } \) \( \text{L. aurata} \) consisted of 80 fish, and so these unexpectedly low values cannot be lightly dismissed. It may be that a minority of fish were actively feeding in these months, but since they moved into the shallower mud flats to feed, were more likely to be captured. It is also possible that the low calorific content of the mud during the winter months stimulated the ingestion of larger amounts (Rozin & Mayer, 1961). The increase in vacuity coefficient in April before the decrease in the summer months may have been the result of the number of empty stomachs being influenced by two opposite effects:

(i) Increase in temperature tending to increase rate of gastric evacuation, i.e. tending to decrease fullness of stomachs

(ii) Increase in feeding intensity tending to increase fullness of stomachs.
Thus, the increase in stomach vacuity coefficient from March to April may have been due to the rise in temperature increasing rate of gastric evacuation whereas by June, despite the higher rate of gastric evacuation, the increase in feeding intensity was sufficient to cause a reduction in the proportion of empty stomachs.

This is speculative, and much more detailed sampling would be required to clarify the seasonal pattern of feeding intensity. The low vacuity coefficient in March was consistent with observations of Le Dantec (1955) on *C. labrosus* in the lagoons at Arcachon, but Hickling (1970b) found that the percentage of *C. labrosus* from South-west England with empty cardiac stomachs but over three month periods was 66% for January - March and 60% for April - June, and did not decrease to 32% until July - September. It is very difficult to assess whether this is due to real differences in feeding intensity between sites or age groups studied, or merely a reflection of the variability of feeding intensity. Other authors do not report a decrease in apparent feeding intensity before the increase in the summer, but most estimate feeding intensity over three month periods when such variations may be overlooked.

Examination of individual samples suggested that the feeding intensity of *C. labrosus* and *L. aurata* + *L. ramada* were similar. Pooled stomach and intestine vacuity coefficients indicated that values for *L. aurata* + *L. ramada* tended to be greater in April and May and lower in July than for *C. labrosus* but the differences between them were small. Similarly Albertini-Berhaut
(1973) found that there was very little difference between the vacuity coefficients of *L. ramada, L. aurata* and *M. saliens.*

Vacuity index was plotted with age group indicated in Fig. 5.2 and 5.3. As very little variation among species had been noted, all species were combined and an overall monthly stomach and intestine vacuity coefficient for each age group was calculated (Fig. 5.5.). It is difficult to assess the validity of observed seasonal variation of vacuity coefficients within each age group. From June to August relatively large numbers of fish were captured from a variety of sites, but from January to May fewer fish were captured, so that each point may represent a single group of fish. For example, in March, group II fish were represented by a single sample of *L. aurata* from the R. Lynher. The intestine vacuity coefficients were similar for groups I, II and III fish. However, the stomach vacuity coefficient of group III fish declined between April and May, earlier in the year than in other age groups, and later increased between June and July. Although O group fish were only captured in July and August, low vacuity coefficients were consistent with those observed for other age groups in these months. In April and May group I fish had higher vacuity coefficients than other age groups. This may have been due to feeding intensity increasing later in the year in younger fish (which was not consistent with the observations of Kennedy & Fitzmaurice, (1969) that the growth of older fish commenced later in the year than that of younger fish) or it may be a reflection of the relatively faster digestion rates of smaller fish (Fange & Grove, 1979).
The vacuity indices were also plotted with site of capture indicated in Fig. 5.2 & 5.3. The stomach vacuity index did not appear to vary consistently among sites. It was not valid to pool samples from the same site because of the irregularity of sampling at the four sites and the seasonal variation in vacuity coefficient.

Reports of other authors suggest that there may be some differences between sites in feeding intensity and its seasonal variation. In the Indian mullet there appears to be a lower feeding intensity in brackish water and estuaries in comparison to just offshore (Kuthalingham, 1966; Sarojini, 1957; Pillay, 1953) found that the months of maximum feeding intensity were different in coastal, estuarine, and freshwater localities. Kennedy & Fitzmaurice (1969) reported a partial winter fast in *C. labrosus* in Ireland which has not been observed in the same species in South-west England (Hickling, 1970b; this study).

Locality differences in environmental factors may affect feeding intensity via composition of the diet, but in addition, factors such as salinity, temperature and fat content of the diet, may also have a direct effect on apparent feeding intensity via gastric evacuation rates (Perera & De Silva, 1978). Comparisons among the findings of different authors working in different regions are difficult because variation with locality cannot be readily separated from variation due to species, age group, method of estimating feeding intensity, time of day and state of tide during sampling, current and preceding weather conditions etc.
5.4. FEEDING CYCLES

5.4.1. Methods

The first attempt to examine the feeding of mullet over a period of time was made on 10th March, 1977. At one site in the R. Lynher, one seine haul was made at approximately 45 minute intervals from low to high water. The captured fish were immediately killed by overanaesthetisation with MS 222. In the laboratory, as soon as possible after capture, species, length, weight, gut weight, fullness of stomach and fullness of intestine were recorded for each fish as in section 1.2.

In July, 1977 the feeding cycle of juvenile grey mullet was examined more thoroughly at St. John's Lake. Seine net hauls were made at 3 hour intervals over a period of 24 hours on two occasions, 6/7th July and 13/14th July. The fish were killed by overanaesthetisation with MS 222 and for each fish the following were recorded on site, as soon as possible after capture: species, length, weight, gutted weight, gut weight, number of pyloric caeca, fullness of stomach and fullness of intestine (section 1.2). At each sampling time the approximate state of the tide and whether it was light or dark were recorded.

5.4.2. Results

On March 10th, 1977 a total of 74 L. aurata were captured in 9 seine hauls. For samples consisting of four or more fish, the percentage of stomachs containing food was plotted against time and state of tide (Figs. 5.6 and 5.7). The weight of gut as a percentage of total weight and percentage stomachs
containing food increased to a maximum at high water.

On the 6/7th July a total of 330 fish were captured of which 193 were group I *C. labrosus*, 137 group 0 *L. aurata* and 3 group 0 *L. ramada*. On the 13/14th July a total of 154 fish were captured consisting of 83 group I *C. labrosus*, 74 group 0 *L. aurata* and 5 group 0 *L. ramada*. Due to the small numbers of *L. ramada* captured analysis was confined to *C. labrosus* and *L. aurata*. The data are summarised in Tables 5.1 and 5.2. The percentage of fish in each fullness of stomach and intestine category and the mean gut weight as a percentage of the total weight were calculated, and are shown in Figs. 5.8 and 5.9.

The original plan was to catch fish every 3 hours, making a total of 9 samples over a full 24 hour period. However, at high water the beach from which seining was normally carried out was submerged and fishing from the opposite bank proved very unsatisfactory. Such difficulties account for the absence of some samples, e.g. 21.30 on 6th July and 04.00 on 14th July, and the delay of others, e.g. 10.00 on 7th July. At 10.30 on the 14th July, in spite of several hauls over a period of 45 minutes, no fish were obtained, possibly due to an abortive first haul, from which the fish managed to escape, the disturbance then frightening all fish away from the area.

6/7th July, 1977:

At 15.30, about an hour before low water, only *C. labrosus* were captured. More than half the stomachs contained food and most of the intestines were full. By 18.30, more of the stomachs and intestines were empty, this trend apparently continuing
in the 00.30 sample. In the 03.30 sample about half the stomachs and more than half the intestines contained food, but by 06.30 most were again empty. This was also true in the 10.00 sample but by 12.30, 75% of intestines contained food, with 23% of them being full. However 85% of stomachs were empty which suggests that either feeding had taken place, but had ceased some time previously, or that the fish were actively feeding but had regurgitated on capture.

Of the _L. aurata_ captured at 18.30, 64% had empty stomachs but 92% of the intestines were full. At 03.30 all stomachs and intestines were empty. In the samples from 06.30 to 12.30 the fullness of stomachs and intestines increased but then declined slightly in the 15.30 sample.

13/14th July:

The investigation was repeated with the time of tides approximately 6 hours later. Fishing was less successful with only about half the numbers of fish being caught. This was possibly related to the lower tidal range on the 13th July, or the very calm weather facilitating net evasion.

At 19.00, in all species, gut weight was relatively high, with a low proportion of stomachs empty. Through the late evening and night the proportion of stomachs and intestines empty increased for _L. aurata_ so that at 01.00 there were 100% stomachs and 90% intestines empty. At 07.30 despite 82% of _L. aurata_ stomachs being empty, only 25% of _C. labrosus_ had empty stomachs, although the stomachs containing food were only \( \frac{1}{4} \) full. At 14.30 only _C. labrosus_ were captured. Feeding was actively occurring as indicated by 83% of stomachs full and 63% of intestines \( \frac{1}{2} \) full.
The weight of the whole stomach + intestine could be measured in the field with reasonable accuracy and consistency. It was more independent of the experimentor's technique and judgement than either emptying each stomach and weighing the contents or categorising the stomachs and intestines as full, ½ full etc. Gut weight was expressed as a percentage of total body weight to minimise the effect of variations in body weight.

The experimental design assumed that the same population of fish was being sampled throughout. However, there was a slight difference in the mean weight of both the _C. labrosus_ and _L. aurata_ captured on 6/7th July and 13/14th July (Table 5.3 a). This was shown to be statistically insignificant, but since gut weight as proportion of total weight does alter as body weight increases, the correlation between mean gut weight as a percentage of total weight and mean body weight was examined to ensure that this was non-significant over the weight range involved, i.e. the observed differences in mean gut weight were not merely a reflection of slight differences in the mean body weight of the samples. The correlations between mean gut weight as % total body weight and percentage stomachs containing food, and percentage intestines full or ½ full were also examined to establish whether gut weight did in fact reflect the fullness of the stomach and intestine. The correlations between mean gut weight and the stomach and intestine vacuity coefficients were also estimated (Table 5.3 b).
In both species, the correlation coefficient for gut weight as a percentage of total weight and body weight was low and non-significant, whereas the correlation between mean gut weight and percent stomachs containing food, and between mean gut weight and percent intestines full or 1/2 full, was much higher and highly significant. The mean gut weight was significantly negatively correlated with both the stomach and intestine vacuity coefficients. There was some tendency, particularly among C. labrosus, for the gut weight to be more strongly correlated with the stomach vacuity coefficient than with the intestine vacuity coefficient.

Variation in gut weight from one sample to the next was tested for significance using t-tests (Table 5.1, 5.2). In the majority of cases, observed change in mean gut weight was statistically significant.

To compare results from the 2 days, the percentages of stomachs containing food were calculated, and plotted against time of the day in Fig. 5.6, and against hours after high water in Fig. 5.7. Sequential samples are connected by a straight line, and samples on different days by a dashed line. Pairs of samples were identified, where sampling had occurred: (a) at approximately the same time of the day on the two days (tide opposite). (b) at the same state of the tide on separate days (time of day c. 6 hours different). (c) at the same state of the tide on the same day (time of day c. 12 hours different). For each pair, a t-test was carried out to compare the mean gut weight. This had been shown to be highly correlated with percent
stomachs containing food. The results of the t-tests are shown in Table 5.4.

5.4.3. Discussion

The existence of a feeding rhythm was indicated by:

(a) The similarity of vacuity coefficients of groups of fish caught at the same time and place irrespective of species and age group.

(b) The variability of vacuity coefficients of groups of fish caught in the same month on separate occasions.

However, opposed to this

(i) In most samples there was a range of fullness of stomachs and intestines.

(ii) The vacuity index of fish from St. John's Lake was not very different from that of fish from other sites in spite of the fact that they were captured at approximately opposite states of the tide.

The initial study on 10th March in the R. Lynher suggested an increase in feeding from low to high water. However, in order to investigate the relative importance of the diel and tidal cycle, a more detailed study of the type executed at St. John's Lake was necessary. St. John's Lake was not an ideal site for this work, as the retention of water behind the ford at low water was not typical, and the salinity varied from almost 0% to 35% over the tidal cycle. However, the wide mud flats more typical of these estuaries meant that it was impossible to sample throughout
the tidal cycle at any other locality.

The results of this study showed that feeding was not solely governed by either time of day or tide. There appeared to be certain differences between the feeding cycles of *C. labrosus* and *L. aurata*.

(a) *L. aurata*: Examination of Fig. 5.6 suggests that feeding occurred during the day. On both days there was little or no feeding during the hours of darkness and the percentage of stomachs containing food increased during the morning. The gut weight was high in the evening and low in the morning. Comparing samples captured at the same time of day, there was no significant difference in gut weight. However, peak feeding appeared to be influenced by high water. This was shown by a similarity in the pattern of percent stomachs containing food with hours after high water in Fig. 5.7. Seven pairs of samples collected at the same state of tide but with the time of capture approximately 6 hours apart were compared and the differences between them were found to be highly significant. However, there was some indication, particularly in the gut weight values, that feeding increased more rapidly in the early morning on the 13th when high water was at dawn.

(b) *C. labrosus*: Examination of Fig. 5.6 suggests two feeding peaks, occurring in the early morning and afternoon irrespective of tide. The gut weight of *L. aurata* reached approximately similar values on both days but the peak gut weight of *C. labrosus* was much greater on the 13th July than on the 6th. This was perhaps related to the fact that the afternoon feeding period
corresponded approximately to high water on the 13th. Reference to Fig. 5.7 suggests that, although on the same day there appeared to be some relation between percent stomachs containing food and hours after high water, the pattern of variation on the 6th and 13th was rather different.

The results of t-tests on pairs of samples of fish collected at the same time of the day, indicated no significant differences in mean gut weight at night and in the early morning, but significant differences in the afternoon and early evening. Closer examination showed that these significant differences arose because although the afternoon peak occurred at the same time on the 6th and 13th, it was far greater and more prolonged on the 13th. When samples collected the same number of hours after high water on the same day were compared, on the 6th, the three pairs of samples were not, or only marginally, significantly different in percent gut weight because the feeding peaks occurred at approximately 12 hours interval i.e: the same periodicity as the tides. On the 13th there were significant differences between samples collected the same number of hours after high water, even though the feeding peaks occurred at approximately the same time of day as on the 6th. This occurred because, on the 13th, when high water corresponded with the afternoon feeding peak, the increase in gut weight was far greater than in the early morning, whereas on the 6th, the increase in gut weight was approximately the same in the two peaks. When samples collected at the same number of hours after high water on different days were compared, the issue was again confused by the fact that the peak in gut

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weight on the 13th was greater and more prolonged than on the 6th.

For juvenile mullet in St. John's Lake, *C. labrosus* appeared to differ from *L. aurata* in having two feeding peaks, one in the afternoon at 1400-1600 and another in the early morning at 0300-0700. The feeding of *L. aurata* occurred during the day with little or no feeding at night. The time of the tide had a marked effect on the size and duration of the feeding peaks. When high water coincided with these feeding peaks the gut weight increased to a higher level, and the feeding peak was of longer duration.

It is difficult to assess the general applicability of these results. Ideally similar studies should have been made throughout the year, at different sites, and on all age groups. Although this study suggested that there were differences in the feeding rhythms of the species, the *C. labrosus* were group I and more than double the weight of the 0 group *L. aurata*. Data presented in section 5.3 indicated that when fish were captured on the same occasion there was little difference in the fullness of stomachs and intestines of fish at different age groups. However, in certain fish e.g. coho salmon (Zorbidi, 1977) feeding cycle has been found to vary with age group and De Silva & Wijeyaratne (1976) found that the feeding of juvenile *M. cephalus* peaked at midday irrespective of the state of the tide, whereas Odum (1970) reported that adult *M. cephalus* fed almost continuously during the day with greatest intensity at high water. This may be a reflection of variation of
feeding cycle with age group although, as the observations were carried out in Sri Lanka and America respectively, environmental effects may also have been important.

In samples captured throughout the year C. labrosus and L. aurata showed some similarity in fullness of stomachs and intestines when captured together. However, the samples were not collected at times when, if the feeding rhythm observed in St. John's Lake was generally applicable, the difference between the species would be most noticeable, i.e. at night. Yashouv & Ben Shachar (1967) suggested that L. ramada discovers food visually. If L. aurata behaves similarly it would explain why, in this study, no feeding was observed during the night, and indicates that C. labrosus possibly locates food in a different manner.

Some authors have found no evidence of a daily feeding rhythm, e.g. Pillay (1953) and Sarojini (1957) working in India on M. tade and M. parsia respectively. Similarly Kuthalingham (1966) in India analysed samples of adult M. cephalus collected at regular intervals during the same day, several times in different seasons, and found no noticeable variation in feeding intensity with time of day. This contrasts with Odum (1970) who found that tide affected feeding intensity, De Silva & Wijeyaratne (1976) who found that juvenile M. cephalus had a major feeding peak at midday irrespective of tide, and Das (1978) who found that the feeding intensity of M. cephalus was higher in the afternoon than the morning. There is too little information to come to any conclusions as to whether this reflects differences
in the pattern of daily feeding activity between species, age groups or localities. De Silva & Wijeyaratne (1976) found some evidence to suggest that the feeding rhythm in April was slightly different from that of July and August indicating that the pattern of daily feeding activity may also vary with season.

5.5. RATE OF DIGESTION

5.5.1. Methods

A determination of the rate of passage of natural food through the gut of juvenile grey mullet was made in the upper part of St. John's Lake on 31st August 1977. St. John's Lake was chosen for several reasons. Fish could be caught in sufficient numbers and, due to the ford, the depth of water enabled fish to be maintained in cages even at low water. 75 fish, mainly I group C. labrosus, with a few II group L. aurata and L. ramada, were collected by seine netting. 15 were killed and examined immediately. The remainder were divided into groups of 15 and placed in 4 submerged wire mesh cages. The cages were supported off the mud layer and positioned so that water could flow freely through them in an undisturbed area, just upstream from where the fish had been caught. After 30, 60, 90 and 120 minutes a cage was removed from the water and the fish killed by overanaesthetisation with MS 222. Immediately after death, species, length, weight, gutted weight, weight of gut, number of pyloric caeca were recorded for each fish as
described in section 1.2. The temperature of the water was recorded at the beginning and end of the experiment.

5.5.2. Results

Some fish escaped through the mesh of the cage during the investigation. For the group of fish in each cage the percentage of stomachs full or ½ full, and those empty or ¼ full were calculated, and similarly, data for the intestines. Gut weight as a percent of total weight was determined for each fish and the means of each group calculated (Table 5.5, Fig. 5.10). The mean body weight of each group was calculated and plotted on the same graph as gut weight.

The gut weight declined with time from 0 to 90 minutes. At 120 minutes there was a slight, statistically non-significant rise. In most fish stomach and intestine contents were evacuated in 90 minutes, although in one fish in each of the 90 and 120 minute samples the stomach was still full or ½ full after 120 minutes. It is possible that these fish were consuming faeces or detritus carried into the cage on the current.

The gut weight as a percent of total weight was calculated for 50 C. labrosus and 50 L. aurata of the same age groups which had empty guts. The mean weight of gut was 11.89% of the total weight. Thus gut weight in excess of 11.89% body weight was an estimate of weight of food in the gut of each fish. The mean gut contents as a percent of body weight were calculated for each sample of fish. The regression of log gut contents on
time was not significant due to a particularly low value at 90 minutes. When this was ignored, the regression was highly significant (p < .001) and log gut contents was highly significantly correlated with time (p < .001). The decline in log gut contents was found to be 0.2841 per hour and it was calculated that 99% of the total initial gut contents would be evacuated in 2.12 hours.

5.5.3. Discussion

The food preference experiment, carried out in the laboratory on group I C. Iabrosus feeding on Artemia and white worms at a temperature of 11-14°C (Section 5.2.) showed that after 6 hours the stomach was empty and the intestines were largely empty after 24 hours. The experiment carried out at St. John's Lake attempted to investigate the digestion rate of the more natural diet of detritus and mud in the natural environment. The temperature of the water was 17-18°C. After 2 hours 83% of the stomachs were ½ full or less, and 92% of the intestines were half full or less.

Rates of digestion and particularly rates of gastric evacuation are of importance partly due to their influence on the fullness of stomachs and intestines in studies of daily and seasonal variation in feeding intensity, and partly, from a practical viewpoint, factors increasing gastric evacuation have a direct effect on the interval between meals in S. gairdneri (Grove et al, 1978). In some fish e.g. channel catfish
there is a linear relationship between food remaining in the stomach and time after feeding. In others e.g. salmonids, perch, cod and flounder, a semilog model of gastric evacuation with time was most satisfactory i.e. rate of stomach evacuation depended on the fullness of the stomach. (Elliott, 1976c; Thorpe, 1977; Jobling and Spencer-Davies, 1979; Kiorboe 1978) Windell 1976 found that this model consistently gave the most significant regressions and the lowest deviations from regression, which is consistent with the observations on juvenile grey mullet in this study. However, Perera & De Silva (1978) found that the stomach contents of juvenile *M. cephalus* declined linearly with time.

Digestion rates obtained in situ, as in this case, are preferable to those obtained in the laboratory because the fish are feeding on the same food at the same temperature, and oxygen concentration as wild fish. However it has generally been found that the rates of digestion of caged fish are lower than those of wild fish, perhaps due to the trauma of capture, the density of fish in the cage, or the cessation of feeding. The rate of stomach emptying during feeding is faster than that measured after feeding has ceased (Moriarty & Moriarty, 1973). In addition this experiment was conducted at low water when the salinity behind the ford approached 0-2%. The digestion rate of juvenile *M. cephalus* declined at low salinities (Perera & De Silva, 1978). Thus rates of digestion obtained in this experiment must be considered as minimum estimates.

Most authors have removed stomach contents for weigh-
ing. The diet of mud and the inevitable spillage from the stomach when the intestine and oesophagus were cut away from it, made the weighing of stomach contents of young grey mullet an impractical procedure under field conditions. The stomach and intestine, including their contents, were therefore weighed entire which could be done with considerable accuracy even in the field and on small specimens containing little food. The gut weight was expressed in terms of per-cent of total body weight because mean weight of fish did vary slightly from sample to sample, although the weight of the gut contents did not significantly affect the weight of the fish, since the mean weight of fish in succeeding samples did not decline significantly.

The rate of evacuation of the gut of young mullet estimated in this study (99% evacuated in 2.12 hours at 17-18°C) was considerably faster than estimates given by other authors for the same species. Hickling (1970b) fed C. labrosus (12-28 cm) a diet of cod roe and mud bound with egg white and found that it passed through the gut in 8-9 hours. The gut of 30-150g C. labrosus fed on artificial paste emptied in 6-10 hours at 8°C (Fange & Grove, 1979). This may be partly due to differences in fish size since the relative digestion rate of mullet decreases with increase in fish weight (Perera & De Silva, 1978). Perera & De Silva (1978) studied the digestion rates of M. cephalus of approx. the same size as the C. labrosus examined in this work. Similar times for gut evacuation were observed at high salinities (125-150 mins) although the time was much longer (c. 330 mins.) at a salinity of less than 1%, despite the considerably higher temperature of
The time for total emptying of the digestive tract recorded for grey mullet - juvenile and adult C. labrosus (see above) adult M. cephalus 4-5 hrs. at 20-26°C (Fange & Grove 1979) - tend to be much shorter than for carnivorous fish e.g. S. gairdneri, 50-30 hrs. at 8-18°C (Grove et al, 1978). Young turbot evacuated meals diluted with kaolin in significantly less time than a control diet (Flowerdew and Grove, 1979). Thus the rapid digestion rate of mullet may be associated with the fact that the cardiac stomach of C. labrosus feeding on mud contained less than 15% organic matter, a proportion of which is indigestible (Hickling, 1970b). Large quantities must be consumed to meet nutritional requirements, and increased food consumption has been shown to stimulate digestion rate (Jobling & Spencer-Davies, 1979; Flowerdew & Grove, 1979). Similarly Yashouv & Ben-Shachar (1967) observed that the time food was retained in the intestine decreased as the amount of food available to M. cephalus and M. capito fry increased.

The rate of passage of food through the gut is affected by both diet and feeding history (Fange & Grove, 1979) and so estimates involving artificial diets (Hickling, 1970b; Fange & Grove, 1979) must be considered of limited value when extrapolating to field conditions. Even when natural food is used, factors such as the trauma of capture and stress associated with handling and confinement in a cage, may have an important effect on the rate of gut evacuation.
5.6. ESTIMATION OF FOOD CONSUMPTION

5.6.1. Calculation of food consumption

Several different methods have been used to estimate daily food consumption of fish.

(a) Bajkov (1935) described a method of estimating food consumption under natural conditions. A number of fish were captured, some of which were preserved immediately, and their stomach contents weighed. The remainder were placed in a food free tank at ambient temperature and sampled at intervals to determine the rate of digestion. The daily food consumption was then calculated from:

\[ \Delta = \frac{A}{n} \]

\( \Delta \) = daily consumption

\( A \) = average amount of food in stomach

\( n \) = number of hours to pass all food from stomach to intestine.

A modification of this method was used to estimate food consumption of mullet.

The calculations depend on the gut contents of the original sample being 'average', and continuous feeding and digestion throughout 24 hours. This method was, therefore, not ideal for fish which were feeding for only part of the day. An 'average' weight of gut contents was calculated for juvenile mullet by pooling samples collected over a 24 hour period, but food consumption would nevertheless be overestimated.
From the feeding investigation on 6/7th and 13/14th July, the mean gut weight was calculated for each species. The mean weight of the empty gut of 50 C. labrosus and 50 L. aurata was 11.89% total body weight, from which gut contents as a percentage of total body weight were estimated. The investigation of the rate of digestion (Section 5.5.) indicated that 99% of the food had passed out of the gut in 2 hours. The temperature of the water was similar on the 6/7th and 13/14th July, varying from 17-19°C. Windell (1976) showed that the effect of temperature on gastric evacuation in the rainbow trout was greatest between 0 and 5°C and least between 15-20°C. Daily food consumption was calculated using the Bajkov formula where

\[ A = \text{average amount of food in entire gut} \]
\[ n = \text{number of hours required to pass all food through the gut}. \]

The results are summarised on Table 5.6 a.

(b) Where feeding is cyclical, as it appears to be in juvenile mullet, Keast & Welsh (1968) estimated food consumption from the difference between the mean weight of stomach contents of the fish at the peak feeding times, and succeeding troughs when the fish were not feeding and the stomachs were assumed to be empty. A modification of this method was used to estimate daily food consumption of juvenile grey mullet in St. John's Lake. Using the data obtained from feeding investigations carried out on 6/7th and 13/14th July, the difference in mean gut weight between the peak and succeeding trough was calculated. It was converted to weight in mg using the mean weight for that group of fish. Where there were two peaks in a day, the differences in
mean gut weight were summed. The results are summarised on Table 5.6 b.

(c) Thorpe (1977) criticised the estimates of Bajkov because periodicity of feeding was not taken into account, and those of Keast & Welsh because of rate of passage of food from the stomach to the intestine was not considered. Thorpe (1977) estimated the rate of disappearence of food from the stomach from samples of fish examined at capture and at known time intervals after capture. An exponential model of the rate of passage of food from the stomach was found to be most appropriate. Fish were seined at 3 hour intervals over 24 hours, and food consumption between netting times was calculated at the increment between successive initial values of stomach contents plus the calculated amount evacuated in the interval.

This method was used on the data collected during the July feeding investigations (section 5.4) and the exponential decline in gut contents calculated from in situ investigations described in section 5.5. The results are summarised in Tables 5.6 c and 5.7.

5.6.2. Discussion

Juvenile mullet appear to have a daily feeding rhythm, but feeding and non-feeding individuals can be found in samples caught at most times of the day. Several methods were used to estimate daily food consumption. Bajkov (1935) assumed continuous feeding and so the estimate obtained by this method was probably an overestimate. The method of Keast & Welsh (1968) was designed
for fish which feed periodically. However, it assumes that no
digestion occurs during feeding and that no feeding occurs
between feeding peaks. Estimates obtained are very low, often
below what is considered a maintenance ration. Nakashima &
Leggett (1978) used the weight of the stomach and intestine
contents in order to account for passage of food from stomach
to intestine during feeding. However, they still assumed
negligible digestion and absorption during feeding which, in
juvenile mullet, with extended feeding periods and such a
fast passage of food through the gut, is almost certainly not
the case. This may account for the fact that the estimate of
daily food consumption obtained by this method was much lower
than by the Bajkov method.

The estimation of daily food consumption by Thorpe
(1977) assumed an exponential decline in fish stomach contents
with time and this is the model which seems most generally
applicable (Elliott & Persson, 1978). In this thesis an exponen-
tial decline of entire gut contents was found to fit the data
reasonably well. Thorpe (1977) captured fish every 3 hours over
24 hour periods and calculated food consumption as the increment
between successive initial values of stomach contents plus the
calculated amount evacuated in the interval. The amount consumed
in each interval was summed to obtain the total daily ration. It
is assumed that any food consumed by the fish during the interval
between netting times is not passed into the intestine. In this
thesis total gut weight was measured so that food passed into the
intestine was taken into account. In spite of this, due to the
fast rate of digestion of juvenile mullet, in a sampling interval of 3 hours food may have passed right through the intestine and, therefore, not have been considered in the calculations at all. In several cases, due to sampling difficulties, intervals between samples were longer than 3 hours. Also, although the exponential decline of gut contents with time may be appropriate for fish prevented from further feeding, it may not decline in this way when fish are actively feeding.

The daily food consumption estimates for juvenile mullet obtained using this method were likely to be underestimates, both for the reasons mentioned above, and because field determination tends to underestimate the digestion rate (section 5.5.). However, the values obtained were 8-19% wet body weight which are higher than the 2.3% wet body weight recorded for _C. labrosus_ of similar size by Flowerdew & Grove, (1980) even taking into account that the diet was dry. They are also higher than the 6% wet weight calculated from data given by Brett (1971) for sockeye salmon of similar size, and the 3.2 -6.5% wet weight obtained by Thorpe (1977) for perch in _L. Leven_. The high food intake may be associated with the poor diet of mullet in St. John's Lake, but De Silva & Perera (1976) reported dry food intakes of up to 26% body weight among juvenile _M. cephalus_ fed on rice bran and fish meal in an experiment carried out in Sri Lanka, which is very high, even allowing for the effects of temperature on maximum ration size.

A more extensive examination of food consumption at different seasons and at different sites was necessary. The food
intake and digestion rates of young *M. cephalus* varied with salinity, tending to increase at higher salinities (Perera & De Silva, 1978). Thus food intake in St. John's Lake might well be exceeded in the more saline waters of the lower estuary. Nakashima & Legget (1978) sampled perch over periods of 48 hours and found considerable variation in the feeding from day to day. Thus two 24 hour periods are an inadequate basis for any firm conclusions. However, the evidence does indicate that a relatively high daily food intake and fast passage of food through the gut may be characteristic of juvenile *C. labrosus* and *L. aurata*. 
Investigations of the effects of parasites on grey mullet have been carried out in Israel where *M. cephalus* and *M. capito* are cultured with carp and tilapia. Under culture conditions reproduction of ectoparasites may become accelerated and their pathological effect becomes more significant.

In fresh water Mugilidae generally suffered less severely than carp or tilapia. Heavy infestations of crustacean parasites (*Argulus* and *Lernaea*) were rare and the mullet did not appear to suffer losses due to monogenean parasites (Lahav, 1974). In brackish water ponds, and in fish ponds fed by streams flowing into the sea/estuary there are records of mullet becoming heavily infected with the parasitic crustacean *Ergasilus* sp. and metacercariae of the trematode *Heterophyes* sp. In addition to mortalities (up to 50%) the high infestations observed would be expected to have some effect on growth of the remaining fish. The fish infected with the *Heterophyes* sp. are harmful to man unless properly cooked. Bromine-50 was used with considerable success to treat mullet heavily infected with *Ergasilus* (although at low salinities, the mullet were sensitive to this insecticide (Lahav, 1974)).

When cultured in sea water ponds, *M. cephalus* suffered major mortality due to heavy infestation with the crustacea *Pseudocaligus apodis* and *Caligus* sp. (Lahav, 1974) and the monogenean *Benedenia* sp.

The only parasite of mullet which has been recorded
as causing mass mortality of mullet in the wild is a Myxosporidian, *Myxobolus exigus* (Shul'man, 1957). This parasite has also been reported from fish ponds in Israel (Lahav, 1974). The Myxosporidia are primarily fish parasites affecting body cavities as well as tissues. The infective stage is the spore (10-20μ). Halliday (1973, 1974) infected rainbow trout with *M. cerebralis* by exposing them to mud taken from earthen ponds which had previously contained infected fish. As the guts of grey mullet are full of mud this seems a likely mode of infection. The action of host digestive juices is thought to cause the spores to extrude polar filaments which anchor the spore to the gut, and to split and release the sporoplasm or amoebula. This then passes between the cells of the intestine and reaches other sites, e.g. the gills or liver, via the blood and lymph. The trophozoite increases in size and undergoes nuclear division to form a relatively large extracellular macroscopic cyst, with a definite limiting membrane. Certain cells, the sporoblasts, become differentiated from the syncytial mass and, after a further period of nuclear division, spores are formed (Grill, 1973). When cysts form in the gut wall or gill filament, spores can be liberated by the bursting of the cysts. Those in the liver presumably depend on the death of the host for release.

6.1. **RESULTS**

Early in this work it was noticed that small white cysts were frequently found on the gut wall and in the liver. These were later also found on the gill. The number of cysts varied from...
one or two to very numerous, covering the entire gut and pyloric caeca (Plates 1.1, 1.2). Dr. R.A. Matthews (Plymouth Polytechnic) identified the cysts as belonging to a Myxosporidian, *Myxobolus exigus*.

The degree of infestation was determined using an arbitrary scale described in section 1.2, which defined levels of infestation from 0 to 3. For each group of fish (fish of same age and species caught on one date, at one location), the percentage containing any Myxosporidian cysts and the percentage falling into each level of infestation was determined. In Fig. 6.1 the percentage of fish containing cysts is plotted against month of the year for each age group. For age groups I, II and III in both *L. aurata* and *C. labrosus*, the percentage of fish carrying cysts declined from 100% in February, March and April to less than 40% by July and August. Group *C. labrosus* contained no cysts when they arrived in the estuary, and the infestation remained negligible until the fish entered the I group. In a group of 0 group *L. aurata* captured in July, only 20% had cysts, a far lower percentage than in older age groups at the same time of the year.

*L. aurata* and *C. labrosus* had a similar annual cycle of cyst frequency, and the percentage of fish carrying cysts was also approximately similar. Only a few groups of *L. ramada* were available for examination, but in these there was some evidence to suggest that the proportion of fish bearing cysts was lower than in the other two species.

The degree of infestation as indicated by the number of cysts is shown in Fig. 6.2. The proportion of fish of each
group on each unit of the infestation scale was plotted according to month of capture, age group and species. The seasonal variation in age groups I, II, and III is somewhat similar. The proportion of fish containing no cysts increased from March to August. The proportion of fish on point 3 of the infestation intensity scale declined from c.20% in February and March to zero by the end of June. The proportion on point 2 declined from c.50% (30% in group I) to 0% in August. The proportion of fish showing grade 1 infestation increased from February to May in groups I and II (very few group III fish were captured early in the year) and then declined from May to August. 0 group fish showed a very low intensity of infestation throughout their first year. The number of cysts and the proportion of fish affected increased during the January, February and March of the following year (year group I) declining in later months in the pattern characteristic of the older age groups.

To compare the occurrence of Myxobolus exiguus in mullet from different sites, the data on the intensity of infestation was replotted according to age group and site of capture (Fig. 6.3). Cysts were found in fish from all sites in this study. The occurrence and intensity of infestation appeared to be approximately similar and follow a similar annual cycle at all sites.

As this study was also concerned with the possible culture of mullet, the effects of so prevalent a parasite on its host fish were of particular interest. Several groups of fish (fish of the same age group and species caught at one site on one
occasion) were selected and the mean weight and mean relative condition of fish on each unit of the infestation scale were calculated (Table 6.1). In certain cases there was some indication that the most heavily infested fish were lighter in weight and had a lower relative condition. Analyses of variance of weight and condition are summarised in Table 6.1. In each case the variance due to the Myxosporidian infestation was not significantly greater than the residual variance.

6.2. DISCUSSION

The decline in the percentage of fish bearing cysts, and in the percentage of fish bearing cysts at levels 2 and 3 on the scale during the summer, may be interpreted in two ways:

(a) The number of cysts in each fish is declining.
(b) The fish bearing most cysts are disadvantaged in some way and thus selected out of the population by, for example, predation. This might occur if the number of cysts was so great as to interfere with digestion in the gut, impair liver function, or significantly affect the surface available for gas exchange in the gills.

Fig. 6.1 shows that the percentage of fish bearing cysts on the gut decreased from 100% to less than 10% over the summer. During this period the total numbers of fish captured were increasing. If the reduction in the proportion of fish bearing cysts were due to predation, then they must have been replaced by fish bearing no cysts. It seems far more likely that disintegration of cysts and release of spores occurred during the
summer and fish were then reinfected by more spores and new cysts developed over the winter. Some idea of the time scale of cyst development can be obtained from examination of the O group _C. labrosus_. When they arrived in the estuaries in July they bore no cysts, as expected since their diet is largely carnivorous up to this time. In July they began to feed at the mud surface and cysts were first noted in the following January. However, the spores may not become infective immediately after they are released as there is some evidence that a period of 'ageing' is necessary (Halliday, 1976).

Control of Myxosporidia includes destruction of infected fish and destruction of the spores in the bottom mud by emptying ponds and treatment with calcium oxide. Griffin & Davies (1978) have recently identified circulating antibody against spores of _M. cerebralis_ in rainbow trout. It may be that, in mullet, infestation is controlled by the immune response of the host fish.

There was no evidence from this study that this parasite has any effect on either growth or condition of juvenile grey mullet in South-west England. However, in the Black Sea, local fishermen report yearly deaths of grey mullet in the spring. Severe bleeding occurs in the gills and this has been shown to be due to haemorrhaging caused by bursting of Myxosporidian cysts. In Kerch Strait a mass mortality of _M. cephalus_ and _L. aurata_ was attributed to a combination of low oxygen concentration and a reduction in gill surface area by Myxosporidian cysts (Shul'man, 1957). Delphy (1916) reported the occurrence of Myxosporidian spores
in *L. aurata*. These spores had only one filament and the cysts were among the muscle fibres causing considerable distortion and disruption. Lahav (1974) recorded *Myxobolus* sp. from the gills and mesentery of *M. capito* and *M. cephalus* in Israel. This Myxosporidian was resistant to chemical agents, e.g. Biomex-50, used to combat other parasites.

Other Myxosporidians found in mullet include *Kudoa tetraspora* on the optic lobes of *M. cephalus* (Narasimhamurti and Kalavati, 1979) *Myxosoma microspora* on the gills of *M. cephalus*, *Myxosoma intestinalis* on the intestinal epithelium of *M. waigensis* and *Myxosoma cephalis* on the gills, jawbone, and intestinal epithelium of *M. cephalus* (Narasimhamurti et al, 1980). The effects of these parasites on survival and growth, and hence their economic significance, is not yet known.
Grey mullet have variously been described as carnivorous, herbivorous and iliophagous (Chapter 5). The diet has generally been considered to be 'poor' but the composition of detritus is very variable, and both the feeding behaviour of *M. cephalus* in selecting the smaller particles (Odum, 1968a) and the rapid passage of food through the gut which seems to be typical of grey mullet, suggest that actual nutrient intake may be high. This stimulated interest in quantifying the basic nutritional requirements of juvenile grey mullet. It was therefore decided to carry out some simple feeding experiments using semi-purified rations to investigate such parameters as protein requirement, apparent digestibility of dietary components, and the effect of varying ration size and energy content on growth and body composition in juvenile *C. labrosus*. 
CHAPTER 7 EXPERIMENTAL METHODS

7.1 EXPERIMENTAL EQUIPMENT

7.1.1 Location

The nutrition experiments were carried out in a room in an oyster hatchery at Steer Point on the River Yealm (Fig. 1.1). The room was lit by two fluorescent strip lights. It had no windows and the walls and ceiling were insulated.

7.1.2 Tanks

Twenty tanks were used. They were circular low density polythene tanks of diameter 610 mm and depth 660 mm with a capacity of 159 l. They were supplied by WCB containers Ltd., Stalybridge, Cheshire (E. 551). They were filled with water and allowed to stand for a few days several times prior to the experiments in order to leach out residual toxic materials.

7.1.3 Water

The water was supplied from the river by a pump. There is a very large tidal range at Steer Point and so pumping was only possible for 2 hours before and after high water. For the purposes of the experiments pumping was restricted to 2 hours, an hour before and an hour after high water, when almost full strength (33-35 °/oo) sea water could be obtained consistently. As the water entered the hatchery it passed through a coarse filter and a fine filter and was then circulated past an ultra-
violet lamp and through a heater. The heater was used in winter to raise the temperature of the water in the estuary to that of the hatchery. Unfortunately parts of this system were not in operation for several periods while experiments were in progress. The water in the experimental tanks was renewed by full changes carried out weekly in the first experiment and twice weekly subsequently. Water could be removed with a bucket because the disturbance at the surface caused the fish to swim to the bottom. When the tank was about \( \frac{1}{4} \) full, the fish were moved to a bucket of clean water with a hand net. The sides and bottom of the tank were cleaned as thoroughly as possible with a brush. The tank was rinsed and refilled to a mark which indicated that it contained 100 l. The fish were returned to the tank as soon as possible. The hand net was immersed in dilute Milton after the transfer of fish from each tank was completed. The salinity of the water in the experimental tanks was measured periodically using a portable refractive index salinometer.

7.1.4. Aeration

The experimental tanks were aerated by a compressor which was situated in the room above. This fed air into a manifold from which a single air line passed to each tank. The air was diffused by an airstone which was weighted to keep it at the bottom of the tank. Air flow was regulated by an adjustable clamp in each air line. A dissolved oxygen meter (Electronic Instruments Ltd., Model 1520) was used to measure oxygen concen-
Temperature

Two tanks were chosen at random and a maximum-minimum thermometer was totally immersed in each. On six days a week the maximum and minimum temperatures were recorded and the thermometers reset. The thermometers were moved to different tanks once a week.

Light

The experimental room was completely enclosed and received no natural light. There was no time switch by which an artificial light/dark cycle could be set up. In the first experiment the fish were exposed to continuous light, and in the second and third to continuous darkness, except for periods of feeding, tank changing etc.

EXPERIMENTAL FISH

Collection

Juvenile *C. labrosus* were obtained from above the ford at St. John's Lake (Fig. 1.1) using a seing net as previously described. They were transported to the oyster hatchery as rapidly as possible and held in 1500 l holding tanks, initially outside, but subsequently inside the hatchery. During this time they were
fed on commercial pond pellets.

7.2.2. Preparation of experimental tanks

About a week after collection the fish were transferred to the experimental tanks. When netting the fish in the holding tank there was a tendency to catch the smaller fish first, and the larger, presumably faster, fish later. In order to obtain an even size distribution among the tanks about 100 fish were transferred from the holding tank to a bucket of water and redistributed in groups of 5 to each experimental tank. This was repeated until all tanks contained 50 fish. 7-10 days were allowed to elapse after transfer before the beginning of the experiment. During this time the fish were fed on commercial pellets. Any fish which died in this period were replaced from the holding tank.

7.2.3. Marking the fish

It was statistically desirable to be able to identify each fish individually. Clipping of pelvic and pectoral fins was not practical as these were very delicate, and subsequent examination of the fins would have been slow and required too much manipulation of the fish. Injected dyes, as used by Riley (1966) and Kelly (1967), were not visible through mullet skin and scales. The method used was a combination of dorsal fin ray clipping and freeze branding. The dorsal fin of juvenile grey mullet has three distinct fin rays and a smaller fourth fin ray. One, or combinations of two, of the first three fin rays of an anaesthetised fish could be clipped with
a small pair of scissors and on later examination it was easy to recognise which had been cut. A freeze branding method of marking was described by Everest & Edmundson (1967) for juvenile salmon, and this was adapted for use on small grey mullet. Two copper wire brands were made, a straight line and a circle, and attached to wooden handles. During the branding procedure the brands were kept with the copper wire submerged in liquid nitrogen in a thermos flask. To mark a fish, the brand was removed, pressed against the side of the fish for 1-2 secs and returned to the liquid nitrogen.

Prior to the commencement of marking of experimental fish, a group of 20 fish were removed to the laboratory. They were marked by various combinations of dorsal fin ray clipping and freeze branding and observed over the next three weeks. There were no mortalities and no apparent difficulties in swimming or maintaining balance, even when most of the first dorsal fin had been removed.

The 50 fish in each tank were divided into groups of 10 by five combinations of fin ray clipping - 1st, 2nd, 3rd, 1st + 2nd, 2nd + 3rd. The 10 fish of each group were individually identified by freeze branding marks (1, 11, X, 0, 1X, 01, 00, 10, X0, XX). These two marking methods were selected because they fulfilled the requirements of being quick to carry out and fast to read and record, thus minimising handling and the length of time an anaesthetised fish was out of water. By using fin ray clipping as well as freeze branding the number of brand marks on any fish could be limited to two. In experiments II and IV, one
tank of fish was not clipped or branded in order to assess the effect of the marking on growth and survival.

7.2.4. Weighing, measuring and marking
At the beginning of each experiment all fish were weighed, measured and marked. The procedure was as follows:
(a) The fish was placed in a 100 ml beaker containing sea water and a few crystals of MS 222.
(b) When the fish began to lose equilibrium it was removed from the anaesthetic. The standard length of the fish was measured, as described in section 1.2, to the nearest mm.
(c) The dorsal fin was gently raised and the appropriate dorsal fin rays were cut about half way down using small pointed scissors.
(d) The fish was blotted dry on a paper towel and weighed on a top pan balance to the nearest $10^{-2}$ g.
(e) The fish was held in a paper towel and freeze-branded as described above.
(f) The fish was allowed to recover in a bucket of sea water and returned to the experimental tank.

Any fish of the _L. aurata_ or _L. ramada_ species, or _C. labrosus_, which showed injury or loss of scales, were discarded and replaced by healthy fish from the holding tank. The weighing, measuring and branding of the 1000 experimental fish was carried out over a period of 4-5 days, at the rate of 200-250 fish per day. The tanks were always treated in the same order so that, for example, in tanks 17 to 20 the experiment started 4 days later and finished.
4 days later than in tanks 1 to 4.

In the first experiment fish were weighed and measured every 4/5 weeks. In subsequent experiments the above procedure was repeated 6 weeks after the beginning of the experiment. Alongside the weight and length of each fish its clipped dorsal fin rays and brand were recorded, and where necessary, renewed. At the end of the 3 month experimental period each fish was killed by overanaesthetisation before being weighed and measured as above.

7.3. EXPERIMENTAL DIETS

7.3.1. Composition of the diets

The experimental diets were based loosely on those used by Halver (1957) and Halver & Shanks (1960) for work on nutrition of salmonids and had the general composition shown in Table 7.1.

In experiment I the protein used was a 1:1 mixture of an animal protein, casein, and a plant protein, gluten. In subsequent experiments casein enriched with certain amino acids (Cowey et al., 1970a) was used as the sole protein source. This was made up as below:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>95 g</td>
</tr>
<tr>
<td>L-arginine</td>
<td>2.5g</td>
</tr>
<tr>
<td>L-methionine</td>
<td>1.0g</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>0.4g</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>1.0g</td>
</tr>
</tbody>
</table>
In experiment I the carbohydrate was a 1:1 mixture of dextrin and maize starch. In subsequent experiments dextrin only was used as the carbohydrate source. The lipid was a 5:2 mixture of corn oil and cod liver oil. The vitamin premix was obtained from Cooper Nutrition Products Ltd.

The mineral mixture was made up as described by Vallet et al (1970) for work on mullet nutrition. Its composition is shown in Table 7.2.

7.3.2. Diet preparation

Several binders have been used in the preparation of experimental fish foods. The following were examined and compared for suitability in this case. Sample diets were made up using (a) water; (b) 5% agar solution and (c) Celacol (carboxymethyl cellulose). Nine aluminium mesh trays were constructed and, using three per diet, approximately equal weights of the prepared experimental foods were divided into 5 mm cubes and placed in the trays. One tray for each diet was reserved as a control and the other two were suspended in seawater at 18°C for one hour. All trays were then dried in an oven at 100°C for 24 hours and cooled in a desiccator.

The percentage dry weight loss from the pellets suspended in sea water was calculated. This was 5-10% for diets bound with agar, 18-21% for diets bound with Celacol, and 25-27% for diets bound only with water. As a result of these tests the following method of diet preparation was adopted. For each diet the dry ingredients were weighed and thoroughly shaken together in
a plastic container with a tightly fitting lid. The oil was added and mixed in as thoroughly as possible. A 5% agar solution was prepared. It was boiled to ensure that all agar was dissolved and allowed to cool until the beaker could be picked up by hand. A volume equal to 1 ml per g dry constituents + lipid was measured out and thoroughly beaten into the dry mixture. The plastic container was sealed with the lid and placed in the refrigerator to cool. An amount sufficient for one week was prepared each time.

7.3.3. **Method of feeding:**

The fish were fed once per day, six days out of seven. The correct amount of diet per tank was weighed out to ± 0.05 g and dropped into the tank as a single lump. Particularly in the case of the poorer diets, the fish surrounded the food immediately and could be seen sucking at it. Although no aggressive behaviour was noted, feeding in this manner may have given rise to competition to reach food, and a hierarchical effect as was observed by Magnuson (1962) in the medaka and Brown (1946a) in brown trout. However, the single lump had a lower surface area from which nutrients could be leached than would several smaller pieces, and for this reason was regarded as preferable. On Saturday a double ration was given.

7.4. **EXAMINATION OF FISH AT THE END OF THE EXPERIMENT**

The fish were not fed the day before the experiment ended. However, probably due to feeding on faeces, not all stomachs and intestines were completely empty by the following day.
At the end of every experiment 13 fish were transferred alive to the laboratory, and on the same day, 3 of these were killed and prepared for histological examination of the gills, liver and gut (7.4.1) and 10 were killed and dissected and relative weights of body, gut and liver determined (7.4.2). In order to select the fish for histological and gut and liver weight examination, at random, the fish from one tank were transferred to a bucket. When so confined, speed of swimming was no longer an advantage in escaping the net, and so selective netting of smaller fish first was minimized. After removal of 13 fish, the remaining fish from each tank were killed, weighed and measured as described in section 7.2. The dead fish were sealed in a plastic bag labelled with experiment number, tank number, and date and stored in a deep freeze for later body composition analysis (7.4.3).

Total fish in each tank c.50.

13 transferred live to laboratory

10 killed + dissected for measurements of total weight, gutted weight, gut weight, + liver weight.

Remaining fish killed, weighed + measured.

3 labelled and prepared for histological examination.
7.4.3. **Body composition:**

(a) **Moisture:** The frozen fish were dried in a freeze drier. After 2 days they were removed to a dessicator, and the total weight of dry fish per tank was recorded. The wet weight of each fish was known and so the average moisture content could be estimated. The dry fish from each tank were then ground to a powder in a Moulinex Coffee Grinder. A standard grinding time of 30 seconds was used. The ground fish were stored in sealed jars in a deep freeze.

(b) **Protein analysis:** The principle of the Kjeldahl method of protein determination is that the protein is digested with concentrated sulphuric acid and a catalyst at high temperatures for several hours to convert nitrogen in the protein to ammonium sulphate. In the second stage of the process the acid ammonium sulphate solution is treated with a strong alkali which results in the release of free ammonia from the ammonium ions. The released ammonia is measured, and from this the amount of ammonium sulphate, and hence the amount of nitrogen in the original sample can be calculated.

Initially, a micro-Kjeldahl method of protein determination was used as described in Munro and Fleck (1969). After digestion in a micro-Kjeldahl flask the digest was transferred to a Markham still. Ammonia was separated by steam distillation. It was trapped in a known volume of standard acid and the amount of ammonia was calculated by back titration with standard alkali. This method of protein determination was tested using known weights.
of tryptophan and a standard urea solution containing $1.98 \text{ mg N per ml}$. In 16 complete determinations the error varied from 1.7 to 24.8% with a mean of 9.2%. As a major source of error was felt to be the transfer of the digest from the Kjeldahl flask to the still, this method was abandoned in favour of the Lernher method. This is an adaptation of the micro-Kjeldahl technique with the advantage that the distillation is carried out from the same tube in which digestion occurred i.e. no transfer of the digest is required. The digestion was carried out in a 'quick fit' test tube using selenium catalyst tablets (from British Drug Houses). When digestion was complete the tube was cooled, a few drops of phenolphthalein added, and it was connected for distillation to a second test tube. A boric acid solution (10 g boric acid per 500 ml) was made up and 5 ml of Tashiros indicator added to it. This solution appeared purple in colour. 5 ml of the boric acid-Tashiro's indicator solution was pipetted into the second test tube and diluted with distilled water until the test tube was $\frac{1}{3}$-$\frac{1}{4}$ full. The boric acid-Tashiro's indicator solution then appeared colourless, or just faintly purple. Concentrated sodium hydroxide was added dropwise to the digestion tube until the contents were thoroughly alkaline. This was indicated by the phenolphthalein which turned a deep crimson colour. As the alkali was added, a water pump was used to maintain an air flow from the digestion tube through the tube containing boric acid. Thus any ammonia released was drawn through the boric acid solution where it was trapped by the formation of ammonium ions.
The boric acid-Tashiro's indicator solution turned green as the pH increased. The digestion tube was heated gently for 15 minutes to ensure that all ammonia had been driven off. The boric acid tube was then disconnected and the air tube rinsed into it with distilled water. The contents were then transferred into a 250 ml Erlenmeyer flask, again rinsing carefully with distilled water. The solution in the flask was titrated with .02N hydrochloric acid until the green colour disappeared and the first slightly purplish tinge appeared. From the titre the amount of nitrogen in the original sample was calculated.

An assessment of the accuracy of ammonia recovery from the digest was made using a solution of ammonium sulphate containing 1.9766 mg N per ml. In 7 completed trials the error varied from .84 to 4.24% with a mean of 2.68%. The accuracy of the whole method, digestion and ammonia recovery, was tested using known weights of tryptophan. In 12 complete determinations the error varied from 2.5 to 10.2% with a mean of 6.3%. It was felt that some of this error must be attributed to the weighing out of such small amounts of tryptophan. Using the Lernher method the nitrogen contents of 25 mg samples of dried ground fish were determined. All determinations were replicated and, where necessary, a third estimation was carried out. Blank determinations, approximately 1 per 12 fish samples, were done so that the nitrogen estimations could be corrected for any nitrogen in the reagents or distilled water.

(c) Fat analysis: A gravimetric method (obtained from the manual of Official Methods of Analysis of the Association of
Official Analytical Chemists) was used. Analysis was carried out on freeze dried ground fish. Samples of approximately .5 g were weighed out and placed in 25 ml Erlenmeyer flasks. 10 ml of analytical grade chloroform were added to each flask, and the flask swirled around several times. The chloroform and lipid in solution were separated from the residue using a Buchner flask and a vacuum pump. The flask and residue were rinsed 3-4 times with chloroform. The filtrate was poured into a weighed foil cup, rinsing the Buchner flask into the cup with chloroform. The cup was placed in a fume cupboard while the chloroform evaporated off, and weighed to constant weight which usually occurred after 24-36 hours. Replicate determinations were made for each tank, and, where necessary, a third determination was carried out. Control determinations were made with no fish sample, to enable correction for any residue from the chloroform or filter paper.

(d) Ash determination: 2000 mg samples of freeze dried, ground fish were ashed overnight in a muffle furnace at 500°C and cooled in a dessicator to constant weight.

7.5. **ASSIMILATION**

Chromic oxide was used as an inert indicator to measure the assimilation of the experimental diets. The assumption was made that chromic oxide was neither absorbed or secreted by the gut i.e. it passed through the digestive system totally unaltered. Assimilation of dietary constituents was estimated by measuring their concentration relative to the concentration of
chromic oxide before and after passage through the gut.

7.5.1. **Collection of faeces.**

Chromic oxide was incorporated into the diets at the level of 1% by weight dry constituents. Other authors have used widely varying levels e.g. Schurch et al (1950) -2%; Dansky & Hill (1952) -0.2%.

Faeces were removed from the tanks once per day by means of a siphon tube, which was moved gently around the base of the tank. Faeces were collected over a period of a week, with the exception of Sunday and Monday, on two separate occasions chosen so as not to occur immediately after the fish had been weighed and measured. The faeces were collected in a sieve, rinsed gently in freshwater; placed in a labelled jar and deep frozen.

Each jar contained daily faeces samples from one tank pooled over a period of a week. 10-20 g samples of the diets used during the period of faeces collection were stored similarly in sealed jars in a deep freeze.

7.5.2. **Analysis**

Prior to analysis the food and faeces samples were freeze dried and ground in a Moulinex Coffee Grinder for a standard time of 30 seconds. The dried ground material was stored in a sealed jar in the deep freeze.

(a) Chromic oxide analysis: This method of analysis
was adapted from Schurch et al (1950), Dansky & Hill (1952) and Fisher, Atkins and Joplin (1972). The principle of the method is that chromic sesquioxide (Cr₂O₃) is converted by fusion with sodium peroxide to the soluble chromate ion (CrO₄²⁻). This is a coloured ion which can be assayed by spectrophotometry.

Approximately 200 mg samples of food or faeces were weighed out into weighed porcelain crucibles, and ashed overnight at 500°C in a muffle furnace. Porcelain crucibles were used because sodium peroxide reacts with both silica and nickel (Fisher, Atkins & Joplin, 1972). After cooling in a desiccator and reweighing the crucible, about 1 g of sodium peroxide was mixed well with the ash, and the mixture overlaid by a further 1 g of sodium peroxide. The crucible was returned to the muffle furnace, heated to about 500°C for 20 minutes, and allowed to cool. When cool enough to handle the crucible was placed on a hot plate and distilled water added cautiously to the white residue. The frothing and effervescence leached the melt off the sides of the crucible. Water was added until the effervescence had stopped, and the yellow solution in the crucible was boiled gently for about 10 minutes. The solution was poured into a beaker, and the crucible rinsed several times with distilled water. The solution in the beaker was filtered into a 500 ml volumetric flask, rinsing the beaker and washing the residue with more distilled water. After the solution had thoroughly cooled it was made up to 500 ml with distilled water. Optical density was measured at 375 μ using a SP200 Unicam spectrophotometer, matched cuvettes and a distilled water blank. The optical density obtained using known amounts of chromic oxide was measured and a calibration
curve calculated. Replicate determinations were always made, and where considered necessary, a third determination was carried out. The method was checked at intervals by determinations with known amounts of chromic oxide.

(b) Protein analysis: Protein determinations were carried out as described in section 7.4.3 b on 100 mg samples of faeces and 25 mg samples of food. Replicate and, where deemed necessary, triplicate determinations were made.

(c) Fat analysis: This was carried out as described in section 7.4.3 c on 500 mg samples of the food and faeces in experiment IV.

(d) Ash determination: Approximately 200 mg samples of food and faeces were weighed out, ashed overnight in a muffle furnace at 500°C and allowed to cool to constant weight in a dessicator.

7.6. **STATISTICAL METHODS**

Data from fish dissection was analysed using a calculator. Analysis of variance was the main statistical technique employed. Data on weights and standard lengths of the fish during the feeding experiments were analysed using a DEC-20 computer. A short FORTRAN - IV program was written to calculate relative condition. Expected weight was calculated from the length-weight relationship estimated for I-group *C. labrosus* from field samples. Use was made of the SPSS package (University of Pittsurge Statistical Package for the Social Sciences, version 6.02C (25th October 1976)) to calculate means, standard deviations, regressions and analyses of variance.
The aim of the first experiment was to quantify the protein requirement of juvenile *C. labrosus*. Dietary protein is necessary for three main purposes:

(i) Maintenance of existing tissues
(ii) Repletion of depleted tissues
(iii) Formation of new tissue or growth.

The amount of protein needed to produce maximum growth is of particular concern when the aim of fish culture is to produce food protein from cheap sources, because protein is usually the most expensive component of the diet. Investigations in chinook salmon (De Long et al., 1958) and in plaice (Cowey et al., 1970a) suggest that the protein requirement of fish is high, about 40-50% dry diet. However, these are carnivorous fishes near the top of the food chain and mullet, with their detritus/herbivorous feeding habits, might be expected to have a lower protein requirement more closely resembling the 25-40% reported for carp (Ogino & Saito, 1970).

8.1. SPECIAL METHODS

8.1.1. Experimental diets

The composition of the experimental diets is shown in Table 8.1. The diets were constructed so that they were approximately isocaloric, and that 10%, 20%, 40% and 60% of the calories respectively were supplied as protein. However, in most studies
protein requirement has been expressed as percent by weight of the dry diet, and in these terms the 4 diets used in this experiment contained 8.5%, 16.8%, 39.1% and 58.5% protein respectively. There was an increase in calories per gram dry diet with increase in protein content but this was small (4.07-4.32 kcal/g).

The mean total live fish weight in each tank at the beginning of the experiment was 50.38g. The high feeding level was designed to approximate to the optimum feeding level described by Vallet et al. (1970) for juvenile *L. aurata* - 6% wet weight fish. After adjustment was made for the fact that their diet contained only 25% moisture this was equivalent to a ration of 4.037g per tank in this experiment. However since:

(i) the temperature in this experiment was generally lower than 15°C used by Vallet et al. (1970).

(ii) for technical reasons it was desirable that the fish should consume the entire ration.

(iii) the effect of dietary protein level on growth would be more likely to be exhibited at a feeding level below optimum than above. At high feeding levels even a low protein diet may be consumed in sufficient quantity for protein not to be a limiting factor on growth.

the high feeding level was set at 3g diet per tank per day. The low feeding level was intended to be just above maintenance. Maintenance requirements for various fish species have been estimated by numerous authors (Davies, 1964; Dawes, 1930; Brown, 1946b) Davis, 1968). These vary widely, but the maximum maintenance est-
imate, after correction for the moisture content of the diet and the mean weight live fish in the tank in this experiment, was approximately 1 g diet per tank per day. This was used as the low feeding level. Each diet was fed at each feeding level to fish in replicate experimental tanks. Treatments were allocated at random (Fig. 8.1). Feeding was carried out as described in Chapter 7.

8.1.2. Experimental fish and procedure

The fish were collected at St. John's Lake during January and February 1976. Mortality was considerable during the first week after capture but the remaining fish appeared healthy and quickly began to feed on commercial fish pellets. On the 2nd March, 1976 the fish were transferred to the experimental tanks and continued to be fed with commercial fish pellets. The dates of commencement, remeasuring, and ending of the experiment are shown below.

<table>
<thead>
<tr>
<th>Date</th>
<th>Tank No.</th>
<th>Date</th>
<th>Tank No.</th>
<th>Date</th>
<th>Tank No.</th>
<th>Date</th>
<th>Tank No.</th>
</tr>
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<tbody>
<tr>
<td>10/3</td>
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<td>14/4</td>
<td>1-3,5</td>
<td>12/5</td>
<td>1-3,5</td>
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<td>1-3,5</td>
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<td>5-8</td>
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<td>14/5</td>
<td>10,11,</td>
<td>18/6</td>
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<td></td>
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<td></td>
<td>13,14,</td>
<td></td>
<td>13,14,</td>
</tr>
<tr>
<td>13/3</td>
<td>13-16</td>
<td>17/4</td>
<td>15-18</td>
<td>15/5</td>
<td>15-18</td>
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<tr>
<td>14/3</td>
<td>17-18</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

157
Post-branding mortality was slight in all tanks except tank 12, in which mortality exceeded 50% and many of the remaining fish were suffering from erosion of the caudal fin and tissues at the base of the fin. Neither the tank nor the remaining fish were used in the experiment and an extra experimental tank (tank 18) was set up. Tank 4 was a control tank in which fish were not fed in order to assess the nutritional importance of any food introduced into the tanks with the seawater, and of algae and bacteria which sometimes grew as a thin film on the sides of the tank between water changes.

The periods of faeces collection were:

(1) 23rd - 30th April, 1976 (excluding Sunday 25th and Monday 26th).

(2) 25th May - 2nd June, 1976 (excluding Sunday 30th May and Monday 1st June).

Sunday and Monday were excluded because the experiment was not visited on Sunday, and although faeces were removed on Monday, they were discarded because they might have been in the tank longer than 24 hours. The tanks were cleaned out and the seawater renewed once a week. The fish were exposed to 24 hours light.

8.2. RESULTS

8.2.1. Temperature

Temperature varied little from tank to tank. Insulation in the walls of the room was sufficient to prevent extreme diurnal fluctuation. The difference between daily maximum and min-
The maximum temperature was rarely greater than 2°C. Weekly maximum and minimum temperatures were calculated Figs. (8.2 - 8.5). Temperature increased slowly from March to April, was relatively constant during April, and increased slowly in May and June.

8.2.2. **Experimental diets**

The diets became less solid as protein content increased. After cleaning the tanks, the water became progressively less clear and more green in colour, particularly in tanks where fish were receiving a high feeding level of a high protein diet. The fish consumed the experimental diets readily. Immediately food was added it was surrounded by fish sucking at the surface of the lump. The ration was completely consumed in all tanks.

8.2.3. **Growth**

The branding marks were very clear after one month, less clear after two months and by the end of the experiment some were faint and difficult to read. The cut dorsal fin rays remained obvious except in a few cases where the second and third fin ray showed some evidence of regrowth. In tank 4, where no food was added to the tank, the fish rapidly became thin and dark. At the end of one month, 20% of the fish had died and the average weight loss of the survivors was 0.204g. These fish were returned to the holding tank. In other tanks mortality was fairly low during the first two months but considerably higher in the third month. Some of the mortality in the last month of the experiment could be attrib-
uted to technical problems with the compressor.

For each fish the following were calculated:

(i) Change in weight during each month of the experiment.

(ii) Total change in weight.

(iii) Instantaneous growth coefficient for each month of the experiment and for the whole experiment.

For each tank the following were calculated:

(i) Mean and standard deviation of length, weight and condition at the beginning of the experiment, and after 1, 2 and 3 months on experimental diets.

(ii) Mean and standard deviation of change in weight during each month of the experiment and for the whole experiment.

(iii) Mean and standard deviation of instantaneous growth coefficient for each month of the experiment and for the whole experiment.

Mean and standard deviations for the first and second months were calculated for all fish which survived until the end of the second month. Means and standard deviations and subsequent statistical analysis for the third month, and the whole experiment, were calculated for fish which survived until the end of the experiment.

Mean length, weight and condition are shown in Tables 8.2. to 8.5. Mean standard length, mean weight and maximum and minimum weekly temperatures are plotted in Figs. 8.2. to 8.5. Some fish gained in weight whereas others lost weight. Where growth occurred it was slow and the standard deviation of weights and
lengths was large. Growth did not proceed at a constant rate throughout the experiment. In the third month there was a marked increase in the mean weight and standard length of fish fed at a high feeding level, even when the fish had previously been losing weight. Initial variation in weight and length made the growth of fish in different tanks difficult to compare. Since change in weight was calculated for each fish individually, this could be used as a more convenient means of comparison without impairing the statistical significance of the data.

Some authors e.g. Cowey et al (1970a) Wohlfarth & Moav, (1972) found that change in weight of fish during a feeding experiment was significantly affected by initial weight, and hence made adjustments for variation in initial weight before the effects of treatments were investigated. Regressions of total change of weight on initial weight were calculated for the mullet from each tank in this experiment. The results are summarised in Table 8.6. In 11 out of 16 tanks variance due to the regression of change of weight on initial weight was non-significant, and amongst the other 5 the regression coefficient varied from -.2184 to +.4268. Initial fish weight did not differ significantly between replicate tanks, between fish fed at different feeding levels or between fish fed diets containing different proportions of protein (Table 8.7.). In view of this, and of the fact that there did not appear to be any consistent relationship between total change in
weight and initial weight, no adjustment for variation in initial weight was made.

Mean instantaneous growth coefficient and change in weight for each month of the experiment and for the whole experiment are shown in Tables 8.8 to 8.11. Mean change of weight for the fish of each tank was plotted against protein content of the diet (Figs. 8.6 to 8.9). The total variance of changes in weight of individual fish was resolved into components due to feeding level, protein content of the diet, the interaction between these effects, and a residual variance. Variance due to treatment (diet and feeding level) was significantly greater than the residual variance, which included variance within tanks and between replicate tanks. However, the interaction of diet and feeding level was highly significant \((p < .001)\) in each month and over the whole experiment. The two main effects were not acting independently, i.e. the effect of diet depended on feeding level, and/or the effect of feeding level varied with diet. The main effects were, therefore, investigated separately.

Variance of weight changes in the first month of all fish fed the 8.5% protein diet was resolved into variance due to feeding level and residual variance. This was repeated for the weight changes in the second and third months and in the whole experiment. Similar analyses were carried out on weight changes of fish fed the 16.8%, 39.1% and 58.5% protein diet. The results are summarised in Table 8.12.

The variance of weight changes in the first month of all fish fed at the low feeding level was resolved into variance
due to protein level in the diet and residual variance. This was repeated for weight changes in the second and third months, and in the whole experiment. Similar analyses of variance were carried out on the weight changes of fish fed at the high feeding level. The results are summarised in Table 8.13.

In the first month (Fig. 8.6) variance of change of weight due to protein level was highly significant at both high and low feeding levels ($p < .001$). On the 8.5% and 16.8% protein diets fish lost weight with slightly greater mean weight losses on the 8.5% protein diet. Variance of change of weight due to feeding level was non-significant among fish fed the 8.5% protein diet and only marginally significant among fish fed the 16.8% protein diet. Among fish fed the 39.1% protein diet, the variance of change of weight due to feeding level was highly significant ($p < .001$). At a high level, there was a slight increase in weight in both tanks whereas at the low feeding level there was a slight decrease in both tanks. There was an increase in weight among fish fed the 58.5% protein diet at both the low and high feeding level. The variance of weight change of these fish due to feeding level was non-significant.

In the second month (Fig. 8.7), the variance of change of weight due to protein level was highly significant at both the high and low feeding level. On the 8.5% protein diet the fish lost approximately the same amount of weight as in the first month. Variance of weight change due to feeding level was non-significant. Among fish fed diets containing 16.8 to 58.5% protein, the variance of weight change due to feeding level was highly significant ($p < .001$).
At the high feeding level there was a mean increase of fish weight, with the increase being greater for the fish fed the 39.1% compared with the 16.8% protein diet, but similar in fish fed the 39.1% and 58.5% protein diets. At the low feeding level there was a slight mean decrease in weight of fish on the 16.8% protein diet and a slight mean increase in fish weight in one tank and a slight mean decrease in the replicate tank where fish were fed a 39.1% and a 58.5% protein diet. Weight increases tended to be greater in the second month than in the first.

In the third month (Fig. 8.8) mean weight increases of fish fed at the high feeding level were considerably greater than in the second month. Variance of change of weight due to feeding level was highly significant \( (p < .001) \) at all protein levels. On all diets fish fed at the high feeding level showed a mean gain in weight whereas fish fed at the low feeding level showed a mean loss in weight. At the low feeding level the variance of weight change attributable to protein level in the diet was not significant \( (p > .05) \) and the mean weight loss was similar in all tanks irrespective of the protein content of the diet. At the high feeding level the variance of weight change due to protein level in the diet was highly significant \( (p < .001) \). Mean weight gain tended to increase with protein content of the diet from 8.5 to 39.1% protein. The mean weight gains of fish fed the 58.5% protein diet were markedly lower, similar to those of fish fed an 8.5% protein diet.

Mean total weight change is shown in Fig. 8.9. The variance in total weight change attributable to feeding level was highly significant at all protein levels \( (p < .001) \). The variance in
total weight change due to protein level was highly significant at both the low and high feeding level. At the low feeding level there was an overall mean loss of weight in all except one tank in which fish were fed the 58.5% protein diet. Mean weight loss tended to decrease with increase of protein in the diet. At the high feeding level increase in protein in the diet from 8.5 to 39.1% caused a marked increase in weight gain. The effect of dietary protein content on weight change was much greater at the high than the low feeding level. The mean weight gains of fish fed the 58.5% protein diet at the high feeding level were considerably lower than those of fish fed the 39.1% protein diet at the same feeding level.

Two methods were used to describe the relationship between dietary protein level and growth:

(1) A discontinuous straight line analysis as used by Hegsted (1948) for the relationship in rats, although later adapted for fish (Zeitoun et al., 1974; Ogino & Saito, 1970). An ascending line to the left and a line parallel to the dosage axis on the right are constructed, and optimum protein level is estimated as their point of intersection. The ascending line was plotted by linear regression to fit the means for groups of fish where an increase in protein level in the diets resulted in a significant increase in weight gain. The intercept of the horizontal line was equal to the average of the means of the gains which did not differ significantly. t-tests were used to compare means. The results are summarised on Table 8.14. The discontinuous analysis proved appropriate for fish fed at the high feeding level in the period 5-9 weeks.
The optimum dietary protein level was 37%.

Polynomial regression: A polynomial curve was more appropriate for the same fish in the period 9-14 weeks and 0-14 weeks (Figs. 8.8., 8.9.). The equations were:

9-14 weeks: \[ y = -0.1274 + 0.0230x - 0.0003x^2 \] (\( p \ll 0.05 \))

0-14 weeks: \[ y = -0.2795 + 0.0381x - 0.0005x^2 \] (\( p \ll 0.01 \))

where \( y \) = change in weight (g) and \( x \) = dietary protein level (% dry weight). Estimates of optimum dietary protein level were the same in both cases, 38%.

Among fish fed at the high feeding level in the first month and the low feeding level in the first and second month, weight gain increased significantly when protein was increased from 39.1% to the highest level of 58.5% and so optimum protein level could not be estimated. In the third month dietary protein level had no significant effect on growth of fish fed at the low feeding level. The regression of weight change over the whole experiment, 0-14 weeks, on dietary protein level for fish fed at the low feeding level did not differ significantly from linearity.

The regression equation was:

\[ y = 0.0036x - 0.2282 \] (\( p \ll 0.01 \)).

8.2.4. Conversion efficiency and protein efficiency ratio

Numerous ratios have been used to express the efficiency with which food is converted into fish flesh. In this work conversion efficiency and protein efficiency ratios were calculated in order to compare the weight of fish flesh produced per unit food and protein consumed respectively. They were defined as:
Conversion efficiency = \( \frac{\text{gain in wet weight fish}}{\text{dry weight food consumed}} \times 100\% \) 

Protein efficiency ratio = \( \frac{\text{gain in wet weight fish}}{\text{dry weight protein consumed}} \times 100\% \)

The change of weight of individual fish was summed to give a total change of fish weight in each tank. The dry weight of food and protein added to each tank are calculated:

<table>
<thead>
<tr>
<th>Dry weight of food added to each tank (g)</th>
<th>1st Month</th>
<th>2nd Month</th>
<th>3rd Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low feeding level</td>
<td>17.0</td>
<td>13.5</td>
<td>17.0</td>
</tr>
<tr>
<td>High feeding level</td>
<td>51.0</td>
<td>40.5</td>
<td>51.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weight of protein (g) added to tanks containing fish fed diets of protein level:</th>
<th>1st + 3rd months</th>
<th>Low feeding level</th>
<th>High feeding level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st + 3rd months</td>
<td>8.5%</td>
<td>16.8%</td>
<td>39.1%</td>
</tr>
<tr>
<td>Low feeding level</td>
<td>10.0</td>
<td>6.7</td>
<td>2.9</td>
</tr>
<tr>
<td>High feeding level</td>
<td>30.0</td>
<td>20.0</td>
<td>8.6</td>
</tr>
</tbody>
</table>

| 2nd month                                                                   |                 |                   |                   |
| Low feeding level                                                           | 7.9             | 5.3               | 2.3               | 1.2               |
| High feeding level                                                          | 23.8            | 15.9              | 6.8               | 3.4               |

A pooled C.E. and P.E.R. were calculated for the fish in each tank in each month of the experiment (Tables 8.15. and 8.16)
CE and PER were plotted against percent protein in the diet in Figs. 8.11 and 8.12. Little information was available about the first month or fish fed at the low feeding level because in relatively few tanks did fish show an overall gain in weight. In the second month conversion efficiency increased with dietary protein level up to 39.1% after which a further increase in protein did not further increase conversion efficiency. In the third month conversion efficiencies were improved. They increased with dietary protein up to 40% but declined sharply among fish fed a 58.5% protein diet.

For reasons stated above PER values for the first month and fish fed at the low feeding level were very limited. In the second month PER showed a slight tendency to decline with increase in dietary protein level. In the third month all PER values increased, and the decline with increasing % protein in the diet was more marked.

8.2.5. Mortality

Apart from tank 12, which was subsequently discarded, mortality was slight in all tanks in the first two months, but increased sharply in the third month reaching 50% in some tanks. Some fish deaths could be attributed to technical problems with the compressor and air supply but some of the dead fish were very dark and thin and were apparently suffering from malnutrition.

In Fig. 8.13, mortality was plotted against protein content of the diet with feeding level indicated. In the first and second month mortality was similar in all tanks irrespective
of feeding level and percent protein in the diet. In the third month mortality of fish fed at the high feeding level did not vary in any consistent way with diet, but at the low feeding level there was a tendency for mortality to decline with increasing percent protein in the diet. Analysis of variance indicated that the variance due to the interaction of feeding level and dietary protein content, and the variance due to feeding level were non-significant, whereas variance due to percent protein in the diet was significant \((p < .05)\) (Table 8.17). The daily ration was not altered in the third month in spite of the varying levels of mortality. Thus, where mortality was high, the remaining fish were receiving relatively more food and might have been expected to grow faster. In Fig. 8.14 mean change of weight of fish in each tank was plotted against the number of fish remaining in each tank at the end of the experiment, using symbols to indicate diet and feeding level. There was no evidence to suggest that a higher mortality resulted in increased growth of the remaining fish. The difference in the mean weight change of fish in replicate tanks could not be attributed to differences in mortality rates, possibly because most mortality occurred during the last 10 days of the experiment.

8.2.6. Relative condition

The mean and standard deviation of the condition of fish in each tank is shown in Tables 8.2 to 8.5 and in Fig. 8.15. The mean condition of the fish in replicate tanks was similar.
The mean condition of fish fed the 8.5% protein diet at both feeding levels tended to decrease during the first and second months of the experiment but in the third month, although the mean condition of fish fed at the low feeding level continued to decrease, mean condition increased at the high feeding level. The mean condition of fish fed the 16.8% protein diet decreased slowly at the low feeding level, and decreased in the first month but tended to increase thereafter in fish fed at the high feeding level. On the 39.1% protein diet the mean condition of fish fed at the low feeding level declined especially in the third month, whereas that of fish fed at the high feeding level increased steadily throughout the experiment. The mean condition of fish fed the 58.5% protein diet was unaltered or increased very slowly at both the high and low feeding levels in the first two months. In the third month the mean condition of fish fed at the high feeding level remained unaltered, but at the low feeding level mean condition declined in both tanks.

The variation of condition with dietary protein level is shown in Figs. 8.16 to 8.19. Fig. 8.16 shows that the condition of fish in all tanks at the start of the experiment was similar, as might be expected if the fish had been randomly distributed to the tanks. The exceptions were tanks containing fish to be fed 58.5% protein diet at the high feeding level, where mean condition was slightly greater than in other tanks. Figs. 8.17 and 8.18 show mean condition at the end of the first and second month. Mean condition tended to increase with percent protein in the diet at both feeding levels. The effect was more marked, and
the difference between the mean condition of fish fed at different feeding levels was greater, at higher protein levels. The increase in dietary protein content from 16.8% to 39.1% had the greatest effect on condition. By the end of the third month (Fig. 8.19) the difference between the mean condition of fish fed at different feeding levels was considerably greater, even among fish fed the 8.5% protein diet. At both feeding levels condition tended to increase with protein content in the diet up to 39.1% but the condition of fish fed the 58.5% protein diet was similar to that of fish fed the diet containing 39.1% protein.

As the condition of each fish had been calculated separately, an analysis of variance similar to that used to examine weight changes was undertaken. The interaction between feeding level and dietary protein content was highly significant (p < .001) showing that these two effects did not act independently. Separate analyses of variance were therefore carried out, and the results are summarised in Tables 8.18 and 8.19. At the end of the first month variance of condition due to feeding level was non-significant among fish fed the 8.5% and 16.8% protein diet, marginally significant (p < .05) among fish fed the 39.1% protein diet, and highly significant among fish fed a 58.5% protein diet. This is of limited importance since variance of initial condition due to feeding level was also significant (p < .01) in the case of fish fed a 16.8% and 39.1% diet. However, examination of the means shows that initially, condition tended to be higher in tanks to be fed at a low feeding level whereas by the end of the first month the reverse was true. By the end of the second month, variance of condition due
to feeding level was highly significant on all diets.

At both the high and low feeding level variance of condition due to protein content of the diet was highly significant (p < .01) both at the end of each month and at the end of the experiment. There was a slight tendency for initial condition to be lower amongst fish to be fed at the higher protein levels (with the exception of those to be fed the 58.5% protein diet at the high feeding level). Thus, the significance of the variance of relative condition due to protein level at the beginning of the experiment does not detract from the significance of the variance due to protein level at the end of the first, second and third months when condition tended to increase with dietary protein content.

8.2.7. Assimilation

In calculating assimilation or apparent digestibility it was assumed that no chromic oxide was lost in solution in the water or by digestion and absorption in the fish. The chromic oxide and moisture content of both food and faeces had been measured, enabling both to be expressed as mg dry weight per mg chromic oxide.

Then:

\[
\text{Apparent digestibility} = \frac{\text{mg dry weight food/mg Cr}_2\text{O}_3 - \text{faeces/mg Cr}_2\text{O}_3}{\text{mg dry weight food/mg Cr}_2\text{O}_3} \times 100\%
\]

Organic material in the food and faeces was taken to be the total dry material minus the residue remaining after ashing. Thus:
The mean nitrogen content of the food and faeces had been measured. This was converted to protein content by multiplying by 6.25 to account for the average nitrogen content of proteins. The assimilation of protein was then calculated as:

\[
\text{Apparent digestibility of protein} = \frac{\text{mg protein in food/ mg } \text{Cr}_2\text{O}_3 - \text{mg protein in faeces/ mg } \text{Cr}_2\text{O}_3}{\text{mg protein in food/ mg } \text{Cr}_2\text{O}_3} \times 100\%
\]

The apparent digestibility of dry material, organic material and protein were calculated for the fish of each tank. Separate calculations were made using data from the food and faeces from each tank sampled during the second period of faeces collection. The results are shown in Figs. 8.20 to 8.22. An analysis of variance of the apparent digestibility of dry material of fish from each tank in the first and second period, was carried out to resolve total variance into variance due to period of collection, feeding level, percent protein in the diet, the interaction between feeding level and dietary protein level, and residual variance which includes variance within tanks and between replicate tanks. Similar analyses of variance were carried out on the apparent digestibility of organic material and protein. The results are shown in Tables 8.20 - 8.22.

There were no consistent differences in the apparent digestibility of dry and organic material in the first and second
periods of faeces collection or at low and high feeding levels. Variance of apparent digestibility of dry and organic material due to period of faeces collection was non-significant. The differences between replicate tanks were quite large but there appeared to be an overall tendency for apparent digestibility of dry and organic matter to decrease with increase in protein content of the diet. The exception was fish fed the 39.1% protein diet at the high feeding level where evidence from the 2nd period of faeces collection indicated that apparent digestibility increased with protein in the diet up to 39.1% but declined when fish were fed a 58.5% protein diet. However, variance due to protein level and feeding level, and the interaction between them, was non-significant.

The pattern of variation of apparent digestibility of protein differed from that of dry and organic material. Overall variability and the variation between replicate tanks tended to be smaller (with the exception of 8.5% protein diet, low feeding level, first period and 16.8% protein diet, high feeding level, first period). There was no consistent variation of apparent digestibility of protein with either period of faeces collection or feeding level, with the possible exception of fish fed a 58.5% protein diet where apparent digestibility was slightly lower at the higher feeding level. Fish in four tanks during the first period seemed to have anomalously low protein assimilation (8.5% protein, low feeding level; 16.8% protein, high feeding level; both tanks containing 39.1% protein, high feeding level), but generally the apparent digestibility of protein tended to increase with protein in the diet up to 39.1%. The protein
assimilation of fish fed the 58.5% protein diet was similar to that of fish fed the 39.1% protein diet. Variance of apparent digestibility of protein due to period of faeces collection and feeding level were non-significant, whereas variance due to dietary protein content was significant at $p < .05$.

8.2.8. **Body composition**

The results of proximate analysis of fish are summarised in Table 8.23 and illustrated in Figs. 8.23 and 8.24. All fish contained small amounts of chromic oxide. In most cases this was probably due to food retention in the gut but it may indicate that chromic oxide was assimilated by the fish to a limited extent. The protein content was obtained by multiplying the nitrogen content by 6.25, a factor which takes into account the mean nitrogen content of proteins.

Moisture, ash, lipid and protein content are plotted against dietary protein level, with feeding level indicated by symbols in Figs. 8.25 and 8.26. An analysis of variance of moisture content of the pooled fish samples was carried out to resolve total variance into variance due to feeding level, percent protein in the diet and the interaction between these two effects. Similar analyses of variance were calculated for ash, protein and lipid content (Tables 8.24 and 8.25). In each case the interaction of feeding level and dietary protein content was non-significant. With a few exceptions, e.g. protein content of fish fed the 8.5% protein diet, the body composition of fish from replicate tanks was very similar.
Moisture content tended to decline with increase in protein level in the diet. The moisture content of fish fed at the high feeding level was consistently lower than that of fish fed at the low feeding level, particularly among fish fed the 58.5% protein diet. Variance due to both feeding level and dietary protein content were significant (p < .01). Ash content showed a very similar pattern of variation to that of moisture content with a marked difference between fish fed at the low and high feeding level. Variance due to feeding level and percent protein in the diet were highly significant.

The lipid content of fish fed at the high feeding level was consistently higher than that of fish fed at the low feeding level. Lipid content increased with dietary protein at both feeding levels. There was some indication, particularly at the high feeding level, that increase of dietary protein from 16.8 to 39.1% had a greater effect on lipid content than the increase from 39.1 to 58.5%. Variance due to feeding level (p < .001), and variance due to dietary protein content (p < .05) were both significant.

There were no significant differences in protein content between fish fed at a high and low feeding level. With increasing dietary protein from 16.8% to 58.5%, the protein content of the fish tended to decrease slightly. The protein content of fish in two of the four tanks containing fish fed the 8.5% protein diet was similar to that of fish receiving the 16.8% protein diet, whereas in the other two tanks it was considerably lower. This was not related to feeding level. The variance due to both feeding
level and dietary protein level was non-significant.

Moisture content has been used to estimate lipid content in fish. There was a highly significant negative correlation between moisture content and lipid content of juvenile C. labrosus \( (r = -0.8768, p < 0.001) \) (Fig. 8.27). The regression equation was calculated as:

\[
y = -1.33X + 108.29
\]

where \( y \) = lipid content (mg/100mg dry wt).
\( X \) = moisture content (mg/100mg wet wt)

Variance due to regression was significantly greater than the variance due to deviations from regression. \( (p < 0.01) \).

Lipid content was also highly correlated with relative condition \( (r = 0.9234, p < 0.001) \) (Fig. 8.27). The regression equation was calculated as:

\[
y = 55.22X - 35.31
\]

where \( y \) = lipid content (mg/100mg dry wt).
\( X \) = relative condition

Variance of lipid content due to regression was significantly greater than variance due to deviations from regression \( (p < 0.01) \).

8.2.9. Variation of gutted weight, gut weight and liver weight

The effect of protein content of the diet and the feeding level on the relative proportions by weight of liver, gut and gutted fish was investigated. As fish under different treatments had grown at different rates, it was not valid to compare weight of gutted fish, liver and gut directly. They were, therefore, expressed as a percentage of the total weight of each fish. The mean and standard deviation of actual and percentage gutted fish, liver and gut weight are shown in Tables 8.26 to 8.28. The variation of mean
and standard deviation of gutted fish, liver and gut weight, as a percentage of total weight, with dietary protein level is shown in Figs. 8.28. to 8.30.

An analysis of the variance of the percent by weight of the various components was carried out to resolve the total variance into variance due to feeding level, protein level, the interaction between these two effects and residual variance which included variance within tanks and between replicate tanks. In each case the variance due to the interaction of protein level and feeding level was significant, i.e. the two main effects were not acting independently, and it was necessary to examine the effects of protein level and feeding level separately. The results are summarised in Tables 8.29 to 8.31.

The percent gutted fish tended to increase with increasing protein level in the diet. Feeding level appeared to have no effect on percent gutted fish weight. The analyses of variance indicated that the variance due to feeding level was non-significant at each protein level, whereas variance attributable to protein level in the diet was highly significant at both the low and high feeding levels (p < .01).

Relative liver weight did not vary consistently with the protein content of the diet. The analyses of variance showed that the variance of percent liver weight due to dietary protein level was non-significant at the low feeding level but marginally significant (p < .05) at the high feeding level. This was probably due to the particularly large livers of fish from one tank fed the 58.5% protein diet. Variance due to feeding level was non-significant
among fish fed the 16.8% and 39.1% protein diet, but significant (p < .01) among fish fed the 8.5% and 58.5% protein diet. Amongst fish fed both the lowest and highest protein level, fish fed at the higher feeding level showed tendency to have relatively larger livers.

Mean percent gut weight tended to decrease with increasing protein in the diet, whereas feeding level appeared to have relatively little effect. Variance due to feeding level was non-significant at all protein levels, but variance due to protein level was significant at both the low feeding level (p < .05) and the high feeding level (p < .001).

Changes in the proportion by weight of gut, liver and gutted fish undoubtedly do occur as fish gain in size. However, reference to Figs. 8.2 to 8.5 showed that the actual weight of the fish fed the 39.1% protein diet was higher than that of fish fed the 16.8% and 58.5% protein diets, which had higher and lower relative gut weights respectively. Also, in almost all cases, the proportions by weight of gutted fish, liver and gut varied very little with feeding level although by the end of the experiment fish fed at the higher feeding level were longer and heavier than those fed the same diet at the lower feeding level. The observed variation in the relative weight of gut, liver and gutted fish could not, therefore, be attributed to differences in body size.

8.2.10. **Histology**

The gills, gut and liver of three fish from each tank were examined histologically. All fish from the same tank, or from tanks receiving replicate treatments, tended to show similar features
although to varying degrees.

(a) Gills: The gills of fish fed the 8.5% protein diet at the low feeding level showed a lifting and thickening of the epithelium, such that some filaments appeared to fuse. Serial sections indicated that this was not an artifact due to sectioning technique (Plate 8.1). There were also sections of apparently healthy gills. At the high feeding level there was some evidence for thickening of the epithelium, and swellings were noted on some filaments (Plate 8.2). Among fish fed the 16.8% protein diet at the low feeding level epithelial thickening and distortion of lamellae (Plate 8.3) was quite common. Swellings similar to those shown on Plate 8.2 were also seen. Fish fed the same diet at the high feeding level had gills in which there were areas of relatively healthy gill and areas showing epithelial thickening and lamellae distortion.

Fish receiving the 39.1% protein diet appeared to have the most healthy gills (Plate 8.4). Plates 8.5 and 8.6 show filaments from fish fed at the low and high feeding levels respectively. At the low feeding level the lamellae tended to be more spindly than at the high feeding level.

Fish receiving the 58.5% protein diet showed the greatest alteration of gill structure at both high and low feeding levels. The lamellae were distorted (Plate 8.7) and swollen (Plate 8.8). In certain fish some filaments appeared to be disintegrating completely and showed signs of bacterial infection (Plate 8.9).

(b) Liver: Myxosporidian cysts were common in liver sections, and present among fish from all tanks except one containing fish fed 16.8% protein diet at the high feeding level, and one
containing fish fed 39.1% protein diet at the high feeding level. Myxosporidian cysts tended to be more common amongst fish fed at the low feeding level irrespective of dietary protein content. The cysts varied in size, but some were quite large and complex (Plate 8.10).

In none of the sections was there obvious deterioration of liver structure. The main differences between the livers of fish receiving different treatments lay in the amount of fat or glycogen deposited in the liver cells. Plates 8.11 and 8.12 show livers of fish fed the 8.5% and 58.5% protein diet at the low feeding level. There was little or no fat/glycogen deposited in the liver cells of either. At the high feeding level there was a little fat/glycogen in the liver cells of some of the fish fed the 8.5% and 16.8% protein diet, but there were relatively large amounts of fat/glycogen in the liver cells (Plates 8.13 and 8.14) of fish fed the 39.1% and 58.5% protein diets.

c) Guts: There appeared to be very little difference in the structure of the guts of fish receiving different treatments. The amount of fat deposited between the pyloric caeca tended to be greater among fish fed at the high feeding level. Myxosporidian cysts were found on the pyloric caeca (Plate 8.15) and intestine wall (Plate 8.16) in some fish from all tanks, irrespective of diet. Trematode worms were found, particularly in the stomach (Plates 8.17, 8.18)
8.3. DISCUSSION

8.3.1. Growth

During the course of the experiment overall mean weight increased from approximately 1.09g to 1.23g, an increase of only 12.7%. However, in many tanks, particularly those fed diets low in protein at the low feeding level, the fish actually lost weight, and the mean weight of fish fed on the 39.1% protein diet increased from 0.87g to 1.65g, a gain of 89.7%. The instantaneous growth coefficient of wild C. labrosus age group I was .221 (Section 3.1.3). In most of the experimental tanks the instantaneous growth coefficient of the fish was well below this, but in the third month in one tank containing fish fed the 16.8% protein diet and in both tanks containing fish fed the 39.1% protein diet, the instantaneous growth coefficient was greater than .221. However, in 1975 I-group C. labrosus from St. John's Lake increased in weight from .655g in February to .908g in April to 2.42g in June. Thus even the fish which showed optimum growth in the experiment grew slower than fish of equivalent species and age group in St. John's Lake. De Silva & Perera (1976) also reported relatively small absolute weight gains in feeding experiments with young M. cephalus, but could not specify the reason. Apart from feeding level and dietary protein level, other factors which may have contributed to the relatively slow growth rate include photoperiod and seasonal variation in metabolism, temperature, salinity, stocking density and its effect on the concentration of oxygen and waste products in the water, anaesthetising, handling and branding of fish and...
dietary factors such as vitamin, mineral, and essential fatty acid requirements, carbohydrate and protein source, and level and frequency of feeding.

(a) Photoperiod and seasonal variation in metabolism and enzyme activity: In the experiment described in this Chapter, growth in the period 9-14 weeks was considerably greater than in either 0-5 or 5-9 weeks and corresponded to the time of increased growth in wild fish. Several authors have reported seasonal variations in growth (e.g. brown trout, Brown, 1946b) and the relationship between growth, net efficiency and food consumption (e.g. sculpins, Warren & Davies, 1967), which are not apparently attributable to existing environmental conditions and seem to indicate a possible seasonal cycle in metabolism. In turbot, pike, perch and bream rates of production of pepsin, trypsin, amylase and lipase also seem to vary seasonally (Ananichev, 1959) although how this is mediated is unclear.

In some species, including coregonids (Hogman, 1968) and brown trout (Swift, 1955), the seasonal growth cycle of wild fish has been found to be far more highly correlated with day length than with temperature. The standard rate of oxygen consumption of brown trout maintained at 10°C but exposed to natural day light increased from 30 mg kg\(^{-1}\) hour\(^{-1}\) in March/April to 63 mg kg\(^{-1}\) hour\(^{-1}\) in late Autumn (Beamish, 1964), although in contrast, sunfish adapted to a 15 hour day had a lower respiration rate than those adapted to a 9 hour day (Roberts, 1964). The growth response to photoperiod also seems to vary somewhat with species. Brown (1946b) found that at 11.5°C the specific growth rate of trout was significantly lower.
when maintained in a regime of 12-18 hours light per day than
less than 12 hours light per day. However, Gross et al (1965) reported
that the growth of green sunfish increased when photoperiods
were lengthened. This was not only due to stimulation of food
consumption, which would be an irrelevant factor in a controlled
ration feeding experiment, since the increasing day length also
found that long day length had a growth promoting effect on
channel catfish although similar results were not obtained by
Tiemeier et al (1970b) working on the same species. Amongst juvenile
sole, photoperiod had no significant effect on survival or
growth (Fuchs, 1978).

It seems that photoperiod interacts with other
factors, particularly temperature and age of the fish, in its
influence on growth, which appears to be mediated via stimulation
of the pituitary to produce growth hormone. Reports of the in-
fluence of photoperiod on growth are somewhat conflicting and it
may be that the direction of change in day length is of greater
significance than day length itself (Gross et al, 1965).

The juvenile C. labrosus in this experiment were
subjected to 24 hours artificial light although it is possible
that some extraneous light reached the room around the door.
Observations in the laboratory showed that in darkness the fish
tended to spread out, hanging motionless in the water, whereas in
light they tended to form a shoal and swim around the tank, gener-
ally showing a higher level of activity. Thus the abnormally long
light period may have depressed growth by stimulating activity and
metabolism, and hence increasing the maintenance requirement and decreasing the energy available for growth. Larval sole grew better in 24 hours light (Fuchs, 1978) but constant illumination has also been found to result in stress and reduced growth in Blennius pholis (Qasim, 1955). Juvenile C. labrosus were observed to feed in the dark and observations in the field suggested that they have a natural feeding peak during the night (Chapter 5). Thus in later experiments, in the absence of means to impose a more natural lighting regime, the fish were subjected to 24 hours darkness. This was not ideal - long term stress effects have also been reported in fish held in continuous darkness (Brett, 1979).

(b) Temperature: Temperature rose steadily throughout the experiment. The temperature records were inadequate in that only maximum and minimum were recorded, and the temperature regime of two days with the same range may be very different. A continuous temperature record or a method of accurately controlling the temperature in the room would have been preferable. The increase in the rate of growth in the third month may have been related to temperature, although the mediation of the effect of temperature on fish growth is unclear.

The effect of temperature on growth and growth efficiency is complex, and depends on factors such as ration size, diet and previous temperature history. The interaction between temperature and ration size was demonstrated by Wurtsbaugh & Davis (1977b) for rainbow trout. At rations near maintenance level elevated temperatures decreased growth but as feeding level increased this effect was reduced, and at ad lib feeding levels elevated tem-
perature up to $17^\circ C$ improved feeding rate and growth. For fingerling sockeye salmon the general shape of the growth-ration curve shifted gradually from a simple concave form at low temperatures to a sigmoid shape at high temperatures (Brett et al, 1969). The optimum temperature for growth declined as ration was reduced in both sockeye salmon and brown trout (Brett et al, 1969; Elliott, 1976c).

Temperature also interacts with ration size in its effect on growth efficiency. Paloheimo and Dickie (1966b) in their review concluded that an increase in temperature increased the overall energy turnover but the relationship between ration size and growth efficiency i.e. distribution of energy, was not affected. However, Wurtsbaugh & Davis (1977) found that in rainbow trout increased temperature resulted in reduced efficiencies at low feeding levels although it had little effect on efficiency at high feeding levels. Similar results were reported by Brett et al, (1969) for sockeye salmon. Among both brown trout (Elliott, 1976c) and juvenile C. _labrosus_ Flowerdew & Grove (1980) increase in temperature resulted in peak efficiency occurring at a higher ration size. A genuine effect of temperature on ration utilisation also seems to be indicated by the work of Hochachka & Hayes (1962) who found that the biochemical pathways involved in glucose metabolism in trout differed at $4^\circ C$ and $15^\circ C$.

The effect of temperature on growth and growth efficiency is also influenced by the composition of the diet. Dupree and Sneed (1966) found that although casein was utilised as
efficiently by channel catfish at 20.6°C as at 24.4°C, wheat gluten was used more efficiently at 24.4°C. The optimum dietary protein level for chinook salmon increased from 40% to 55% when temperature increased from 8.3°C to 14.5°C (De Long et al, 1958).

On ad lib feeding increase in temperature causes an increase in food consumption but in the experiment described in this chapter the fish were fed a fixed ration throughout. However, mullet are iliophagous, i.e. they consume their own faeces. Thus it is conceivable that, at the higher temperature in the third month, the fish were consuming a larger ration by increased ingestion of faeces. Another way in which effective ration size may have been increased is by improved digestibility of food as was observed in Tilapia mossambica at higher temperatures (Mironova, 1976). However in Experiment I there was no evidence to suggest that digestibility during April differed from that at the end of May. Bacterial growth on the faeces and sides of the tank may also be a source of additional nutrients. Bacterial growth on the tank was shown not to be important in the first month since starved fish lost weight rapidly, but the higher temperatures in the third month and the nutrients leached from food and faeces may have promoted some bacterial growth in tanks where fish were fed. Bacterial action on faeces of brown trout over 48 hours at 3.8°C - 17.1°C (Elliott, 1976b) and of Carassius auratus over 14 days at 21.5°C (Davies, 1964) was found to be negligible. Other authors have reported similarly in both freshwater (Gerking, 1955b) and seawater (Wood, 1958). In
Experiment I faeces were generally removed after 24 hours and on only one day a week after 48 hours. It, therefore, seems unlikely that increased bacterial growth on the faeces would be sufficient to account for increased growth in the third month.

(c) Salinity: Paloheimo & Dickie (1966b) in their review of results of various feeding experiments concluded that salinity altered the position and slope of the relationship between metabolism and body weight, and growth efficiency and ration size, i.e. changed the basic distribution of energy. In support of this in M. acrolepis the relationship between oxygen consumption and body weight varied from $Q = 0.756W^{0.832}$ at 7% to $Q = 0.538W^{0.876}$ at 20% and $Q = 0.486W^{0.893}$ at 30% (where $Q =$ oxygen consumption and $W =$ body weight) (Mathew, 1976).

Kinne (1960) found the growth efficiency of desert pupfish was at a maximum at 15%, and declined at 35%, and in freshwater, and the growth of Paralichthys lethostigma post larvae varied with salinity (Deubler, 1960; Stickney & White, 1974). Tilapia nilotica grew as well in 18% as in freshwater (Chervinski, 1961) but catfish grew less well and converted less efficiently at 10% compared with 2% (Arunachalam & Reddy, 1979).

Mullet can characteristically withstand wide salinity fluctuations but it appears that this involves a metabolic cost which is reflected in reduced growth rates (Holt & Strawn 1977). The metabolic cost of osmoregulation of M. cephalus at different salinities, measured by oxygen uptake, was determined by Nordlie & Leffler (1975). They found that M. cephalus were excellent ionic
regulators and their metabolic rate in freshwater was not very different from that in an isosmotic medium. In a hyperosmotic medium, metabolic rate was greatly elevated because the fish drank the medium to replace osmotic water losses, which involved active transport of sodium ions in the gut and gills. The osmoregulation of C. labrosus and L. ramada in estuarine fish ponds in the Bay of Arcachon was examined by Lassere & Gallis (1975). Whereas L. ramada maintained plasma sodium over a range 0.5% to 35%, the equivalent range for C. labrosus was 5% to 35%. At lower and higher salinities plasma osmotic pressure decreased and increased respectively. De Silva & Perera (1976) found that when M. cephalus were fed ad lib. under a variety of salinity conditions, growth was greatest at 20%, slightly less at 1% and 10%, and declined markedly at 30%. Conversion efficiency was salinity dependent, being highest at 10%, and declining at both lower and higher salinities. In L. aurata growth and food conversion were optimum at 20% and declined at lower and higher salinities (Vallet et al, 1970).

Unfortunately in Experiment I pumping from the estuary could only be done at high water when the pump inlet was submerged. The only freshwater supply was a small erratic well. Thus, C. labrosus were maintained at 33-35% which is near the limit of their capacity for ionic regulation. Although the plasma osmotic pressure would be maintained, the metabolic cost would be expected to be relatively high. This may be one reason for the low growth observed in the experiment in comparison to growth in the less saline environment of St. John's Lake.
Salinity also interacts with factors such as temperature and feeding level. For example in the hogchoker maximum growth occurred at 25°C in a salinity of 30% but 15°C at 0%. (Peters and Boyd, 1972). The work of Lasserre & Gallis (1975) and that of Nordlie (1976) on *M. cephalus* suggested that the metabolic cost of osmotic regulation was higher at lower temperatures. Similarly Mires et al (1976) reported that the mortality of *M. cephalus* exposed to an osmotic stress was higher at 10°C than at 17°C. One factor contributing to the increased growth observed in the third month of Experiment I may have been a reduction in the metabolic energy required for osmoregulation. Among juvenile *M. cephalus* maximum growth occurred in salinities of 20%, but the salinity of maximum conversion efficiency varied with feeding level. Thus on excess diet conversion efficiency was greatest at 10%, whereas on a fixed ration conversion efficiency decreased with increasing salinity (De Silva & Perera, 1976). Salinity stress may have been particularly acute after weighing and measuring since treatment of salmon smolts with MS222 resulted in a marked reduction of their ability to tolerate seawater (Bouck & Johnson, 1979).

The scope for increasing growth via the manipulation of environmental salinity was illustrated by Brett & Sutherland (1970) who reported that the growth of sockeye salmon was improved from 14 to 24%/day by using isosmotic salinity and increasing photoperiod. The increased growth was not only due to increased food intake since conversion efficiency was improved at each ration
level. Also important, particularly with respect to Experiment I, is the fact that the dietary protein level which promoted maximum growth of rainbow trout was lower at lower salinities (Zeitoun et al., 1973). It has been suggested that the higher mineral turnover in fish maintained in seawater caused the increased amino acid, and hence protein, requirement (Lall & Bishop, 1979).

(d) Stocking density: The review of Paloheimo & Dickie (1966b) suggested that stocking density, like salinity, had a fundamental effect on the partition of energy in the fish. De Silva & Perera (1976) suggested that confinement in experimental tanks caused stunting in juvenile *M. cephalus*. Several authors have reported that growth and/or growth efficiency of a variety of fish species was affected by stocking density (e.g. carp, Mislov (1973), Kawamoto, 1961, Andrews et al. (1976); rainbow trout, Refstie (1977), Kilambi et al. (1977); Atlantic salmon, Refistie & Kittelsen (1976)). In most cases growth and growth efficiency declined with increase in stocking density, although in other species, such as the estuarine grouper *Epinephelus salmoides*, density up to a threshold level of 60 fish/m$^3$ had no effect on growth or food conversion (Teng and Chua, 1978). In sable fish fastest growth occurred at intermediate stocking densities with slow growth at low and high densities (Kennedy, 1970). He suggested that a degree of social interaction stimulated food consumption. The growth and growth efficiency of isolated *Dicentrarchus labrax* was poor compared with fish maintained in groups (Stirling, 1977) and Brown (1946b) found that two year
old trout grew more slowly at densities 31 and 501 per fish than at 121, 231 and 351 per fish.

Olla & Samet (1974) showed that juvenile *M. cephalus* had a high level of visually mediated fish to fish attraction. In the presence of a feeding group of the same species feeding was facilitated. In the presence of a non-feeding group it was inhibited. Thus at low densities the presence of other fish of the same species may stimulate growth by depressing metabolic energy requirement and facilitating feeding, but as density increases growth may be limited by oxygen concentration and accumulation of metabolic products such as ammonia.

*M. cephalus* greater than 2g were good oxygen regulators with oxygen uptake independent of external oxygen concentration from 1.5 - 5.5 ml O$_2$/l (Nordlie & Leffler, 1975). However in smaller individuals (0.45 - 1.25g) the slope of the regression of oxygen uptake on external oxygen concentration was 0.158, 2.5 times greater than that of larger fish. Thus smaller fish were much more dependent on external oxygen concentration. Kutty & Mohamed (1975) found that *Rhinomugil corsula* showed increased anaerobic metabolism and protein utilisation at low oxygen concentrations with no apparent oxygen debt. Although one would expect metabolism, energy production and hence growth to be depressed during anaerobic respiration the authors suggested that biochemical pathways yielding energy more efficiently than conventional glycolysis might be in operation in mullet during hypoxia.

When oxygen concentration was measured it was uniformly high, approaching 100% saturation in tanks where the
airstone was bubbling strongly. However, occasionally the air system became unbalanced or leaks occurred, and then the oxygen concentration in the affected tank or tanks fell dramatically to less than 20% saturation, which may have had an adverse effect on growth and conversion efficiency, since both were depressed in juvenile coho salmon at low oxygen concentrations. Where oxygen supply was interrupted for any length of time, as in power cuts, some mortality occurred although this was not obviously related to size, diet, or feeding level.

Wood (1958) examined the nitrogen excreted by several fish and found that in the stony flounder, sculpin, and sea perch respectively ammonia made up 84%, 63% and 48% of the total. Urea and ammonia together made up 75-90% of the total excreted nitrogen. The toxic form is unionised ammonia which passes easily through cell membranes. It is thought to act intracellularly, possibly by interfering with osmotic balance (Lloyd & Orr, 1969). Both temperature and pH affect the \((\text{NH}_3) \rightleftharpoons (\text{NH}_4^+)\) equilibrium and hence toxicity. Thus toxicity is increased at high pH when a greater proportion of ammonia exists in the unionised form and is also affected by salinity due to ionic exchange relationships between \(\text{Na}^+\) and \(\text{NH}_4^+\) (Sousa et al, 1974).

Hampson (1976) reported that the toxicity threshold for trout was 0.3 ppm N in water as unionised \(\text{NH}_3\). Carp are more resistant than trout (Kawamoto, 1961) but prolonged exposure to sublethal concentrations caused marked regressive changes including tissue disintegration, especially directly ex-
posed tissues, e.g. gills, gut, skin, lesions in the blood vessels and capillaries, extensive haemorrhages and abundant secretion of mucus. In Dover sole *Solea solea* (L), and turbot *Scophthalmus maximus* (L), the threshold concentration of unionised ammonia below which there was no detectable effect was 0.066 mgN/l and 0.110 mgN/l respectively. Above this level growth was depressed linearly with increase in unionised ammonia with no growth occurring at concentrations of 0.77 - 0.38 mgN/l for sole and 0.90 - 0.30 mgN/l for turbot (Alderson, 1979). Similarly in channel catfish, *Ictalurus punctatus*, growth was depressed linearly with increase in ammonia concentration, no growth occurring at 0.967 mgN/l.

In trout ammonia levels greater than 0.5 ppm and oxygen levels below 5 ppm caused a decline in growth rate, damage to gill tissue, and occasionally to kidney and liver tissue (Larmoyeux & Piper, 1973). From his investigations in carp ponds, Kawamoto (1957) concluded that concentration of waste products such as ammonia was more commonly the limiting factor than oxygen concentration, but these two effects may not be independent since a decrease in dissolved oxygen increased the toxicity of unionised ammonia in rainbow trout (Merkens & Downing, 1957).

In the juvenile grey mullet in this experiment histological studies of the gills indicated abnormalities in some fish which may have been due to the concentration of ammonia. This is discussed in more detail later in this section but the accumulation of waste products may have contributed to the generally poorer growth in the experimental tanks compared with St. John's Lake.
There is also evidence that, at least in some species, living space per se, independent of oxygen or excretory product concentration, has an effect on growth rate. Chen & Prowse (1966) divided ponds unequally with wire netting and stocked them with Puntius gonionotus and Tilapia mossambica at the same stocking density. Water flowed freely between the two areas but there was significantly higher growth rate in the larger area. Similar results were obtained when Tilapia mossambica was grown in ponds of different sizes - the fish grew largest in the largest ponds.

Several attempts have been made to derive formulae for calculation of maximum permissible stocking densities. Most are designed for salmonids and apply to rearing troughs with a continuous water flow. Haskell (1955) also included formulae for rearing ponds which more closely approximates to this experiment situation, and using his data the maximum permissible stocking density of the smallest size brown trout ($< 2.5\text{cm}$) at the highest recommended feeding level, and equivalent temperature is 350-400g/100l. This is 7-8 times the stocking density used in this experiment, and so it seems unlikely that density was the factor limiting growth. However as there was evidence for gill damage which may have been due to ammonia it was evident that this tank system was not ideal. A flow-through system would have been better but was not feasible with the facilities available. In later experiments the water in the tanks was changed more frequently (twice a week) in an attempt to improve water quality.
Handling, anaesthetisation and branding: It is possible that stress to the fish due to handling, anaesthetising and branding in some way inhibited growth. The use of an anaesthetic enabled the weighing, measuring and branding procedure to be carried out quickly and carefully, minimizing loss of scales. It is possible to weigh fish without the use of an anaesthetic by using for example a pre-weighed beaker of water on the balance thereby avoiding post anaesthesia stress. However, handling had a profound effect on the routine oxygen consumption of *M. cephalus*, *L. dumerili* and *L. richardsoni*, lasting approximately 8 hours, (Marais, 1978). Thus, in the case of juvenile mullet, it was felt that the stress caused by handling and removing excess water from the fish, and the risk of the fish jumping out of the beaker was such that the use of an anaesthetic was preferable.

Dick (1975) reported that *M. chelo* showed no evidence of shock after anaesthetisation with tricaine methanesulfonate. However, treatment with MS222 reduced salinity tolerance of coho salmon smolts (Bouck & Johnson, 1979) and Goddard et al (1974) found behaviour abnormalities in yearling lake trout persisting for five days after anaesthetisation with 150 ppm MS222. In this experiment the grey mullet were anaesthetised with 100-200 ppm MS222. They recovered quickly after anaesthetisation and fed on the same day. There was no evidence of abnormal behaviour or other detrimental after effects but in all species so far examined, MS222 caused a degree of chemical stress and haemoconcentration (Smit et al, 1979a,b).
The method of marking the fish was selected to cause the minimum effect on fish growth. Tagging was discounted due to the size of the fish and its detrimental effects (Roberts et al., 1973a, 1973b, 1973c). Marking fish by injection with liquid latex dyes was attempted (Riley, 1966) but proved unsatisfactory because the dye was not visible through the skin. Freeze branding has been used successfully with salmonids (Laird et al, 1975; Everest & Edmundson, 1967), and was quick and simple to carry out, and therefore suitable for the large numbers of fish involved in this experiment. Laird et al (1975) reported that in salmonids the skin wound caused by freeze branding healed rapidly and that the inflammatory reaction was of short duration and had no long term deleterious effects. Post-branding mortality among the mullet was less than 2% and most fish showed no apparent ill effects. The mark grew darker over the first 5-6 days and thereafter faded slowly. After 9 weeks the marks were distinct but by the end of the experiment considerable fading had occurred. Laird et al (1975) found freeze branding suitable for marking salmonids as small as 5cm. Some of the mullet in this experiment were even smaller than this.

In order that the maximum number of brands per fish was limited to two, the 50 fish were identified by a combination of freeze branding and fin ray clipping. Coyle (1967) investigated the effect of fin clipping on yellow perch and found that, although it had some effect on survival in the wild, in most groups it had no effect on growth. There was no evidence
that losses were caused by the invasion of pathogens. Coble (1967) suggested that there might be a long term effect on growth if, for example, fin clipped fish expended more energy in swimming or maintaining position. The dorsal fin of a fish like the mullet is used in turning but fish with the first, second, and third fin ray cut were observed to swim and turn with no apparent difficulty.

While there was no direct evidence it was felt that the trauma of fin ray clipping and branding on such a small fish might have contributed to their slow growth. Therefore, in subsequent experiments the fish in one tank were not marked by either branding or fin ray clipping, and growth was compared with that of fish in the replicate tank where fish were marked in the normal way.

(f) Vitamin requirements: The vitamin requirements of *C. labrosus* have not been investigated in detail and although the fish in this experiment did not show symptoms of acute vitamin deficiency, retarded growth is a common result when fish are fed diets containing inadequate vitamin levels (Halver, 1964, 1972). Essential vitamin requirements have been investigated for several species including trout (Poston, 1976; McClaren et al., 1947), carp (Parova, 1976; Zobairi, 1956; Aoe et al., 1967), silver salmon (Coates & Halver, 1958), chinook salmon (Halver, 1957), eels (Arai et al., 1972a), channel catfish (Lovell, 1973; Andrews & Murai, 1975, 1979; Dupree, 1966), turbot (Adron, et al. 1978). Among these species essential vitamins tended to be similar, and included thiamin, riboflavin, pyridoxine, choline, niacin, pantot-
henic acid, inositol, biotin, folic acid, vitamin B₁₂ and ascorbic acid. Attempts have been made to estimate quantitative requirements, but vitamin deficiency in the diet may be partly offset by microorganisms in the gut, such as *Pseudomonas* sp. which in carp are an important source of the B vitamin complex.

In addition the sparing action of one vitamin on another has not been thoroughly investigated and requirement may vary with diet. For example thiamin requirement is related to carbohydrate intake, pyridoxine requirement is related to dietary protein level, and biotin requirement to dietary lipid level (Robinson and Lovell, 1978).

As information on the vitamin requirement of grey mullet was not available, a premix containing all the above vitamins was incorporated in the diet at a level similar to that used by Halver (1972) for chinook salmon and by Cowey et al. (1970a) for plaice. However, the diet of grey mullet is very different from that of plaice and chinook salmon and so one might expect their vitamin requirements to differ. Vallet et al. (1970) used a similar vitamin mixture in diets for *L. aurata* and obtained faster growth than was observed in this experiment, although his experiments were of relatively short duration.

Vitamin inadequacies may, therefore, have contributed to the slow growth observed in juvenile mullet but this seems unlikely, and they were not so severe as to cause acute vitamin deficiency symptoms.

(g) Mineral requirement: Until recently, comparatively little was known about either macromineral or trace mineral require-
ments of fish. Ogino & Kamizono (1975) showed that dietary salt mixture level had an effect on growth and mortality of trout and on growth of carp. Under the trial conditions 4-5% was the optimum concentration of the salt mixture in the diet of both species, although the requirement of eels has since been shown to be considerably greater, at approximately 8% (Nose & Arai, 1979). Vallet et al. (1970) found that addition of a salt mixture stimulated growth of L. aurata. In the experiments described in this thesis the salt mixture of Vallet et al. (1970) was used at 4g per 100g dry diet.

The importance of the ratio of one mineral to another is indicated by the fact that absorption and turnover of phosphorous in red sea bream is highest at calcium : phosphorus ratios of 1:2 whilst calcium absorption is highest at ratios of 1:2 and 1:4 (Sakamoto & Yone, 1973). Marine fish derive some minerals from seawater. Atlantic salmon could derive calcium from seawater provided phosphorus was supplied in the diet, but dietary zinc, copper, cobalt, manganese, iron and iodine were necessary for maximum growth and food utilization.

As minerals often have specific coenzyme functions it is likely that lack of specific minerals will lead to specific metabolic failures, as in rats where lack of copper impaired absorption of protein (Koreleski, 1971). Growth of channel catfish on a purified amino acid test diet only occurred when the original salt mixture was replaced by a low magnesium, low sulphate mixture. This suggested that dietary salts had an effect on amino acid availability or absorption. Zinc affected the digest-
ibility of protein and carbohydrate in rainbow trout (Ogino & Yang, 1978) and diets poor in iron resulted in lower haemoglobin and more immature erythrocytes in the blood of carp (Sakamoto & Yone, 1978b). Low levels of phosphorous in carp (Ogino & Takeda, 1976) and cobalt in M. parson (Ghosh, 1975) caused retardation of growth. Excess dietary sodium chloride reduced growth rate in salmon (Hastings, 1969) although levels up to 8.5% had no effect on food intake or conversion in rainbow trout (McCleod, 1978). Ion exchange across the gills complicates the quantitative determination of mineral requirements in fish (Lall, 1979). However, the phosphorus and magnesium requirements of rainbow trout have been estimated at .7 - .8% dry diet and .06 - .07% dry diet respectively, (Ogino & Takeda, 1978; Ogino et al, 1978) and the iron requirement of red sea bream was reported as .15% dry diet (Sakamoto & Yone, 1978a).

It seems unlikely that growth of juvenile grey mullet in this experiment was impaired by lack of essential minerals since the same mineral mixture supported good growth in L. aurata (Vallet et al, 1970). In addition they were maintained in seawater from which marine fish are able to derive at least part of their mineral requirement (Cowey & Sargent, 1979).

(h) Lipids and essential fatty acids: Lipids fulfill two major roles in fish.

(i) They are involved in maintaining the structural integrity of a wide variety of biological membranes between and within cells.

(ii) They play a major role in the provision of energy. Fish tend to rely on lipids, and possibly protein, for energy
All lipids contain fatty acids which may be of different chain lengths and different degrees of saturation. They are linked to compounds such as glycerol, sugar etc. which characterise the different lipid types. The structure of an unsaturated fatty acid is described by the number of carbon atoms in the chain, the number of double bonds, and the inclusive number of carbon atoms from the terminal methyl to the carbon atom of the first double bond from the methyl end. This last number, which is not affected by further desaturation reactions, is called the omega ($\omega$) number and characterises polyunsaturated acid families. There are four common families in fish - palmitoleic ($\omega 7$), oleic ($\omega 9$), linoleic ($\omega 6$) and linolenic ($\omega 3$). Although precise functions of specific fatty acids are unknown, chain length tends to be shorter, and the ratio of $\omega 3$ to $\omega 6$ fatty acids lower, in freshwater fish than in marine fish. Decreased temperature tended to cause increased desaturation of fish lipids in several fish, and increased desaturation of intestinal lipids is associated with an increased rate of transfer of amino acids across the intestinal mucosa (Castell, 1979). While all bio-membranes are rich in phospholipids there is considerable evidence that specialisation of membrane function e.g. ion exchange across gills, ability to detect pressure change, ability to elaborate light reflecting surfaces, is characterised by unique lipid class composition (Cowey & Sargent, 1972).

Fish derive fatty acids from two main sources - from their food and by biosynthesis. Dietary fatty acids may be altered by the fish by increasing chain length and desaturation.
However, it appears that certain fatty acids cannot be synthesised and must be supplied in the diet as "essential fatty acids".

The influence of essential fatty acids (EFA) on growth has been demonstrated in several species. In rainbow trout Lee et al. (1967) found that a diet containing corn oil did not support rapid growth and both growth rate and food utilisation were improved when salmon oil or linoleic acid (ω6) was added to the diet. Other workers (e.g. Watanabe et al., 1974a, 1974b, Vu & Sinnhuber, 1975) have investigated the effect of linolenic and linoleic acid on the growth of rainbow trout. In all experiments 1% dietary linolenic acid (ω3) was sufficient to prevent the slow growth, high mortality, fin erosions and fatty livers characteristic of fish fed diets containing none or very low levels of this essential fatty acid. The effect of linoleic acid on growth was not as great, but carp required linoleic and linolenic fatty acids for maximum growth (Takeuchi & Watanabe, 1977). The growth and feed efficiency of red sea bream was markedly improved by the addition of 1% linolenic acid or 2% of a mixture of longer chain polyunsaturated fatty acids of this family to a diet containing corn oil.

It has been shown that non-marine fats such as lard or hydrogenated oils may be used as the main dietary lipid without adverse effects on the growth of carp or trout provided they are supplemented with a marine oil e.g. herring/pollack oil which contains essential fatty acids (Yu et al., 1977; Takeuchi et al., 1978). However, EFA requirement appears to vary with species since Stickney and Andrews (1972) observed high weight gains in
channel catfish fed diets containing beef tallow, olive oil, or menhaden oil i.e. either ω3 or ω6 fatty acids appeared to meet the EFA requirement of channel catfish.

Reiser et al (1963) demonstrated that M. cephalus and Fundulus grandis only elongated the carbon chain of dietary linolenic acid when it was present at relatively low dietary levels. This, and other work (Cowey, et al., 1972) indicated that the ω6 : ω3 ratio in dietary fatty acids was very important. This was probably due to competitive inhibition of chain lengthening such that the chain elongation of one series e.g. 18.3.ω3 was inhibited by the presence of another e.g. 18.2.ω6.

In the diets for juvenile C. labrosus, corn oil, rich in ω6 fatty acids and cod liver oil containing ω3 fatty acids were used in the ratio 5 : 2. The ratio of ω3 : ω6 fatty acids was, therefore, similar to that used by Lee et al. (1967) to promote good growth in trout. Mullet differ from trout in several ways which may influence their EFA requirement, including diet and environmental temperature, but trout pellets supported growth in juvenile C. labrosus (Flowerdew & Grove, 1980). Thus, the low growth rate of juvenile C. labrosus observed using this experimental diet may have been attributable to some essential fatty acid deficiency.

In addition to supplying EFA, dietary fat is an important energy source. The experimental diet fed to juvenile grey mullet in this work contained 7% fat. Similar fat levels have promoted good growth in experimental diets for salmonids (Halver, 1957, Lee, et al. 1967). However, some fish can utilise 20-30%...
dry diet as fat provided adequate amounts of choline, methionine and tocopherol are present (Lee & Sinnhuber, 1972) although fat levels as high as this may cause gross alteration of carcass composition and a reduction in efficiency (Cowey & Sargent, 1979). The effect of dietary energy level on growth of juvenile C. labrosus was investigated in Experiment IV.

(i) Carbohydrate source: In the experimental diets devised for juvenile C. labrosus the carbohydrate consisted of varying levels of a 1:1 mixture of dextrin and maize starch, with 3% dry diet as cellulose. Cellulose was included because there was evidence that inclusion of some non-assimilable material increased the growth rate in other animals (Peterson et al., 1954). However, Vallet et al. (1970) found that 10% cellulose in the diet impaired growth and food conversion of L. aurata, presumably by diluting more easily digested dietary components. Cellulose levels up to 14.5% had no effect on growth or protein efficiency ratios in yellowtail fingerlings (Hosokawa et al., 1979) and the optimum dietary cellulose level for channel catfish was found to be 21% (Dupree & Sneed, 1966). Halver (1957) and others included cellulose flour at 3% dry diet with no detrimental effect on growth of other species of fish. It seems unlikely that the level of cellulose in the experimental diets could have caused the low growth rates observed in juvenile grey mullet in this experiment.

In spite of early suggestions to the contrary, it is now apparent that fish can utilise relatively high quantities of carbohydrate. It is mainly used as an energy source, but in plaice (Cowey et al., 1975) and carp (Creach et al., 1973) $^{14}$C labelled carbon injected as glucose was also incorporated into
It appears that fish can utilise some carbohydrates better than others. Dextrin has been widely used in diets for a variety of fish (Halver, 1957; Cowey et al., 1972; Nose et al., 1974). Maize starch is less commonly used, but there are numerous reports of mullet being fed diets containing large quantities of plant material, e.g. rice bran (De Silva & Perera, 1976), which are rich in starch. In most fish investigated, glucose, maltose and sucrose were better used than dextrin, and starch was relatively poorly utilised, (Buhler & Halver, 1961; Singh & Nose, 1967) although Koops et al., (1974) reported that maize starch was well utilised by rainbow trout.

The utilisation of both dextrin (Cowey & Sargent, 1972; Dupree & Sneed, 1966; Singh & Nose, 1967) and starch (Singh & Nose, 1967; Inaba et al., 1963) declined as dietary level increased. In Experiment I the combined dextrin and maize flour content reached 78% in the lowest protein diet and, extrapolating from Singh & Nose (1967), only 30-40% of the carbohydrate would be assimilated at this level. However, most investigations have involved carnivorous fish which would normally consume very little dietary carbohydrate. In an experiment with young carp, dietary starch levels up to 60% (with equivalent reduction in protein content) did not impair growth. Maltase, amylase and protease activities adapted according to diet within a week. Chiou & Ogino (1975) reported that in carp the digestibility of α-starch was 85% and did not alter as starch increased from 19% to 48% of the diet. Digestibility of β-starch declined slightly
from 60% to 50% as the amount of starch in the diet rose. In
grass carp fed on animal diet assimilation of carbohydrates was
92.3%, although this fell to 75.4% on a high carbohydrate plant
diet (Fischer, 1972). It appears that carp and grass carp
(and perhaps grey mullet) utilise starch better than plaice
and salmonids, possibly due to the production of different
enzymes since Tiews et al. (1972) attributed poor utilisation
of maize starch in trout to lack of amylase activity.

In juvenile C. labrosus apparent digestibility
of organic material was relatively high in the high carbohydrate
diets and tended to decline with increase in dietary protein
level. This is the reverse of what would be expected if mullet
were unable to assimilate high levels of carbohydrate. However,
results from assimilation studies were very variable and are
discussed in more detail in Section 8.3.2. In view of the facts
that there was some doubt about the utilisation of starch and
that dextrin, a partially hydrolysed starch would be expected to
be better assimilated, dextrin only was used as the carbohydrate
source in subsequent experiments.

(j) Protein source: The growth of plaice varied with
protein source, being highest when protein was supplied as freeze dried
cod muscle and declining as the proportion of plant protein in the
diet increased (Cowey et al., 1971). Similarly growth of rainbow
trout declined when the proportion of total protein which was of
animal origin was reduced from 75% to 35% (Steffens & Albrecht,
1977). Dupree & Sneed (1966) found that weight gain and feed con-
version of channel catfish was superior on casein diets compared with those containing wheat gluten or soybean proteins. However, when certain proteins were mixed (Fowler & Banks, 1976; Kesamaru et al., 1972; Fukada & Kesamaru, 1972) or supplemented with amino acids (Koops et al., 1976; Rumsey & Ketola, 1975; Dabrowska & Wojno, 1977) growth and food conversion were improved. This was interpreted as reflecting an essential dietary requirement for particular amino acids.

Two methods have been used to investigate the essential amino acid requirements of fish. Halver (1957) devised a diet in which all the protein was supplied by highly purified amino acids which could be excluded one at a time. In a more recently developed technique $^{14}$C labelled glucose was injected into the fish. Any $^{14}$C labelled amino acids later isolated from the fish had obviously been synthesised, enabling the essential amino acids, which cannot be synthesised, to be identified (Cowey et al., 1970b; Creach et al., 1973). Using these techniques the essential amino acids of chinook salmon (Halver, 1957), eel (Arai et al., 1972b), rainbow trout (Shanks et al., 1962), channel catfish (Dupree & Halver, 1970), carp (Creach et al., 1973), sole and plaice (Cowey et al., 1970b) have been identified. All fish species examined so far require the same ten indispensable amino acids for growth. These are arginine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, leucine and valine. The determination of quantitative requirements is complicated by the sparing action of, for example, cystine on methionine and lysine on the phenylalanine requirement. In
addition, inhibition of growth occurred when isoleucine : leucine was greater than 3:1 or less than 1:3 and when there was excess valine in the diet (Chance et al., 1964).

The situation is further complicated by the fact that channel catfish and carp appear to differ from salmonids in being unable to utilise free amino acids (Andrews et al., 1977). A diet of pure amino acids would only support growth of channel catfish when the salt mixture was replaced by a low magnesium, low sulphate mixture (Dupree & Halver, 1970). The ability of an amino acid diet to support growth depended on the pH of the diet in both carp (Nose et al., 1974) and catfish (Wilson et al., 1977). The preparation of protein may also have an important effect on the availability of constituent amino acids to different species of fish. Thus although trout grew well on hydrolysed casein, carp grew better on casein or wheat germ than the hydrolysate of either protein (Aoe et al., 1974). Similarly in catfish it was found that supplementation of a casein containing diet with arginine as gelatin stimulated growth, whereas addition of free amino acids had little effect on either food conversion or growth (Andrews et al., 1977). Cowey et al. (1971, 1974) found that plaice grew better on diets where protein was supplied by freeze dried cod muscle than a fish protein concentrate or low temperature dried cod meal despite, apparent similarities in amino acid content.

Vallet et al. (1970) investigated the effect of protein source on weight gain in juvenile L. ramada. The results are difficult to interpret since protein level, carbohydrate level
and vitamin level also varied among the diets. Smaller fish (21mm) appeared to grow better on fish or yeast protein whereas larger fish (36mm) grew better when protein was supplied as egg albumen. Albertini-Berhaut & Vallet (1971) found that half the required nitrogen of juvenile grey mullet could be supplied as non-protein nitrogen such as urea. This was possibly due to the presence of intestinal bacteria which may also modify the dietary amino acid requirements of mullet. In Experiment I the protein for juvenile C. labrosus was supplied as a 1:1 mixture of gluten and white fish meal supported growth in Plecoglossus altivelis (Kesamaru & Fukuda et al, 1972). From their natural diet one might expect that mullet would be able to utilise plant proteins as well as animal proteins. However, when the amino acid composition of casein and gluten were compared with freeze dried cod muscle, gluten was poor in most essential amino acids and casein was poor in arginine and lysine. Casein has been widely used as a protein source in fish diets but in comparison with the estimated amino acid requirements of chinook salmon, is relatively poor in arginine and methionine. Atlantic salmon grew better on diets where casein was supplemented with essential amino acids (Rumsey & Ketola, 1975).

The slow growth of juvenile grey mullet observed in Experiment I may have been due to dietary deficiency or the unavailability of certain amino acids. Egg albumen supplies all essential amino acids and was very efficiently used for growth by carp (Ogino & Chen, 1973), and L. ramada (Vallet et al., 1970). Therefore, in subsequent experiments gluten was omitted from the diet and casein was supplemented with arginine, cysteine,
methionine and tryptophan so that it more nearly resembled whole egg protein.

(k) Feeding frequency: In Experiment I the mullet in each tank were fed the whole daily ration at one time. There is some evidence that feeding frequency affects growth of some fish with the growth of smaller fish in particular being stimulated by an increase in feeding frequency (Shelbourn et al., 1973; Murai & Andrews, 1976). This is as expected since optimum feeding frequency is determined by stomach size (Andrews & Page, 1975). Murai & Andrews (1976) found that an increase in feeding frequency improved food consumption and growth of channel catfish, but variations in the feeding frequency of trout from once every two days to six times per day did not affect either specific growth rate or body composition (Grayton & Beamish, 1977).

Improved growth was partly attributable to increased food consumption, but in carp, increasing the feeding frequency from 2 to 5 times per day resulted in improved growth even where total daily ration was identical, (Wohlfarth, et al, 1971). Greenland & Gill (1979) reported improved food conversion in catfish fed at a higher frequency. The single feeding per day may have been contributed to the slow rate of growth of juvenile C. labrosus observed in this experiment.

(l) Feeding level: The inadequacy of the daily ration size, even at the highest feeding level, may have been an important factor in limiting the growth of the juvenile grey mullet in this experiment. It was well below the daily food intake calculated for fish of similar size in St. John's Lake at a time of fast growth (Section 5.6.) but the natural diet included relatively
large quantities of indigestible matter such as sand grains which were not present in the experimental diets. Excessive feeding rates were deliberately avoided, firstly because the effect of protein content in the diet would be expected to be greater at a lower feeding level, and secondly, from a practical viewpoint, it was preferable that the entire ration be consumed as soon as possible after being added to the tank. However, young mullet are characterised by high activity and a high level of energy metabolism (Alekseeva, 1978) which would necessitate a high energy intake. The maintenance requirement calculated for L. aurata by Vallet et al. (1970) was 2.2g dry weight per 100g living fish, suggesting that the lowest feeding level in this experiment was below maintenance. De Silva & Perera (1976) found that a ration of 5% body weight was inadequate to support growth of M. cephalus at most salinities, and that growth of fish on a ration of 8% body weight was similar to that of fish given excess food. However both these experiments were carried out at higher temperatures when maintenance requirement would be expected to be higher (Brett et al., 1969). At 13°C the maximum ration of trout food consumed by juvenile C. labrosus was 0.8% dry weight food : wet weight body (Flowerdew & Grove, 1980). After correcting for the moisture content, the experimental diets used in Experiment I were of approximately similar caloric content, to that of Flowerdew & Grove (1980) and so the low ration (c.1% dry weight food : wet weight body) would have been expected to promote growth. The effect of ration on growth of juvenile C. labrosus was investigated in more detail in a subsequent experiment (Chapter 9).
All these factors may have influenced growth. However, most, including photoperiod, salinity, etc. affected all tanks equally. Others, such as ammonia concentration, varied with both feeding level and dietary protein content and were taken into consideration when comparing growth on different diets. Slow growth of experimental fish in feeding experiments compared with equivalent fish in the wild has been observed by several authors, including Cowey et al (1970a) for plaice, and De Silva & Perera (1976) for M. cephalus. However, in spite of the slow growth, the variance of change in weight due to treatment (diet and feeding level) was highly significant.

The experiment was designed to minimise variation among tanks in all factors affecting growth except protein content of the diet and feeding level. Treatments were distributed to the tanks at random and there was no evidence of a temperature gradient within the room. However, there was differential mortality in the tanks with resultant differences in feeding level and stocking density. Comparison of growth in replicate tanks containing different numbers of fish at the end of the experiment indicated that increase in feeding level and volume per fish due to mortality, did not result in consistent increases in growth rate. This may have been due to the fact that most of the mortality was due to technical problems which arose in the last two weeks of the experiment. There was some evidence to suggest that mortality declined with increasing percentage protein in the diet, particularly at the low feeding level. Fish on the 8.5% protein diet might, therefore, be expected to have a slight stocking density and feeding level
advantage over fish on higher protein diets. However, as growth and condition tended to increase with dietary protein level, mortality was not considered to be an important factor in the interpretation of the results.

Hierarchical effects on fish growth have been observed by several authors in a variety of fish species (Brown, 1946a, 1946b; Magnuson, 1962; Cowey et al., 1970b) including Cephalus (De Silva & Perera, 1976). The effects of a hierarchy on growth, which might be expected to be particularly marked where fish are competing for a limited food supply, have important consequences both for the practical husbandry of the fish concerned and the use of statistics based on the normal distribution in the analysis of fish weight gain. In most tanks the standard deviation of weight of the fish either did not increase during the course of the experiment or increased slightly as might be expected as a result of the smaller numbers of fish in the tank. In a few tanks there was a marked increase in the standard deviation of weight in the third month. In five out of the sixteen tanks there was a significant correlation between weight gain and initial weight although the regression coefficient varied from -.22 to +.43. In only two of these, those containing fish fed a 39.1% protein diet at the high feeding level was the regression coefficient greater than .16. In most tanks there was no significant correlation between the initial weight and the total weight change of each fish indicating no significant hierarchical effect on growth.

Although the diets were designed to be approximately isocaloric, the calorific value selected for protein (4.5 kcal g⁻¹)
may have been the cause of additional discrepancies between the diets. The calorific value of protein used by other authors has varied from 4.0 kcal g⁻¹ (Hastings, 1979) to 5.7 kcal/g (Brett et al., 1969). The metabolisable energy of protein varies according to the relative amounts of urea and ammonia produced as the end product of protein metabolism. If it is entirely ammonia or entirely urea, the energy derived is 5.7 and 4.3 kcal/g respectively. Wood (1958) measured the nitrogen excretion of three marine species and found that the proportion of ammonia varied from 48% to 84% of the total excreted nitrogen. In bass temperature has a differential effect on ammonia and urea excretion, and, more importantly for this study, as ambient ammonia concentration increased, the proportion of urea excreted increased (Guerin-Ancey, 1976b). Thus, as ambient ammonia increased, the amount of energy derived from 1g of protein tended to decline. In this work, in the absence of detailed knowledge of the excretion of C. labrosus, a value of 4.5 kcal g⁻¹ was used, as calculated by Smith (1971) for trout at 15°C and subsequently used by several authors for other fish, e.g. Adron et al. (1976) for turbot. Even on this basis the diets were not exactly isocaloric but increased in metabolisable energy content from 4.07 kcal g⁻¹ in the 8.5% protein diet to 4.32 kcal g⁻¹ in the 58.5% protein diet. If 4.5 kcal g⁻¹ protein proved to be an underestimate, then the difference in metabolisable energy of the low and high protein diets would be increased.

In addition, the work of Austreng (1978) on rainbow trout, suggested that protein and energy digestibility increased
with increasing protein level. In this work apparent digestibility of protein by juvenile _C. labrosus_ increased with percent protein in the diet from 8.5 to 39.1% which suggested that more energy was available from the protein in more protein rich diets.

Carbohydrates were assumed to have a metabolisable energy content of 40 kcal g⁻¹ (Smith, 1971). However several authors have reported that the digestibility of dextrin and starch declined with increasing proportions in the diet which would result in overestimation of the metabolisable energy of low protein diets. Singh & Nose (1967) found that the assimilation of dextrin and potato starch fell from 77% and 69% respectively at 20% of the diet to 45% and 26% at 60% of the diet. In this experiment the assimilation of both dry matter and organic matter by juvenile grey mullet was very variable, but there was a tendency for assimilation to decline with increasing protein and decreasing carbohydrate in the diet. This was the reverse of what was expected. Although at least some of this difference may be due to higher solubility of the carbohydrate or the preferential bacterial action on the carbohydrate in the faeces, there was no evidence to suggest that the assimilation of carbohydrates was reduced at high concentrations in the diet. Odum (1968b) suggested that gut microflora played a special role in the utilisation of carbohydrates by _M. cephalus_, possibly as an adaptation to a herbivorous diet relatively rich in carbohydrate compared to the carnivorous diet of salmonids. Thus, although work on other species would suggest that metabolisable energy of the low protein, high carbohydrate diets was over-
estimated, this was not supported by the observed digestibilities of dry and organic matter by *C. labrosus* in Experiment I.

Many experiments have been carried out to determine the protein requirement of fish, which is of interest both nutritionally and because protein is often the most costly component of the diet. Comparison of results obtained for different species by different authors is complicated by the fact that optimum protein level is affected by most of the factors affecting growth mentioned above, particularly temperature, body size, ration size, protein source and dietary energy level and source.

De Long et al. (1958) found that increase in temperature from $8.3^\circ C$ to $14.4^\circ C$ raised the optimum protein level for chinook salmon fingerlings from 40% to 55%. For channel catfish of 114-500g the optimum protein level was 25% but for smaller channel catfish (14-100g) the optimum protein level was 35% (Page & Andrews 1973). In small *L. ramada* (mean length 12mm) increase in protein content of the diet from 25% to 72% resulted in significantly faster growth whereas in a slightly larger group (mean length 36mm) diets containing 25% and 75% protein resulted in similar growth (Vallet et al, 1970).

The ration level used in experiments to investigate protein requirements varied from ad lib (Ogino et al., 1976; Lall & Bishop, 1977; De Long et al., 1958; Zeitoun et al., 1974; Cowey et al., 1970a, 1972) to some form of restriction, e.g. 2% body weight per day (Zeitoun et al., 1976), 6% body weight per day (Dupree & Sneed, 1966), 70% and 85% of the ad lib intake (Cho et al., 1976). In Experiment I there was a highly significant ($p < .001$) interaction between the effect of dietary protein level
on growth of juvenile _C. labrosus_. The level of food intake affected optimum dietary protein level in rainbow trout, with restriction of feeding level tending to increase optimum dietary protein level (Cho et al., 1976). Ad lib feeding has the advantage that it is more natural and enables the effect of dietary protein level on appetite to be examined, which is of considerable practical importance. The amount of food consumed tended to decline as the protein content of the diet increased in plaice (Cowey et al., 1972) and coho salmon (Zeitoun et al., 1974). Ration size, independent of diet, has important effects on factors such as conversion efficiency and so it becomes very difficult to distinguish effects due to dietary protein level and ration size. By using two (more would have been preferable) fixed feeding levels, as in Experiment I, it was hoped to gain more information about each of the variables (protein level and ration size) and their interaction than would be possible using an ad lib feeding regime.

In most cases feed restriction was based on body weight. However, since the maintenance requirement increases proportional to some power of body weight less than 1, e.g. $BW^{0.75-0.77}$ (Elliott, 1976c), if ration size increases proportional to body weight, which is usually the case, then as fish increase in weight they have an increasing advantage over fish which grow at a slower rate. In Experiment I it was decided to keep the ration size constant throughout, irrespective of the growth of the fish, firstly because mullet are a slow growing fish and so large increases in body weight were not expected, and secondly because, on a constant
ration fish which grew fastest would be at a disadvantage, thus tending to minimize any advantage due to diet.

In some of the reported experiments fish were fed once per day as in this experiment, but in others fish were fed to satiation 3-4 times per day (Lall & Bishop, 1977) or the restricted ration was divided into several meals (Dupree & Sneed, 1966). The effect of feeding frequency on growth and conversion efficiency was discussed in Section 8.3.1.(k).

Another factor to be taken into consideration when comparing results from different experiments is the protein source used in the experimental diets. This has varied from relatively pure protein sources such as casein and gluten (Dupree & Sneed, 1966; Nail, 1962; Ogino et al., 1976; Cowey et al., 1970a) to freeze dried cod muscle (Cowey et al., 1972), herring meal (Austreng, 1978), and mixtures of proteins such as herring and soybean meal (Cho et al., 1976) and menhaden meal, soybean meal and corn gluten (Page & Andrews, 1973). Even in experiments where casein was used as a protein source, in some cases it was supplemented with amino acids so as to resemble more closely the amino acid composition of whole egg protein (this work; Cowey et al., 1970a; De Long et al., 1958) whereas in others it was not (Ogino & Saito, 1970; Nail, 1962; Ogino et al., 1976). Proteins differ in digestibility (Cowey et al., 1974) and in their amino acid composition and availability (Section 8.3.1.(j)), which may influence optimum dietary protein level. Dupree & Sneed (1966) found that in channel catfish optimum growth on a gluten diet occurred at a dietary protein level of 35% but optimum growth on a casein diet occurred at a dietary protein level of 41%.
In addition to the protein source, the energy content of the diet also had an influence on optimum dietary protein level. Hastings (1979) reported an experiment with channel catfish in which optimum growth was achieved on a 29% protein diet when total energy was 2.20 kcal g\(^{-1}\), but when energy was increased to 2.86 kcal g\(^{-1}\) growth was superior, and optimum growth occurred on a 42% protein diet. In several of the experiments designed to investigate the effect of dietary protein level diets containing different amounts of protein have also contained different amounts of energy. In the work of Dupree & Sneed (1966) and Nail (1962) on channel catfish, the protein in high protein diets was replaced by water and Alphacell (which was not assimilated) in lower protein diets. In these diets energy content increased markedly with increase in dietary protein level. In other experiments (De Long et al., 1958; Zeitoun et al., 1974; Cowey et al., 1970a) protein was replaced by an equivalent weight of carbohydrate. This was commonly dextrin (Zeitoun et al., 1974; Lall & Bishop, 1977; Dupree & Sneed, 1966) but maize meal (Austreng, 1978), glucose (Adron et al., 1976) and sorghum grain and wheat bran (Tiemeier et al., 1969) have also been used. According to Smith (1971) the metabolisable energy content of carbohydrate of 4.0 kcal g\(^{-1}\) is lower than the 4.5 kcal g\(^{-1}\) of protein, although other authors estimate the metabolisable energy of protein as nearer 4.0 kcal g\(^{-1}\). However, in experiments with carp, when protein in the diets was replaced by an equal weight of dextrin, increase in body weight was proportional to the protein content of the diet, whereas in later experiments, where diets were adjusted to be more nearly
isocaloric, the optimum dietary protein level was 35% (Ogino & Saito, 1970; Ogino et al., 1976).

Diets varying in protein content have also been designed by replacing protein with a mixture of carbohydrate and lipid. These include maize meal and capelin oil (Austreng, 1978), dextrin, maize oil and cellulose (Cowey et al., 1974) and dextrin, glucose and capelin oil (Adron et al., 1976). However in the work of Tiemeier et al. (1969) and Austreng (1978) energy content of the diet increased with protein level despite the replacement of protein by a mixture of carbohydrate and lipid. Even among isocaloric diets, dietary energy source may have a significant effect on protein requirement. Ogino et al. (1976) described an experiment in which trout were fed diets of varying protein content in which the protein was replaced isocalorically by either lipid + cellulose or dextrin. When the protein was replaced by lipid, the 30-35% protein diet supported optimum growth, but when protein was replaced by dextrin, optimum growth occurred on the 40% protein diet (Ogino et al., 1976). This suggested that the diets were not isocaloric to the fish, perhaps because, as observed by Kitamikado et al. (1964), increase in dietary starch caused a decrease in protein digestibility which was not observed with increase in dietary lipid level. Dietary energy levels and energy source, and their sparing effect on protein, are examined more thoroughly in a later experiment (Chapter 10).

Thus temperature, size of fish, ration size, frequency of feeding, protein source, energy level and energy source must be taken into consideration when comparing the response
of fish to varying levels of dietary protein as reported in different experiments.

Most experimenters have used growth in weight as a measure of growth. This has been criticised for its poor statistical reliability in fish (De Silva & Perera, 1976; Austreng, 1978). Atherton (1975) measured the growth in length of rainbow trout because this showed less fluctuation than growth in weight, which may vary widely with the amount of fat and moisture stored in the body. This is distinguished from true growth (Maynard & Loosli, 1969), which is why some authors have preferred to measure growth in terms of nitrogen or protein (Iwata, 1970; Birkett, 1969; Gerking, 1955a; Ogino & Saito, 1970). In the experiments described in this thesis weight and length were measured. Change of weight was used in statistical analyses because, with a standard method of removing surface moisture and the use of a top pan balance, it was felt that this could be measured with more accuracy and objectivity than length. However, body composition was estimated at the end of the experiment to examine the relative proportions of fat and protein of fish fed on different diets.

The relationship between growth and dietary protein level has been described as a curve in which increasing levels of protein result in a steadily declining increase in growth until a plateau is reached, and further increase in protein level causes no further increase in growth. Such a curve was reported for rats (Hegsted & Chang, 1965), blue-gills (Gerking, 1955a), carp (Ogino & Saito, 1970), chinook salmon (De Long et al., 1958), coho salmon (Zeitoun et al., 1974) and channel catfish (Nail, 1962). Hegsted
(1948) fitted two straight lines to his data on rats, with an ascending line to the left and a line parallel to the axis on the right. This type of discontinuous analysis has also been used for fish (Zeitoun et al., 1974; Ogino & Saito, 1970; De Long et al., 1958). The protein requirement was determined as the point of intersection between the two lines. The ascending line was plotted to fit the means for groups of fish where an increase in protein level in the diet resulted in a significant increase in weight gain. The intercept of the horizontal line was equal to the average of the means of the percentage gains which did not differ significantly (Zeitoun et al., 1974). This type of analysis was criticised by Cowey et al. (1972) on the grounds of being arbitrary and statistically inefficient. In addition, it was not suitable for analysis of a curve in which weight gain increased with dietary protein up to a certain optimum level but declined when dietary protein was further increased. This type of curve was reported for plaice (Cowey et al., 1972), rainbow trout (Zeitoun et al., 1976), channel catfish (Dupree & Sneed, 1966) and carp (Ogino et al., 1976). Cowey et al. (1972) and Zeitoun et al. (1976) fitted a second order polynomial equation to the curve. This had the advantage of being continuous, more accurate where intervals between experimental dietary nutrient concentrations were wide, and well adapted to economic analysis.

Growth of fish fed at the high feeding level improved as the experiment progressed, with highest weight gains in the third month. This may have been due to 'adaptation' of the fish to the experimental diets (Brown, 1946 b) but, since fish fed at
the low feeding level lost more weight in the third month, it seems more likely that this is an illustration of the interaction between temperature and ration size in their effect on growth (8.3.1 b).

In the first and second month growth of fish fed at the high feeding level increased to a maximum at 39.1% protein in the diet with no further increase of growth on the 58.5% protein diet. However, in the third month growth increased with dietary protein level up to 39.1% but was depressed at the higher protein level. In channel catfish food consumption decreased when dietary protein level exceeded 45% (Lovell, 1979) but in Experiment I ration was already restricted and there was no evidence to suggest that juvenile C. labrosus did not fully consume the 58.5% protein diet. Protein assimilation of juvenile grey mullet tended to increase with protein content of the diet, as has been observed for other fish (Austreng, 1978; Kim, 1974; Lall & Bishop, 1977) up to 39.1%, and although there was no further increase, there was no decrease in assimilation from the 58.5% protein diet. However, the apparent digestibility of dry matter and organic matter in the second month tended to decrease with increasing protein in the diet. In contrast, among other fish, reduced assimilation of low protein diets has been reported, and attributed to large amounts of carbohydrates in the diet (Austreng, 1978). In the third month apparent digestibility of dry and organic matter increased with dietary protein level up to 39.1% but declined markedly at 58.5%. Thus although growth depression could not be attributed to reduced food consumption or protein assimilation from the 58.5% protein diet, there was some
evidence to suggest that assimilation of other dietary constituents from the 58.5% protein diet was reduced, which may have contributed to growth depression.

When examined histologically the gills of *C. labrosus* fed on the 58.5% protein diet showed considerable degeneration, which contrasted with the healthy gills of the fish fed the 39.1% protein diet. The gill damage included various stages of disintegration of the gill lamellae and evidence of bacterial infection. Certain nutritional deficiencies, including pantothenic acid deficiency, may result in hyperplasia of gill epithelium and clubbed gills in salmonids (Sniesko, 1972) and in channel catfish (Murai & Andrews, 1979). Since the same quantity of vitamins, including panththenic acid, was incorporated into all diets, this was not a likely cause of the observed gill damage among mullet fed the 58.5% protein diet. Ammonia is known to be highly toxic to fish (Downing & Merkens, 1955; Hampson, 1976; Wilson et al., 1969). Flis (1968a, 1968b) studied the anatomic and histological effects of ammonia on carp and observed disintegration of gill epithelium at sublethal concentrations which became more severe as the concentration of ammonia increased. Among juvenile mullet, as protein content in the diet increased at the expense of carbohydrate, it was necessary to metabolise more protein for energy, resulting in increased ammonia excretion and a build up of ammonia in the tank. The subsequent damage to gill epithelium may have contributed to the observed depression of growth among mullet fed the 58.5% protein diet. De Long et al. (1958) attributed depression of growth of chinook salmon fed diets containing more than 40% protein at 8°C to stress due to the effect of increased ammonia.
concentration on the gills. Flis (1968b) found degenerative changes in the gut epithelium of carp exposed to sublethal concentrations of ammonia. This may have contributed to the reduced apparent digestibility of organic matter observed in mullet fed the highest protein diet. Andrews et al. (1976) found that the conversion efficiency and weight gain of channel catfish deteriorated as the concentration of metabolic end products increased.

As external ammonia concentration increased an increased proportion of nitrogen was excreted as urea rather than ammonia by young bass (Guerin-Ancey, 1976b). If this is also true of young C. labrosus, the increased ammonia concentrations may have resulted in a reduction in the amount of energy derived from protein, and thus a reduction in the total amount of energy available to the fish. Evidence that depression of growth of fish fed high protein diets was due to the environment was derived from a comparison of optimum protein levels of channel catfish stocked in aquaria and ponds (Hastings, 1979). Aquaria fish attained maximum growth on a 40% protein diet and diets containing more protein depressed growth. In ponds no maximum protein level was found and growth increased with increasing protein level, although to a lesser degree on diets exceeding 30% protein.

Growth depression of mullet fed the 58.5% protein diet occurred in the third month only, which may have been associated with the slightly higher temperatures during this period. Weight gain of juvenile channel catfish at 69°F increased with casein in the diet up to 35-40%, with no further increase of
weight gain on a higher protein diet. At 76°F weight gain increased with the proportion of casein in the diet up to 40% but growth on higher protein diets was depressed (Dupree & Sneed, 1966). Ammonia and urea excretion increase with temperature in bass (Guerin-Ancey, 1976a), and increased temperature increased the toxicity of ammonia to rainbow trout and channel catfish (Wilson et al., 1969). In addition, the higher temperatures would have caused a reduction in the dissolved oxygen in the tank. The toxicity of ammonia to rainbow trout increased as the concentration of dissolved oxygen decreased (Downing & Merkens, 1955). However, in contrast, De Long et al. (1958) reported that, in chinook salmon, growth depression in fish fed high protein diets occurred at 47°F but not at 58°F. They also found that it was limited to fish of about 2g, and was not observed with older fish weighing c. 6g. Thus although a relatively high concentration of ammonia in the tank appears to have contributed significantly to reduced growth of juvenile C. labrosus fed the highest protein diet in Experiment I, it may not be the only cause of growth depression among fish fed high protein diets.

At the lower feeding level interpretation of results was complicated by the fact that most fish lost weight. Although statistical analyses of negative weight changes are valid, care must be taken in their biological interpretation. Starving fish undergo certain changes in metabolism and ammonia excretion (Phillips, 1972) so that observed differences in degree of weight loss cannot be considered equivalent to differences in weight gain. However, excluding the third month, dietary protein
level had a highly significant effect on change in weight
\((p < .001)\). The weight gains of fish fed the 58.5% protein
diet, and the progressive increase in loss of weight as
protein content of the diet decreased to 8.5% suggested that
the optimum protein level for \(C. \text{labrosus}\) at the lower
feeding level was in excess of 58.5%.

In the third month variance of change of weight
due to protein level was not significant at the lower feeding
level and fish lost approximately the same amount of weight on
all diets. A possible explanation is that the increase in tem­
perature in the third month of the experiment so increased the
energy requirement of the fish for activity, metabolism etc.
that all dietary protein was metabolised for energy. The fact
that weight loss was similar on all diets suggests that they
were approximately isocaloric, as intended.

The relationship between dietary protein level
and growth of fish fed at the high feeding level was best
described by discontinuous straight lines in the second month,
and a polynomial curve in the third month, with estimated optimum
dietary protein levels of 37% and 38% respectively. There was a
highly significant interaction between protein level and feeding
level, with the estimate of optimum dietary protein level for fish
fed at the low feeding level in the second month being greater
than 58.5%. Cho et al. (1976), working with rainbow trout, reported
a similar increase in optimum dietary protein level when feed in­
take was restricted. A wider range of feeding levels were
examined in Experiment II. At zero growth protein intake of
groups of juvenile mullet fed at the low and high feeding levels
were .24 and .63g day$^{-1}$ respectively which prompted further investigation of protein : energy ratios in Experiment IV.

The high protein requirement of juvenile C. Iabrosus observed in this experiment is typical of fish and considerably higher than that observed for most warm-blooded animals e.g. ducks, 18%; pigs, 15-18% (Nail, 1962). Among fish, reported optimum protein levels are remarkably consistent considering the differences in diet formulation and experimental technique. Cowey et al. (1970a) found that growth of young plaice increased linearly with percent protein in the diet to 70%. However, protein in the diet was replaced by dextrin on a weight basis, and so the metabolisable energy of the diets probably increased with protein content. In a later experiment in which protein was replaced by dextrin, maize oil, and cellulose and the diets were more nearly isocaloric, maximum growth occurred in plaice fed a 50% protein diet (Cowey et al., 1972).

Among salmonids optimum dietary protein levels of 40-50% have been reported for rainbow trout (Zeitoun et al., 1976; Austreng, 1978; Lall & Bishop, 1977); coho salmon (Zeitoun et al., 1974) and chinook salmon (De Long et al., 1958). Pfeffer & Becker (1977) reported no obvious effect of dietary protein level on growth of rainbow trout, but the protein levels in their experimental diets (39-55%) were probably at, or in excess of, the protein requirement for maximum growth. Cho et al. (1976) reported that increased protein level in the diet caused reduced weight gain in rainbow trout. However, since the lowest dietary protein level used in this experiment was 40%, his results are consistent with growth depression observed in fish fed diets con-
taining protein in excess of the optimum protein level. 

Ogino et al. (1976) reported an optimum dietary protein level of 35% for carp. The optimum dietary protein level of channel catfish was also reported to be approximately 35% (Dupree & Sneed, 1966; Nail, 1962; Tiemeier et al., 1969). Among other fish the dietary protein requirement for optimum growth has been estimated as 48% for eels (Nose & Arai, 1972), 35-40% for sea bream (Luquet & Sabaut, 1974), 35-40% for Tilapia zillii (Teshima et al., 1978), 50% for the grouper Epinephelus salmoides (Teng et al., 1978), 40% for the milkfish Chanos chanos (Lim et al., 1979) and 47-50% for the bass Dicentrarchus labrax (Metailler et al., 1977).

The optimum dietary protein level observed for juvenile C. labrosus appears to be similar to that of other fish. In view of all the factors which have been shown to affect the response of fish growth to protein level in the diet, including size of fish, protein source, and amount and source of dietary energy, an estimate of optimum dietary protein level at only two ration levels is of limited value. The optimum dietary protein level of fish in St. John's Lake might be expected to be lower than that of fish in Experiment I, both because feeding level in the experiment was restricted, and because low salinity has been shown to decrease the dietary protein level for maximum growth of rainbow trout (Zeitoun et al., 1974; Lall & Bishop, 1979). Ideally the variation of optimum dietary protein level for C. labrosus with all these factors, as well as environmental factors such as temperature and salinity, should be examined.
8.3.2. Assimilation

An inert indicator such as chromic oxide has been used to measure apparent digestibility in various species of fish, including rainbow trout (Nose, 1967; Inaba et al., 1963; Singh & Nose, 1967), carp (Kim, 1974) and channel catfish (Page & Andrews, 1973), although the assumption that chromic oxide moves through the gut at the same rate as other food components has recently been questioned (Bowen, 1978). The use of an inert indicator has the great advantage of not requiring quantitative collection of faeces. Page & Andrews (1973) collected faeces from the posterior portion of the gut by dissection. Singh & Nose (1967) and Cowey et al. (1974) collected faeces without killing the fish by applying gentle pressure to the surfaces of the fish in the rectal region. However, the number and size of mullet involved in Experiment I prohibited collection of faeces directly and faeces were siphoned out of the tank once a day as described by Iwata (1970). Bacterial action and solution of faecal material during the period before collection might be expected to cause overestimation of apparent digestibility. The effect of bacterial action on faeces has been studied in freshwater (Iwata, 1970; Elliott, 1976b; Davies, 1964) and seawater (Wood, 1958). In each case it was concluded that bacterial action on faecal matter over a few days was negligible.

There was no significant difference between the apparent digestibility of dry matter, organic matter or protein by mullet fed at different feeding levels. This is consistent with observations on the effect of feeding rate on assimilation.

Assimilation of food and energy increased with temperature in *I. mossambica* (Mironova, 1976) and rainbow trout (Brocksen & Bugge, 1974). The slight increase in temperature observed in the third month of the experiment did not have a statistically significant effect on apparent digestibility of dry matter, organic matter or protein in mullet. However, the increased temperature in the third month may have resulted in increased production of ammonia (Guerin-Ancey, 1976a) and via damage to the gut epithelium (Flis, 1968b) contributed to the observed reduction in apparent digestibility of dry matter from the 58.5% protein diet.

The overall trend was a decline in assimilation of dry matter and organic matter with increase of protein in the diet, although this was not statistically significant due to high variability between replicate tanks. Most authors have reported that apparent digestibility decreases with increasing amounts of carbohydrate in the diet (Singh & Nose, 1967; Inaba et al., 1963) but the reverse seemed to be true in mullet. Solution of unassimilated dextrin from the faeces may have exaggerated the digestibility of low protein diets, but Ogino et al. (1976) reported that carp utilised carbohydrates more effectively than rainbow trout. It is possible that mullet, being adapted to a herbivorous diet which is relatively rich in carbohydrates, have an enzyme system or specialised gut bacteria which enable them, in contrast to carnivorous fish, to assimilate high levels of carbohydrate.
Apparent digestibility of organic matter varied from 52% to 87%. Hickling (1970b) found that *C. labrosus* assimilated 45% of the organic matter from pellets made of sand and cod roe. On a natural diet *Liza falcipinnis* assimilated 51.7% of the available organic matter (Payne, pers. comm.). Vallet et al. (1970) reported that indigestible matter in the food, such as cellulose, interfered with absorption in *L. aurata*. A diet of semi-purified rations might be expected to be absorbed better than the natural diet, which contains a high proportion of indigestible matter.

In calculating digestibility of protein it was assumed that all nitrogen was protein nitrogen. However, the faeces were collected from seawater, and in spite of being rinsed in freshwater, some inorganic nitrogen probably remained. Protein digestibility may, therefore, have been slightly underestimated in all tanks. Excluding a few abnormally low values, the apparent digestibility of protein varied from 80-95%.

Comparable values have been reported for channel catfish, 80-90% (Page & Andrews, 1973) and rainbow trout, 92.8% (Inaba et al., 1963), 79-96% (Kitamikado et al., 1964), 73-93% (Windell et al. 1978). Apparent digestibility of protein increased with protein level in the diet up to 39.1% but increasing dietary protein level to 58.5% caused no further increase in apparent digestibility. Nose (1967) reported similar changes of apparent digestibility of protein with dietary protein level in young rainbow trout, although maximum apparent digestibility occurred at a lower dietary protein level, approximately 20-30%. Increase of apparent digestibility of protein with increasing protein level
in the diet has been reported for carp (Kim, 1974), channel catfish (Page & Andrews, 1973) and rainbow trout (Lall & Bishop, 1979; Inaba et al., 1963; Kitamikado et al., 1964; Austreng, 1978). This may be due to dilution of the protein in the diet rendering it less susceptible to the action of proteolytic enzymes of the gut. Nose (1967) measured the metabolic faecal nitrogen of young rainbow trout and found that it formed a higher proportion of faecal nitrogen at lower dietary protein levels, although the exact relationship varied with the type of protein. He concluded that decrease in apparent digestibility of protein at low dietary protein levels was due to disregard of metabolic faecal nitrogen, and that the digestibility of protein was constant regardless of protein level. Kim (1974) estimated metabolic faecal nitrogen in carp and came to the same conclusion. Thus the increase of apparent protein digestibility with dietary protein level observed in mullet may be attributable to the disregard of metabolic faecal nitrogen in its estimation.

Factors such as water temperature, fish size, protein source, frequency of feeding, stocking density and previous level of nutrition may also affect protein assimilation (Nose, 1967; Kitamikado et al., 1964; Cowey et al., 1974; Hastings, 1969) but in Experiment I these were, as far as possible, constant in all tanks.

8.3.3. Conversion efficiency and protein efficiency ratio

The net growth efficiency, defined as the efficiency with which an animal utilises for growth that food consumed over
and above the amount it would require for maintenance, has
been considered more useful than gross growth efficiency
(conversion efficiency) (Warren & Davies, 1967) because it
is not affected by variations in maintenance requirements due
to temperature, activity etc. However, estimates of main­
tenance requirement are complex since a fish which is not
changing in weight may be altering significantly in chemical
composition, for example increasing water content at the
expense of fat (Cowey & Sargent, 1972). Edwards et al
(1972) suggested that increases in food intake were divided
between metabolism and growth i.e. metabolic rate increased
with increased food intake so that maintenance requirement
increased with ration size. Due to these difficulties and
because, maintenance requirement may alter with the dietary
protein level, gross growth efficiency only was estimated.
Expression of both food and growth in terms of energy would
have been preferable since, although the experimental diets
were similar in water and energy content, growth of fish
on different diets, as illustrated by the body composition
analysis at the end of the experiment, differed in the pro­
portion of moisture and fat.

Gross growth efficiency of juvenile
C. labrosus increased with protein content of the diet from
8.5% to 39.1%. This could not be attributed to differences
in the composition of growth, since, at the end of the ex­
periment, fish receiving the higher protein diets tended to
have a higher lipid content. Although the slight increase in
metabolisable energy content of the diet with increase in per­
centage protein would tend to result in an increase in gross growth efficiency, the tendency towards reduction in apparent digestibility would be expected to counteract this. Lall & Bishop (1979) found the growth efficiency of rainbow trout improved when dietary protein level was raised from 40% to 50%. Possible reasons for the decline of growth and gross growth efficiency of fish fed the 58.5% protein diet in the third month have already been discussed. A similar decline of growth efficiency among fish fed diets containing more than 40% protein was reported for channel catfish fry (Dupree & Sneed, 1966) and *Chanos chanos* fry (Lim et al., 1979).

The important relationship between conversion efficiency and ration size has been studied mainly among salmonids (Brett et al., 1969; Elliott, 1976c; Huismann, 1976). Conversion efficiency increased steeply from zero at maintenance ration to a maximum at 'optimum ration', thereafter decreasing with further increase in ration size. In Experiment I conversion efficiency of fish fed diets containing 8.5% to 39.1% protein was greater at the higher feeding level, which suggested that the lower feeding level (approximately 1% dry weight food per wet weight fish per day) was below 'optimum ration'. This was consistent with the observations of Flowerdew & Grove (1980) that conversion efficiency of juvenile *C. labrosus* increased with ration size up to the highest ration used which was 2.3% wet body weight per day. Among fish fed the 58.5% protein diet the conversion efficiency of those fed at the high and low feeding levels
was similar, which may indicate that the 'optimum ration' decreases as protein content in the diet increases.

The maximum conversion efficiency observed in juvenile *C. labrosus* in Experiment I was 19% (wet weight growth/dry weight ration X 100) for fish fed the 39.1% protein diet at the high feeding level in the third month. The mean conversion efficiency calculated by De Silva & Perera (1976) for *M. cephalus* of similar size on a diet containing approximately 60% protein in a salinity of 30% was about 3%. Both these estimates are far below those obtained by Flowerdew & Grove (1980) for 5-10g *C. labrosus*. They reported maximum conversion efficiencies (dry weight growth/dry weight ration X 100) of 21% - 24% which, allowing for a mean water content of approximately 68% was equivalent to a conversion efficiency (wet weight growth/dry weight ration X 100) of 65% to 75%. This may have been due to a more suitable diet - starter trout pellets rather than experimental rations (this thesis) or a rice bran and fish meal mixture, (De Silva & Perera, 1976) - or to better environmental conditions in a laboratory aquarium rather than tanks and ponds. Improvement of conversion efficiency with increasing oxygen concentration has been demonstrated (Brett, 1979) and Andrews et al. (1973) found that the conversion efficiency of channel catfish deteriorated as the concentration of metabolic end products increased.

Although growth efficiency was very variable (De Silva & Perera, 1976), at a constant feeding level, mean growth efficiency of juvenile *M. cephalus* tended to decrease
with increase of salinity above 10%. Similarly the feed intake/unit gain of rainbow trout was higher in seawater than in freshwater (Lall & Bishop, 1979). Although this does not explain the large differences in conversion efficiency as estimated by Flowerdew & Grove (1980), De Silva & Perera (1976) and this experiment, since salinities of 30 - 35% were used in all three studies, it does suggest that a significant improvement of conversion efficiency might be achieved at intermediate salinities.

Both the gross growth efficiency and the protein efficiency ratio of fish fed at the high feeding level tended to increase throughout the experiment with the highest values occurring in the third month. In contrast, the growth efficiency of fish fed at the low feeding level declined in the third month. The increase in temperature observed during the last month of the experiment was probably an important influence.

In studies on other fish, at each ration, increase in temperature resulted in an increase in efficiency to a maximum level after which efficiency declined with further increase in temperature (Brett & Groves, 1979; Elliott, 1976c). The 'optimum ration', at which conversion efficiency was a maximum, increased as temperature increased both in C. labrosus (Flowerdew & Grove, 1980) and other species (Elliott, 1976c; Brett & Groves, 1979). Illustrated in the diagram below, this provides an explanation of how conversion efficiency of fish fed at the higher feeding level increased with increase in temperature (CE₂ > CE₁), whereas conversion
efficiency of fish fed at the lower feeding level decreased with increase in temperature ($CE_1 > CE_2$).
However, this must be considered as speculative, particularly since many of the fish fed at the lower feeding level actually lost weight.

As a measure of the efficiency with which protein is converted into weight gain, the protein efficiency ratio has many limitations. It is assumed that all protein is used for growth and takes no account of protein requirement for maintenance. It also assumes that gain in body weight is constant in composition which is unlikely to be true. In addition, PER has been shown to vary with age, length of assay period, feeding level, the amount of moisture in the diet, and the protein and energy source of the diet (McLaughlin & Campbell, 1969; Ogino et al., 1976; Cowey et al., 1974). Comparison of actual values obtained under other experimental conditions is therefore of very limited value, but within one experiment in which these factors were constant, PER could be used to compare the efficiency of protein utilisation among fish fed diets differing in protein content at the same feeding level.

There were very few PER values for fish fed at the low feeding level, but at the high feeding level PER tended to decline with increasing protein level in the diet in both the second and third month. In diets low in protein content, most, if not all, of the maintenance energy was supplied by carbohydrates and lipids and so the gain per g protein would be relatively high. As the protein content of the diet increased, carbohydrate content decreased, and so a larger amount of maintenance energy would be derived from protein. Thus, in spite
of increase in weight gain, the weight gain per g protein consumed declined. It is possible that protein exerts a specific dynamic action in fish resulting in elevation of metabolic rate with increasing protein level in the diet (Cowey & Sargent, 1972). This may contribute further to the decline of PER with increasing dietary protein level.

A similar relationship between PER and dietary protein level has been observed in channel catfish (Tiemeier et al., 1969), carp (Ogino & Saito, 1970; Ogino et al., 1976), rainbow trout (Cho et al., 1976) and turbot (Adron et al., 1976). However, in other experiments involving rats (Hegsted & Chang, 1965), plaice (Cowey et al., 1972) and coho salmon (Zeitoun et al., 1974), PER increased to a maximum and then declined as dietary protein level increased. The dietary protein level resulting in maximum PER was approximately 40% for plaice and coho salmon which was considerably higher than the 10% reported for rats. Both Cowey et al. (1972) and Zeitoun et al. (1974) fed fish to satiation whereas Tiemeier et al. (1969), Cho et al. (1976) and Adron et al. (1976) all imposed some kind of fixed ration. Zeitoun et al. (1974) suggested that the increase of PER with increase in dietary protein level among fish fed lower protein diets may be due to the fact that the higher intake of the lower protein diets so increased maintenance requirement that PER was depressed. However, Ogino et al. (1976) found that, when dextrin replaced protein in low protein diets, the PER of young rainbow trout was low and increased slightly with increased protein in the diet. When protein was replaced by lipid
and cellulose, PER was much higher at low dietary protein levels and decreased as the dietary protein content increased. Poor assimilation of the high levels of dextrin in low protein diets effectively results in those diets being lower in metabolisable energy, which has been shown to result in lower PER in turbot (Adron et al., 1976). However, this does not explain the results obtained by Cowey et al. (1972) with plaice since in these diets protein was replaced by maize oil and dextrin, and dextrin did not exceed 24% of the diet. The increase of \( \alpha \)-cellulose with decrease of protein level in the diet may have impaired the assimilation of the other components of the diet (Vallet et al., 1970).

In carp, Ogino et al. (1976) reported that replacement of protein by dextrin in low protein diets did not depress PER, as was observed in trout. This suggests that carp and, from the results reported in this thesis, juvenile grey mullet, in contrast to fish such as plaice, rainbow trout and coho salmon, can effectively utilise high levels of dietary carbohydrate for energy to spare protein for growth. This is consistent with the data on assimilation of organic matter from diets of differing protein levels by juvenile grey mullet obtained in this experiment.

Cowey et al. (1972) suggested that the high PER of carp on low protein diets was due to growth with a low protein content. Unlike both carp and rainbow trout, in which the increase in PER was accompanied by an increase in the % lipid in the fish (Ogino et al., 1976), the lipid content of mullet increased with increase in PER and decreasing dietary
protein level, although in one group of fish fed the 8.5% protein diet at the high feeding level, fish protein content was particularly low.

Juvenile *C. labrosus*, in common with carp, appear to utilise high levels of carbohydrate effectively as an energy source. Efficiency of conversion of protein into weight gain tended to increase with decreasing protein content to a maximum at 8.5% or less, although there must be a limiting level of dietary protein below which the protein turnover and the amino acid reservoir cannot be maintained and growth cannot occur.

8.3.4. **Condition and body composition**

Relative condition was described in Chapter 4, and compares weight at a given length with mean weight of fish at the same length. Tiemeier et al. (1970a) found that the condition of channel catfish improved when fish were given supplementary food, and similarly, the relative condition of juvenile *C. labrosus* was higher in fish fed at the higher feeding level. At both feeding levels relative condition increased with dietary protein level.

Hart et al. (1940) reported that the condition of herring was positively correlated with the oil content of the fish, and condition was one of the factors used to estimate body composition of bluegills, *Lepomis macrochirus* (McComish et al. 1974). There was a highly significant correlation between the mean relative condition of *C. labrosus* in each tank and their lipid content. This suggested that the observed variation of
condition of fish on diets of different protein content may have been related to variation in their body lipid content.

Diet has been considered the most important factor affecting body composition in fish (Wood et al. 1957). It is of interest both in estimating the energetic cost of growth and because the fat content of fish flesh is an important factor in determining its processing and consumer acceptibility (Buckley & Groves, 1979). In calculating the protein content of mullet, all nitrogen was assumed to be protein nitrogen. In marine fish non protein nitrogen has been estimated as 9 to 18% of the total nitrogen content. However, Gerking (1955a) found that rate of feeding did not influence the proportion of non protein nitrogen in bluegills and it has become standard practice to base protein determinations on total nitrogen determinations.

Among C. labrosus in Experiment I, an increase in feeding level resulted in a significant increase in the proportion of body lipid, a significant decrease in the proportion of body moisture and ash, but had no significant effect on the proportion of body protein. Perera and De Silva (1978b) found that ration size had no effect on body composition of juvenile M. cephalus, but they were comparing two relatively high feeding levels (ad lib and 8% body weight/day). Increase in ration size resulted in an increase in the proportion of lipid and a decrease in the proportion of moisture in brown trout (Elliott, 1976a), sockeye salmon (Brett et al. 1969) and Tilapia mossambica (Pandian and Raghuraman, 1972). Expressed on a wet weight basis, fish protein content has been reported
to increase with ration size (Elliott, 1976a; Brett et al. 1969), although expressed on a dry weight basis the reverse occurred (Elliott, 1976a).

The decline in the proportion of ash with increasing protein in the diet in mullet fed at both the high and low feeding levels was probably at least partly due to an increase in other body constituents, such as fat. A similar decline in ash content was observed in rainbow trout as the carbohydrate content of the diet increased (Austreng, 1976).

In most fish, the protein content is the least influenced by factors such as dietary fat and protein levels and ration size (McLaughlan and Campbell, 1969; Elliott, 1976a). Among juvenile C. labrosus there was a slight decline in protein content with increase in dietary protein level above 16.8% although this was not significant. In two tanks in which fish were fed the 8.5% protein diet there was a particularly low percentage protein in the body. This may indicate that the protein content of this diet was inadequate even to meet requirements for maintenance of circulating protein and amino acid pools for the fish in these tanks, necessitating utilisation of body protein (Idler and Bitners, 1959). Cowey et al (1972) found that the proportion of protein in young plaice increased with dietary protein level but other authors have reported that dietary protein level had little effect on the proportion of protein in the fish (De Long et al. 1958; Lall and Bishop, 1979; Adron et al. 1976; Meske & Pfeffer, 1979).
In contrast, the lipid content of fish is highly variable and readily altered by nutritional factors (Gerking, 1955a; Hart et al, 1940; Albertini-Berhaut, 1975; Elliott, 1976a). The lipid content of C. labrosus showed significant variation with dietary protein level, tending to increase with increase in the level of protein in the diet. Lall and Bishop (1979) reported that the lipid content of rainbow trout increased with dietary protein level up to 50%, but this contrasts with reports on carp (Ogino et al., 1976; Hepher and Chervinski, 1965), plaice (Cowey et al., 1972) and rainbow trout (Ogino et al., 1976; Cho et al., 1976; Austreng 1978) in which lipid content declined with increase in percent protein in the diet. Recalculation of data presented by Cowey et al. (1972) indicated that this was not due to expression of body composition in terms of wet rather than dry weight. Nor could it be attributed to the fact that on ad lib feeding, intake decreased with increase in dietary protein level (Cowey et al., 1972; Zeitoun et al., 1974), since in most cases energy content of the diet increased with protein content so that intake of metabolisable energy was probably approximately constant. The increase in the lipid content of C. labrosus with increasing dietary protein level was not due to utilisation of body lipid by fish fed the lower protein diets because evidence from moisture content suggested that the proportion of fat in all fish at the end of the experiment was greater than that of a sample taken just before the experiment began. The increase in lipid content with increase in dietary protein
level suggests that the available energy from the diets did increase significantly with dietary protein level, despite evidence to the contrary from apparent digestibility studies and growth of fish fed at the low feeding level in the third month.

Moisture content of *C. labrosus* decreased with increase in *dietary* protein level, there was a highly significant negative correlation between lipid and moisture content. This has been observed in many species of fish including brown trout (Elliot, 1976a), rainbow trout (Lall and Bishop, 1979), sockeye salmon (Brett et al. 1969), *Tilapia mossambica* (Pandian and Raghuraman, 1972), channel catfish (Murray et al., 1977), plaice (Cowey et al., 1972) and *C. labrosus* (Flowerdew & Grove, 1980). It was attributed by Gerking (1955a) to the fact that 0.1g of water is associated with 1g of fat, whereas about 3.0g water are associated with 1g of protein. The mean moisture content of fifty fish collected from the tanks before Experiment I started was 76.95% which indicated a fat content of 4.5mg/100mg dry weight fish. This was lower than the fat content of any of the fish at the end of the experiment, although the fish were collected at a time of year when their fat content might be expected to be particularly low. In plaice (Cowey et al., 1972), herring (Balbontin et al, 1973), salmonids (Wood et al., 1957) and *M. cephalus* (Perera & De Silva, 1978b), wild fish had a lower lipid content than experimental fish although the reverse was found in turbot (Adron et al., 1976). It seems likely that the differences in body composition between
Experimental and wild fish are mainly due to diet and feeding level but other factors of importance include restriction of activity within the experimental tanks (Brankovic & Dimic, 1972) and salinity (Cowey et al., 1972).

8.3.5. Histology and comparison of gut, liver and gutted fish weights

The increase in the proportion of gutted fish weight and the decrease in the proportion of gut weight as protein content of the diet increased may be a reflection of variation in fat content. Many fish do not possess an adipose tissue in the mammalian sense, but store large quantities of lipid in their livers and muscles (Cowey and Sargent, 1972; Dawson & Grimm, 1980). Thus, mullet which contained more fat as did those fed the higher protein diets might be expected to have a relatively larger gutted weight. In addition, starving fish utilise muscle protein (Sniesko, 1972; Idler and Bitners, 1959) which may be a reason for the lower proportion of gutted weight in fish fed low protein diets. However, there was no significant difference between the proportion of gutted weight or gut of fish fed on the high and low feeding levels, as might be expected from their difference in energy intake and lipid content. The gut as a percent of body weight was found to decrease with increase in size in rainbow trout (Denton and Yousef, 1976) but comparison of actual size of mullet at the end of the experiment indicated that variation in percent gut weight could not be attributed to size alone.

In largemouth bass relative liver weight was positively correlated with food intake (Heidinger and Crawford,
but variance of relative liver weight of *C. labrosus* due to feeding level was only significant among mullet fed the 8.5 and 58.5% protein diets. Relative liver weight was not significantly affected by dietary protein level in fish fed at the low feeding level. This was consistent with the histological studies of the livers of these fish in which structure differed little with dietary protein level. Among fish fed at the high feeding level the liver weight did not vary very much with dietary protein content, except that livers of fish fed the 58.5% protein diet in one tank were particularly large. However, histological studies indicated that with increasing protein in the diet the livers became increasingly infiltrated with fat or glycogen. Since there was a corresponding increase in the proportion of lipid in *C. labrosus* and juvenile *M. cephalus* did not store carbohydrate in any quantity (Perera and De Silva, 1978b), the substance stored in the liver was probably lipid. McLaren et al. (1946) found that rainbow trout fed diets rich in carbohydrates developed enlarged, yellow livers. Buhler and Halver (1961) found no such changes with 48% dextrin in the diet and suggested that liver damage observed by McLaren et al. (1946) was due to dietary imbalance rather than high carbohydrate levels. However, Austreng et al. (1977), using diets fulfilling all known requirements of rainbow trout, reported discolouration, enlargement and increase in glycogen in livers of fish fed diets rich in carbohydrates for twelve weeks, although these differences had largely dissipated after 24 weeks. Judd and Cross (1966) found that the relative liver weight of channel catfish was
inversely correlated with dietary protein level which is not consistent with observations on *C. labrosus*.

Judd & Cross (1966) observed distortion of cell membranes and loss of cytoplasm and nuclei in liver cells which they attributed to damage caused by infiltration of fat and glycogen, although Flis (1968b) found that the liver of carp was similarly affected by sublethal concentrations of ammonia. Liver damage of this type resulting from nutritional defects is called liver lipoid disease. Its causes are complicated, poorly understood and probably multiple (Sniesko, 1972, Watanabe et al, 1974a). The livers of mullet fed the 58.5% protein diet at the high feeding level were more infiltrated with lipid, and evidence from gill structure suggested that ammonia levels may have been increased. However, the limited histological study undertaken was not sufficient to determine the degree to which liver damage contributed to the reduced growth rate observed in these fish.

Gill damage among fish fed the 58.5% diet at the high feeding level and its possible relationship with the concentration of unionised ammonia has already been discussed. This effect may be even more important at lower salinities since the toxicity of ammonia to salmon has been found to decline with increase in salinity, (Alabaster et al. 1979). *C. labrosus* receiving low protein diets, particularly at low feeding levels, also suffered gill damage. In addition to thickening of epithelium and fusion of distal lamellae there was also 'plaque' cells present, which
suggested bacterial infection. The gill damage sustained by *C. labrosus* on diets of 8.5 to 39.1% protein increased with decrease in feeding level and decrease in dietary protein level. The symptoms were very similar to those of nutritional gill disease which affects salmonids, and possibly carp (Sniesko, 1972). It has been attributed to pantothenic acid deficiency (Ashley, 1972) and, more recently biotin deficiency (Castledine et al., 1978). Both pantothenic acid and biotin were included in the diet but the quantitative requirements of grey mullet for these vitamins are unknown.
The results of Experiment I indicated that ration size had an important effect on growth, growth efficiency, protein utilisation, relative condition, and body composition of juvenile C. labrosus. Paloheimo and Dickie (1966b) found that the basic distribution of energy intake for fish could be described by a knowledge of the rate of intake, and two parameters relating ration size and gross growth efficiency. They also concluded that ration size had an important effect on the relationship between metabolism and body weight. Wurtsbaugh and Davies (1977a,b) emphasized the importance of employing a range of ration sizes in growth experiments since (i) fish in nature subsist on rations below maximum levels (ii) ration level influences food conversion efficiency.

It was, therefore, decided to examine the effect of reaction size on growth of juvenile C. labrosus. Two diets differing in protein content were used since the response of fish to feeding level in Experiment I had been influenced by dietary protein content. The 39.1% protein diet was chosen because it appeared to be optimum under these conditions. The 8.5% protein diet was used because the study of protein efficiency ratios suggested that C. labrosus might be similar to carp in its ability to utilise low protein diets, which is of considerable economic significance.
9.1. SPECIAL METHODS

9.1.1. Experimental diets.

Two experimental diets were each fed at each of five ration levels to the fish in two replicate tanks. Treatments were allocated at random (Fig. 9.1.).

The composition of the experimental diets was as described for the 8.5 and 39.1% protein diets in the previous experiment (Section 8.1.1.), except that (a) the carbohydrate was supplied by dextrin only instead of dextrin and maize starch because of doubt about the ability of fish to assimilate starch (Singh and Nose, 1967) (b) the gluten: casein mixture was replaced by casein supplemented with amino acids as indicated below (Cowey et al 1970b).

- casein 95g
- arginine 2.5g
- cystine 1.0g
- methionine 1.0g
- tryptophan 0.4g

The poor growth of fish in the previous experiment may have been partly due to the use of gluten as a protein source (Cowey et al, 1971; Dupree & Sneed, 1966) which is poor relative to cod muscle in several essential amino acids, including lysine, arginine, and methionine.

The feeding levels selected were 2.5 (I), 5.0 (II), 7.5 (III), 10.0 (IV), and 12.5 (V) g wet weight diet per tank per day.
which corresponds approximately to 2.5, 5.0, 7.5, 10.0, and 12.5% body weight (dry weight food/wet weight fish x 100%). These were higher than generally used for fish but Odum (1968a) suggested that mullet might be adapted to utilising large quantities of a poor quality diet, and the previous experiment had indicated that the 'high' feeding level was below maximum.

Feeding was carried out as described in Chapter 7.

9.1.2. Experimental fish and procedure

Fish were collected from St. John's Lake in August 1976 and maintained in a large holding tank at Steer Point. They were fed on commercial fish pellets. On the 3rd September they were transferred to the experimental tanks. The dates on which fish in each tank were weighed and measured are shown below.

<table>
<thead>
<tr>
<th>Tanks</th>
<th>Initial branding, weighing &amp; measuring</th>
<th>Rebranding, weighing &amp; measuring</th>
<th>Final weighing &amp; measuring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>13/9</td>
<td>25/10</td>
<td>6/12</td>
</tr>
<tr>
<td>6-10</td>
<td>14/9</td>
<td>26/10</td>
<td>7/12</td>
</tr>
<tr>
<td>11-15</td>
<td>15/9</td>
<td>27/10</td>
<td>8/12</td>
</tr>
<tr>
<td>16-20</td>
<td>16/9</td>
<td>28/10</td>
<td>9/12</td>
</tr>
</tbody>
</table>

In an attempt to assess the effect of branding on growth and mortality the fish in tank 20 were unmarked, although each fish was weighed and measured as normal.

Immediate post-branding mortality was approximately 2%.

As some of the marks had been faint at the end of Experiment I, fish
were rebranded after 6 weeks.

Faeces were collected over the period 12th October - 19th October (excluding 17th and 18th October).

As water quality may have been a limiting factor on growth in Experiment I the water in the tanks was changed twice a week in this experiment, except for those occasions, particularly in the second half of the experiment, when daylight and tide times made more than one change per week impractical.

9.2. RESULTS

9.2.1. Temperature

Weekly maximum and minimum temperatures are shown on Figs. 9.2 - 9.5. Temperature declined during the course of the experiment, being particularly low during the last 3 weeks.

9.2.2. Experimental diets

The only tanks in which food was ever found on the following day were 5 and 6 (39.1% protein, feeding level V) towards the end of the experiment and, on two occasions, tank 12 (85% protein, feeding level V). No attempt was made to quantify the amount of uneaten food because it had become hydrated and no doubt altered by solution of soluble dietary components. In all tanks fish surrounded the food immediately it was placed in the tank. Observation of feeding behaviour did not indicate the existence of a hierarchy. Large and small fish fed at the same time and no aggression between fish was observed.
9.2.3. Growth

Brand marks were clearly distinguishable at 6 weeks and at the end of the experiment. The cut dorsal fin rays remained obvious except in a few cases.

For each fish (except unmarked fish in tank 20) the following were calculated:

(i) Change in weight during periods 0-6 weeks and 6-12 weeks.
(ii) Total change in weight.
(iii) Change in log weight in periods 0-6 weeks and 6-12 weeks.

For fish from each tank the following were calculated:

(i) Mean and standard deviation of length, weight and condition at the beginning of the experiment and after 6 and 12 weeks.
(ii) Mean and standard deviation of change in weight during each period of the experiment, and the whole experiment.
(iii) Mean instantaneous growth coefficient of fish in periods 0-6 weeks and 6-12 weeks (calculated as the mean change in log weight divided by the time in months).

Means and standard deviations and statistical analyses for the first period were calculated for all fish which had survived up to 6 weeks. Means and standard deviations and subsequent statistical analysis for the second period and the whole experiment were calculated for fish which survived until the end of the experiment.
To assess the effect of branding and fin clipping, mortality and weight of the unbranded fish in tank 20 was compared with fish in tank 11 which had received the same diet at the same feeding level. Seven fish from tank 11 died compared with only three from tank 20, although mortality as low or lower than this was also observed in some tanks containing branded fish. The mean weight of fish in tank 20 at 6 and 12 weeks was higher than in tank 11, in spite of being lower at the beginning of the experiment. However, when compared using t-tests, there was no significant difference in the mean weight of the branded and unbranded fish at 0, 6 or 12 weeks (t = .3464, .6993, 1.1088 respectively).

Mean length, weight and condition of fish from each tank are shown in Tables 9.1 to 9.5. Means and standard deviations of standard length and weight of fish in each tank are plotted in Figs. 9.2. to 9.5. There was a small gain in mean length of fish fed the 8.5% protein diet in all tanks except number 11 (ration level III). Mean weight varied less consistently. Fish fed at ration level I and one group of fish fed at ration level II lost weight in the period 0-6 weeks, but despite this, in most tanks there was an increase in mean weight over the whole experiment.

Among fish fed the 39.1% protein diet increase in mean weight differed markedly from increase in mean length. With the exception of fish fed at ration level I, and one group of fish fed at ration level II, mean standard length increased fairly steadily throughout the experiment, with increase in the period 0-6 weeks tending to be slightly greater than the increase in the period 6-12 weeks. In contrast, mean weight increased rapidly in
the period 0-6 weeks but showed little or no further increase in the period 6-12 weeks. The exception was fish fed at ration level I in which mean length and weight increased faster in the second period than the first.

Regressions of total change of weight on initial weight were calculated for the fish from each tank and are summarised in Table 9.6. In half the tanks the variance due to the regression was significant (p < .05) but among these the regression coefficient varied from -.1376 to +.2249. Analysis of variance in initial weight (Table 9.7), indicated a significant interaction between diet and feeding level (p < .05). When these were examined separately variance of initial weight due to ration level was non-significant among fish to be fed both the 8.5 and 39.1% protein diets. Variance of initial weight due to dietary protein level was non-significant at ration levels I-IV, but at the highest ration level fish to be fed the 39.1% protein diet were initially smaller than those to be fed the 8.5% protein diet. Thus although there were significant correlations between initial weight and change of weight within tanks, particularly among fish fed the 39.1% protein diet, there was no evidence to suggest that observed differences in mean change of weight were the result of variations in the weight of fish in different tanks at the beginning of the experiment.

The instantaneous growth coefficients are shown in Table 9.8 and Fig. 9.6. In the second period (6-12 weeks), the instantaneous growth coefficient was low in all tanks and did not vary consistently with either ration size or dietary protein level. In the first period (0-6 weeks) the instantaneous growth coefficient was
consistently higher amongst fish fed the 39.1% protein diet compared with those fed the 8.5% protein diet at the same feeding level. The instantaneous growth coefficient of fish fed both diets increased with feeding level from 2.5 to 10 g tank\(^{-1}\) day\(^{-1}\). Increasing the feeding level to 12.5 g tank\(^{-1}\) day\(^{-1}\) caused no further increase in instantaneous growth coefficient of fish fed the 85% protein diet and a decline amongst fish fed the 39.1 protein diet.

The mean and standard deviation of change of weight of fish in each tank are shown in Table 9.9 and Figs. 9.7 - 9.9. Analysis of variance of weight change indicated that the interaction between diet and feeding level was highly significant \((p < .001)\) and these two effects were, therefore, examined separately. The results of the analyses are summarised in Tables 9.10 and 9.11. Variance due to feeding level was significantly greater than residual variance \((p < .01)\) among fish fed both diets in the periods 0-6 weeks, 6-12 weeks and over the whole experiment. Variance due to dietary protein level was highly significant \((p < .001)\) in the period 0-6 weeks and over the whole experiment. In the period 6-12 weeks it was significant among fish fed at feeding levels II and III, but non-significant at feeding levels I, IV and V.

In the period 0-6 weeks (Fig. 9.7 ) the mean change of weight varied with ration level as described for the instantaneous growth coefficient, with growth on the 39.1% protein diet being greater than on the 8.5% protein diet at equivalent feeding level. Curvilinear regression was used to describe the relationship between growth and ration size. For fish fed both the 8.5 and 39.1% protein diets, the curvilinearity of the regressions was significant \((p < .05,\)
p < .01). From curves plotted on Fig. 9.10 the ration at which maximum growth occurred was estimated as 11.4g tank\(^{-1}\) day\(^{-1}\) for fish fed the 8.5% protein diet, and 8.9g tank\(^{-1}\) day\(^{-1}\) for fish fed the 39.1% protein diet. The ration at which growth was zero, i.e. maintenance ration, was estimated as 4.7 and 1.9g tank\(^{-1}\) day\(^{-1}\) for fish fed the 8.5 and 39.1% protein diets respectively.

In the period 6-12 weeks (Fig. 9.8.) the differences between mean change of weight of fish fed varying ration and protein levels were very much lower. At feeding levels II and III, where variance due to protein level was significant, change of weight tended to be greater amongst fish fed the 39.1% protein diet. As feeding level increased, weight change of fish fed the 39.1% protein diet increased slightly up to ration level III (7.5g tank\(^{-1}\) day\(^{-1}\)) and then decreased as ration level increased further. Amongst fish fed the 8.5% protein diet, the reverse pattern occurred, with a mean weight loss in both tanks of fish fed at ration level III.

Neither variance due to linear regression nor variance due to curvilinearity of regression was significant among fish fed the 8.5% protein diet in the period 6-12 weeks. In the same period the curvilinearity of the regression relating growth of fish fed the 39.1% protein diet to ration size was significant (p < .05). From the curve plotted on Fig. 9.10 the ration resulting in maximum growth was estimated as 5.8g tank\(^{-1}\) day\(^{-1}\). Maintenance ration could not be estimated since the equation suggested that growth of .0024g would have occurred even on zero rations. This is most unlikely, but the maintenance ration was obviously low.

At each feeding level total change of weight of fish fed
the 39.1% protein diet was higher than that of fish fed the 8.5% protein diet. Total weight change of fish fed the 8.5% protein diet increased with feeding level up to 10g tank\(^{-1}\) day\(^{-1}\), with no further increase at 12.5g tank\(^{-1}\) day\(^{-1}\). Total change of weight of fish fed the 39.1% protein diet increased with feeding level up to 7.5g tank\(^{-1}\) day\(^{-1}\), but progressively decreased when the feeding level was further increased to 10 and 12.5g tank\(^{-1}\) day\(^{-1}\). Over the whole experiment, the linear regression of growth on body weight among fish fed the 8.5% protein diet was significant (p < .01) and the best fit straight line is drawn on Fig. 9.10. Maintenance ration was estimated as 5.4g tank\(^{-1}\) day\(^{-1}\). Among fish fed the 39.1% protein diet the curvilinearity of the regression was significant (p < .01). The curve was plotted on Fig. 9.10. The ration resulting in maximum growth was estimated as 8.6g tank\(^{-1}\) day\(^{-1}\) and maintenance ration as 1.5g tank\(^{-1}\) day\(^{-1}\).

9.2.4. Mortality

Mortality (Table 9.12, Fig. 9.11) tended to be greater in the period 6-12 weeks than in the period 0-6 weeks, and to decline with increase in ration size. The greatest mortality was observed among fish fed the 8.5% protein diet at the lowest feeding level. The results of an analysis of variance of mortality are summarised in Table 9.12. The interaction between diet and feeding level was significant (p < .05). Variance of mortality due to feeding level was non-significant among fish fed the 39.1% protein diet, but highly significant (p < .001) amongst fish fed the 8.5% protein diet. Mortality was plotted against mean total
change of weight of the fish in each tank (Fig. 9.12). There was no significant correlation between change in weight and mortality amongst fish fed the 39.1% protein diet but a marginally significant (p < .05) negative correlation among fish fed the 8.5% protein diet. A positive correlation might have been expected if mortality resulted in a significant increase in the ration size of surviving fish. Thus the observed negative correlation probably arose indirectly from the fact that fish fed the most inadequate diets tended to show both the greatest mortality and the lowest weight gain. Comparing replicate tanks; in 4 cases increase in mortality was coupled with increase in weight gain of remaining fish, in 5 pairs of replicates the reverse was true. There was no evidence to suggest that differences in mortality contributed significantly to observed differences in growth via increased ration size of the surviving fish.

9.2.5. Relative Condition.

Relative condition was calculated for each fish individually at each time of weighing and measuring using the length-weight relation of captured fish of the same age group (Chapter 4). The mean and standard deviation of relative condition of fish in each tank are shown in Table 9.13 and Figs. 9.13 - 9.15. In an analysis of variance of relative condition, the interaction between diet and feeding level was significant (p < .01) and so these effects were analysed separately, and the results summarised in Tables 9.14 and 9.15. At the beginning of the experiment mean condition was similar in all tanks (Fig. 9.13). Variance due to
feeding level was marginally significant amongst fish fed the 8.5% protein diet \((p = .048)\) and significant among fish fed the 39.1% protein diet \((p < .01)\). However, mean condition tended to be slightly lower for fish to be fed the higher protein diet and at higher feeding levels and so later variation in condition could not be attributed to variation present at the beginning of the experiment.

After both 6 and 12 weeks relative condition tended to be higher amongst fish fed the 39.1% protein diet. The difference was greatest at the lowest feeding level and least at the highest feeding level, but variance due to protein content of the diet was significant at all feeding levels.

Variance in relative condition at 6 and 12 weeks due to feeding level was highly significant among both fish fed the 8.5 and 39.1% protein diets \((p < .001)\). After 6 weeks on the 8.5% protein diet relative condition tended to increase with feeding level up to 10 g tank\(^{-1}\) day\(^{-1}\), with no further increase in fish fed 12.5 g tank\(^{-1}\) day\(^{-1}\). After 12 weeks the situation was similar, except that no further increase in relative condition was observed at feeding levels greater than 7.5 g tank\(^{-1}\) day\(^{-1}\). After both 6 and 12 weeks on the 39.1% protein diet relative condition increased with ration size up to 7.5 g tank\(^{-1}\) day\(^{-1}\), but was progressively lower at higher ration levels.

Mean relative condition of fish in each tank over the course of the experiment is plotted in Fig. 9.16. The trend was for a slight overall increase in condition, with the exception of fish fed the low protein diet at low feeding levels, which showed a slight
overall decline. In most tanks mean relative condition of fish increased from 0-6 weeks but declined from 6-12 weeks. To ensure that the observed decline in condition was not due to the death of a few fish with a particularly high relative condition, mean condition was re-calculated using only fish which survived until the end of the experiment (Fig. 9.17). The variation of mean relative condition over the course of the experiment was very similar, indicating that mortality was not a significant factor in this case.

9.2.6. Apparent digestibility

Apparent digestibility of dry matter, organic matter and protein was estimated as described in Section 8.2.7. The apparent digestibility of dry matter is shown in Table 9.16 and Fig. 9.18. Variation between replicate tanks was large and there was no consistent relationship with feeding level. Apparent digestibility of dry matter tended to be marginally greater amongst fish fed the 39.1% protein diet. Variance due to protein level and feeding level were non-significant (Table 9.16).

The apparent digestibility of organic matter also varied little with feeding level or dietary protein level, although at higher feeding levels digestibility of organic matter from the 39.1% protein diet appeared to be slightly greater (Table 9.17, Fig. 9.19). Variation between replicate tanks was large. Variance of apparent digestibility of organic matter due to feeding level and dietary protein level were non-significant, (Table 9.17).

The apparent digestibility of protein differed little
with feeding level, but was markedly higher amongst fish fed the 39.1% protein diet (Table 9.18, Fig. 9.20). Variation between fish from replicate tanks was not as great as for the apparent digestibility of dry and organic matter. Variance of apparent digestibility of protein due to feeding level was non-significant, but variance due to protein level was highly significant \( (p < .001) \) (Table 9.18).

9.2.7. **Gross growth efficiency and protein efficiency ratio.**

Gross growth efficiency and protein efficiency ratio were calculated for the groups of fish which showed an overall gain in weight, as described in section 8.2.4. (Tables 9.19, 9.20, Figs. 9.21, 9.22).

Gross growth efficiency was greater in the period 0-6 weeks than 6-12 weeks. In both periods differences between replicate tanks were not large. Amongst fish fed the 39.1% protein diet in the period 0-6 weeks, gross growth efficiency increased when ration was increased from 2.5 to 5.0g tank\(^{-1}\) day\(^{-1}\) but declined with further increase in ration level. In the period 6-12 weeks growth efficiency declined with all increases of ration level above 2.5g tank\(^{-1}\) day\(^{-1}\) (ration level I). The gross growth efficiency of fish fed the 8.5% protein diet tended to be lower and appeared to vary little with feeding level. The analysis of variance of gross growth efficiency, summarised on Table 9.19, showed that variance due to feeding level was only significant for fish fed the 39.1% protein diet in the first period \( (p < .05) \).

Protein efficiency ratios are shown in Table 9.20, and
plotted against ration size in Fig. 9.22. Among fish fed the 39.1% protein diet in the period 0-6 weeks, PER increased with ration size from 2.5 to 5.0g tank\(^{-1}\) day\(^{-1}\) but declined with further increase in ration size. The maximum PER of fish fed the lower protein diet occurred at a ration level of 10g tank\(^{-1}\) day\(^{-1}\) with PER declining at both lower and higher feeding levels. The maximum PER was higher among fish fed the lower protein diet.

In the period 6-12 weeks PER values tended to be lower, and with the exception of a slight decline with increase in feeding level among fish fed the 39.1% protein diet, PER varied little with either dietary protein or feeding level.

Analysis of variance indicated that variance of PER due to feeding level was only significant (p \(<\) .05) among fish fed the 39.1% protein diet in the period 0-6 weeks.

9.2.8. **Body composition.**

The results of proximate analysis of fish are shown in Table 9.21. The protein content was estimated by multiplying the nitrogen content by 6.25, a factor derived from the mean nitrogen content of proteins. The composition of dried fish from each tank is shown in Figs. 9.23 and 9.24. In most cases the body composition of fish from replicate tanks was similar. The proportion of lipid in the body tended to increase with feeding level, particularly among fish fed the 8.5% protein diet. The proportion of ash tended to be higher at lower feeding levels, and higher in fish fed the 8.5% protein diet.
Moisture, ash, protein and lipid content are plotted against feeding level in Figs. 9.25 and 9.26. Analysis of variance of moisture content was carried out to resolve total variance into variance due to feeding level, dietary protein level and the interaction between them. Similar analyses were carried out for ash, protein and lipid content. The results are summarised in Tables 9.22 and 9.23.

Moisture content declined with increase in ration size, with moisture content of fish fed the 8.5% protein diet tending to be greater than that of fish fed the 39.1% protein diet at the same feeding level. Variance of moisture content due to feeding level was non-significant, but variance due to dietary protein level was significant (p < .05).

There was a significant interaction between the effect of protein level and feeding level on the variance of ash content. Among fish receiving the 8.5% protein diet, ash content declined with increase in ration size and variance due to feeding level was highly significant (Table 9.22, Fig. 9.25). Fish receiving the 39.1% protein diet tended to have a lower ash content, which varied little with feeding level above 5g tank\(^{-1}\) day\(^{-1}\). The variance of ash content due to feeding level was non-significant.

The lipid content of fish fed the 39.1% protein diet was higher than that of fish fed the 8.5% protein diet at all feeding levels except the highest. On both diets lipid content increased with feeding level up to 10g tank\(^{-1}\) day\(^{-1}\). At the highest feeding level lipid content was approximately the same amongst fish.
fed the 8.5% protein diet, but slightly lower amongst fish fed the 39.1% protein diet (Fig. 9.26). Variance of lipid content due to both feeding level (p < .001) and dietary protein level (p < .01) were highly significant (Table 9.23).

Protein, as a proportion of dry fish weight, declined with increase of feeding level up to 10g tank\(^{-1}\) day\(^{-1}\). Further increase in feeding level caused no further decline in protein content. There was no apparent difference between the proportion of protein in fish receiving diets differing in protein level. Variance of fish protein content due to feeding level was significant (p < .05) but variance due to dietary protein level was not.

There was a highly significant negative correlation between lipid content and moisture content (p < .001) (Fig. 9.27). The best straight line was estimated using the method of least squares and the regression equation calculated as:

\[
\text{lipid content} = -1.76 \times \text{moisture content} + 150.56
\]

% dry weight % wet weight

There was a highly significant positive correlation between lipid content and condition (Fig. 9.27). The best straight line was estimated using the method of least squares and the regression equation calculated as:

\[
\text{lipid content} = 67.31 \times \text{relative condition} - 41.45
\]

% dry weight
9.2.9. Variation of gutted weight and liver weight.

As described in Section 8.2.9, gutted weight and liver weight were expressed as a percentage of total weight of each fish so as to enable comparisons between fish of different sizes (Tables 9.24 and 9.26). Mean and standard deviation of gutted fish and liver weight were plotted against feeding level (Figs. 9.28 and 9.29). Analyses of variance indicated that variance of both gutted weight and liver weight due to the interaction of feeding level and protein level were significant (p < .001). The variance due to protein and feeding level were therefore analysed separately and the results summarised on Tables 9.25 and 9.27.

Mean gutted fish weight was higher amongst fish fed the 39.1% protein diet at all feeding levels, but, at both protein levels, varied little with feeding level. Variance due to feeding level was non-significant at both protein levels whereas variance due to protein level was significant at all except the IIIrd feeding level (p < .05).

Variance of liver weight due to dietary protein level was non-significant except at the lowest feeding level, when the mean liver weight of fish fed the 39.1% protein diet was proportionately larger, (p < .001). Among fish fed the 8.5% protein diet mean liver weight tended to increase with feeding level up to 7.5g tank\(^{-1}\) day\(^{-1}\), but declined as feeding level further increased. Variance due to feeding level was significant (p < .01). The liver weight of fish receiving the 39.1% protein diet appeared to be unaffected by feeding level up to 10g tank\(^{-1}\) day\(^{-1}\), but was re-
duced amongst fish fed at 12.5g tank$^{-1}$ day$^{-1}$. Variance due to feeding level was non-significant.

9.2.10. **Histology.**

The gills, gut and liver of 3 fish from each tank were examined histologically. Fish from the same tank tended to show similar characteristics although to differing degrees.

(i) Gills: Thickening of gill epithelium was characteristic of all experimental fish, but was particularly marked amongst fish receiving the 8.5% protein diet, where fusion of lamellae and even filaments was observed (Plate 9.1). Encapsulation of bacteria was observed in fish fed the 8.5% protein diet at all feeding levels and in one fish receiving the 39.1% protein diets at levels III, IV and V (Plate 9.2.). Among fish receiving the 8.5% protein diet at feeding levels IV and V the gill epithelium showed some evidence of disintegration, and at intervals along the filaments of some fish (Plate 9.3) there were swollen rounded lamellae in which the epithelium enclosed a disorganised mass of erythrocytes.

Among fish fed the 39.1% protein diet there was more severe thickening of the epithelium of gills of fish fed at the lowest and highest feeding level (Plate 9.4.) compared with those of fish fed at intermediate feeding levels (Plate 9.5.).

(ii) Guts: Amongst fish fed both diets, the amount of fat around the pyloric caeca tended to increase with
feeding level, and the pyloric caecae were more vacuolated than in the previous experiment, particularly at the higher feeding levels (Plates 9.6 and 9.7). Myxosporidian cysts appeared to be more common amongst fish fed at the higher feeding levels. Trematode worms (Plate 9.8) were observed in the pyloric caecae in 40% of fish examined which had been fed the 8.5% protein diet, and 22% of fish examined which had been fed the 39.1% protein diet. However, as only 3 fish were examined per tank, and only part of the gut was sectioned, this cannot be taken as an estimate of this abundance.

(iii) Liver: The amount of fat/carbohydrate stored in liver cells varied markedly with feeding level among fish fed both the 8.5 and 39.1% protein diets. Plates 9.9 and 9.10. show sections of the livers of fish fed the 8.5% protein diet at ration levels II and V. Plates 9.11., 9.12., and 9.13. illustrate the progressive increase in storage of fat/carbohydrate in liver cells of fish fed the 39.1% protein diet at increasing feeding levels.

9.3. DISCUSSION

9.3.1. Growth.

The mean weight of all fish increased from 1.046 to 1.221 during the course of the experiment an increase of only 16.7%. However, fish on less favourable feeding regimes either lost weight or grew very slowly. Whereas those fed the 39.1% protein diet at the higher feeding levels showed a weight
gain of 25-35.3%. This is small compared with the 123% increase observed in St. John's Lake among fish of the same age group over the slightly longer period of August to December 1975. When allowance was made for growth in August, this was reduced to 45.5%, but nevertheless indicates that fish which showed maximum growth in the experiment grew slower than fish of the same species and age group in St. John's Lake. Possible reasons have been summarised in Section 8.3.1.

As in Experiment I there was a marked dissimilarity of growth in different experimental periods. In both cases the growth pattern of experimental fish resembled that of wild fish. Amongst fish in Experiment II, 77.28% of all growth from September to December occurred in the period up to mid October (0-6 weeks). This is reflected in the instantaneous growth coefficients. Similarly in St. John's Lake, 83.6% of the total gain in weight from August to December occurred in the period up to mid October. Food was totally consumed in most tanks during both 0-6 weeks and 6-12 weeks. Unfortunately separate data for assimilation in the two periods was not obtained, but from results of the previous experiment one would not expect the differences to be large. However, both gross growth efficiency and PER tended to be higher in the period 0-6 weeks. It seems likely that temperature was an important factor since this was not controlled and, therefore, decreased during Experiment II. Kennedy & Fitzmaurice (1969) found that C. labrosus ceased feeding when temperature fell below 8°C. In most tanks fish seemed to feed
throughout Experiment II in spite of the low temperatures. The decline of growth in weight may be connected with the observation of Swift (1961) on brown trout, that growth rate is slower when temperature is falling than when it is rising. The retardation of growth, irrespective of ration size and dietary protein content is consistent with growth being limited in all fish by some other factor, such as pituitary growth hormone synthesis and secretion (Kayes, 1977). In sculpins, Warren and Davies (1967) observed that the relationship between food consumption and growth was also affected by the season in which the experiments were carried out.

There was a marked difference between the weight and standard length growth curves of fish fed the 39.1% protein diet. Standard length gain lagged behind weight gain and increased at a more constant rate. The possibility of technical errors in weighing, for example inadequate removal of surface moisture, was considered. However, the disproportional increase in weight in the first period was limited to fish receiving the 39.1% protein diet and the 8.5% protein diet at high feeding levels, whereas in other tanks the reverse was observed. Errors in the balance or weighing technique would have been expected to affect all fish. The out of phase increase of length and weight was reflected in the relative condition factor which, among fish fed the 39.1% protein diet and the 8.5% protein diet at higher feeding levels, increased during the first 6 weeks of the experiment, and declined during the second 6 weeks. Alternate periods of fast and slow growth
and alternate periods of growth in length and weight were also noted in trout (Brown 1946b) although the time periods were rather shorter (c. 2 weeks).

In about half the tanks there was a significant regression between change in weight and initial weight of individual fish. Although statistical analysis of initial weights indicated that no correction for differences in initial weight were necessary, the regressions, and the slight increase in standard deviation of weight and length during the experiment suggested the possible existence of hierarchical effects.

Although the food ration was not proportionately reduced when mortality occurred there was no evidence to suggest that differences in mortality contributed significantly to observed differences in growth of the surviving fish. The variance of mortality due to feeding level was non-significant among fish fed the 39.1% protein diet. Although highly significant among fish fed the 8.5% protein diet, the negative regression coefficient yielded by the regression of growth on mortality indicated that any advantage to surviving fish was outweighed by other experimental factors. The reasons for the high mortality among fish fed the 8.5% protein diet at the lowest feeding level are unknown. Some showed 'tail rot' suggesting that their low nutritional state had made them vulnerable to attack by pathogens such as Vibrio sp. Although thickening of gill epithelium and fusion of lamellae were observed in these fish, this was more severe among fish fed at
the higher feeding levels where mortality was lower. Other authors have reported that ration size has little or no effect on survival (Tiemeier et al., 1969, Andrews and Stickney 1972).

A curvilinear response of growth to ration size has been observed in many fish including rainbow trout (Huisman, 1976), plaice (Rafail, 1968), channel catfish (Andrews and Stickney, 1972), perch (Solomon and Brafield, 1972), cod (Edwards et al. 1972), Carassius auratus (Davies 1964), and Tilapia mossambica (Pandian and Raghuraman, 1972). Direct comparisons with other work are difficult because of the expression of ration in several ill specified terms, and the use of a wide variety of experimental foods and conditions. For example, the effect of oxygen tension, light regime and temperature on growth vary considerably with ration size (Elliott 1976c; Wurtsbaugh and Davis, 1977a; Brett, 1979). In addition large mackerel appear to show a relatively smaller increase in growth than small mackerel for the same relative increase in ration size (Hatanaka et al., 1957) indicating the importance of body size in the growth-ration relationship.

Flowerdew & Grove (1980) reported that the maximum ration of trout pellets that C. labrosus (c. 6g) were able to ingest was 0.8, 1.4 and 2.3% wet body weight at 13, 18, and 23°C respectively. Compared with this the c.10% body weight (dry weight food: wet weight body) per day ration which was observed to result in maximum growth in this experiment does appear to be large, but compares well with the 7-10% ration (rice bran
+ fish meal) reported for _M. cephalus_ of 0.5.-3.0g by Perera & De Silva (1976). The growth of _L. aurata_ (c.10g) increased with ration size (artificial diet) to the largest ration used (7.5% dry weight food: wet weight fish) suggesting that maximum growth occurred at a higher ration (Vallet et al 1970). This is also consistent with the high food consumption of juvenile mullet observed in the field (Chapter 5) although this was probably also related to the dilution of the diet by sand and mud grains (Rozin and Mayer 1961). Vallet et al (1970) suggested that grey mullet expend a lot of energy in search of food - behaviour reflected in the high basic metabolic rate, and the fact that mortality was higher on rations below maintenance than on zero rations. This was not observed when juvenile _C. labrosus_ (Experiment I) were starved, but the high metabolic rate of mullet has been noted by several authors (Kutty and Mohamed, 1975; Belokopytin, 1968) and may partly account for the necessity of a large food intake.

Maximum growth in the 39.1% protein diet in Experiment I was higher than that observed in Experiment II in spite of the higher feeding levels (maximum growth coefficients .219 and .206 respectively). This suggested that the modifications in diet did not confer any significant advantage, and that juvenile _C. labrosus_ were able to utilise maize starch as well as dextrin, and gluten as well as casein. It is possible that juvenile _C. labrosus_, like carp and catfish (Andrews et al, 1977; Aoe et al, 1970), differ from salmonids and terrestrial animals in that they are unable to utilise free amino acids.

As in Experiment I the protein level in the diet
had a highly significant effect on growth, with the 39.1% protein diet promoting greater growth than the 8.5% protein diet at all feeding levels. Growth increased with ration level, but among fish fed the 39.1% protein diet, growth and relative condition were depressed at the Vth and possibly also the IVth feeding level. The body composition of fish fed at feeding levels III, IV and V was very similar, with even a slight decline in fat content at the higher feeding levels, which suggested that the depression of growth was not due to the laying down of energetically more costly fat rather than lean. In spite of the water in the experimental tanks being changed more frequently in Experiment II than Experiment I in an attempt to reduce any effects of accumulated waste products, the fish receiving the 39.1% protein diet at the highest feeding level which were examined histologically showed changes in the gill epithelium characteristic of exposure to sublethal ammonia concentrations. The various ways in which ammonia concentration may affect fish growth have been discussed in the previous chapter (8.3.1.).

However, C. labrosus fed the 8.5% protein diet at the highest feeding level also showed evidence of gill damage although there was no marked effect on growth in this case. Depression of growth at high ration levels was also recorded in carp (Huisman, 1976), rainbow trout (Staples and Nomura, 1976), and juvenile C. labrosus (Flowerdew & Grove, 1980). Huisman (1976) attributed the depression of growth at high ration levels to the increase of metabolic rate with ration size but Cowey et
al (1972) reported that a curvilinear dose response plot was found to be linear over the same range of nutrient intake for the same species under different experimental conditions. One would expect there to be a physiological limit to the response of growth to nutrient level or ration size. However, it is apparent that the limits observed in experiments may frequently be those imposed by the experimental conditions or hormonal state of the fish rather than the nutrient of ration size under examination. This is corroborated by the fact that growth rates observed in such experiments are often below maximum (Cowey et al., 1970a; De Silva & Perera, 1976).

Results from all experiments should be examined in terms of their environmental factors, and in this context the lack of temperature control was an obvious handicap in these experiments on juvenile grey mullet. In view of this, and the inter-relationships between temperature, ration size, body weight, metabolism and growth (Elliott, 1976c; Wurtsbaugh and Davis, 1977a,b; Solomon and Brafield, 1972), mathematical relationships between ration size and growth were developed to describe the relationship under these specific conditions. This point is illustrated on Fig. 9.10 where mean change of weight over 14 weeks of fish fed 8.5 and 39.1% protein diets during experiment I were plotted for comparison. Although similar overall relationships applied, growth of fish in Experiment I was greater at a ration level of 3g tank\(^{-1}\) day\(^{-1}\) and lower at a ration level of 1g tank\(^{-1}\) day\(^{-1}\) than would be predicted from the results of Experiment II. The reasons for this are unclear. There were differences in diet and
lighting regime and the lower temperatures during Experiment II were undoubtedly of significance. As Experiments I and II were carried out during Spring and Autumn respectively, day length and the hormonal state of the fish may also have been important.

However, within the limitations of Experiment II, the equations developed to describe the relationship between growth and ration size, did enable some useful comparisons between this relationship in fish fed diets differing in protein content. There was a significant interaction between the effects of dietary protein content and ration level on growth. Maximum growth was lower among fish fed the 8.5% protein diet. The ration level supporting maximum growth was $\geq 12.5\text{ g tank}^{-1}\text{ day}^{-1}$ and $8.6\text{ g tank}^{-1}\text{ day}^{-1}$ for fish fed the 8.5 and 39.1% protein diets, respectively. Similarly the ration at which growth was zero was lower for fish fed the 39.1% protein diet ($1.5\text{ g tank}^{-1}\text{ day}^{-1}$) than for fish fed the 8.5% protein diet ($5.4\text{ g tank}^{-1}\text{ day}^{-1}$).

The difference in the ration size which would maintain body weight was not only due to the difference in dietary protein level because at zero growth protein intake of fish fed the 8.5% protein diet was 200g compared with 371g for groups of fish fed the 39.1% protein diet. This difference is made even greater by the reduction of apparent digestibility of protein from lower protein diets but as this may be due to the excretion of metabolic nitrogen it was not taken into account (Nose, 1967). The calories consumed by fish fed the higher ration of the 8.5% of protein diet therefore spared protein, and it was calculated that 1 kcal
spared 0.031g protein. The protein sparing effect of increased calories was more fully investigated in Experiment IV.

The maintenance requirement of juvenile _C. labrosus_ of the size used in Experiment II, calculated from equations derived by Flowerdew and Grove (1980), was 23–30 cals fish⁻¹ day⁻¹ at 18°C. From the results of Experiment II the maintenance requirement of juvenile _C. labrosus_ was 82 cals fish⁻¹ day⁻¹ and 200 cals fish⁻¹ day⁻¹ for fish fed the 39.1 and 8.5% protein diets respectively. These are considerably greater, in spite of the fact that Flowerdew & Grove's calculations were for total energy whereas energy in experimental rations was calculated as metabolisable energy. Possible reasons include inadequacies in the experimental diets rendering parts of it, for example the free amino acids, unavailable; the lower temperatures in Experiment II increasing energy requirement for osmoregulation; and the build-up of ammonia in the experimental tanks increasing the production of urea, thereby reducing the amount of energy derived from each g protein (Guerin-Ancey, 1976b).

9.3.2. **Apparent digestibility**

The apparent digestibility of protein was particularly low in fish fed the 8.5% protein diet, but as described in Section 8.3.2, this may be due to endogenous nitrogen excretion rather than true differences in digestibility. Although the apparent digestibility of protein from the 39.1% protein diet was similar to that observed for other fish (eg. Cho, Slinger and Bayley, 1976; Phillips and Brockway, 1959) the apparent digest-
ibility of dry matter and organic matter tended to be lower (Pandian, 1967a; 1972; Cho, Slinger and Bayley, 1976). The average digestibility of organic matter of 56.2% for juvenile C. labrosus in this experiment compares well with the estimates of 51.7% for Liza falcipinnis on natural food (Payne, pers.comm.) and 45% for C. labrosus fed pellets of sand and cod roe (Hickling, 1970b). These fish were considerably larger, but body size has little or no effect on apparent digestibility in other species, (Staples and Nomura, 1976; Pandian, 1967a; Kelso, 1972).

The apparent digestibilities of protein, dry matter and organic matter were similar to those observed in Experiment I which suggested that gluten protein was as well digested as casein and that, contrary to observations on salmonids (Singh and Nose, 1967; Inaba et al. 1963), maize starch was digested as well as dextrin. Since algae are of such importance in the diet, it is perhaps not surprising that mullet should have become adapted to utilise complex carbohydrates. Stickney and Shumway (1974) experimentally demonstrated the presence of cellulase activity in the stomach of M. cephalus.

Ration size had no significant effect on apparent digestibility of dry matter, organic matter or protein in juvenile C. labrosus and so observed differences in growth cannot be attributed to differences in assimilation. Similarly, protein absorption in bluegill sunfish (Gerking, 1955a) and assimilation efficiency of rainbow trout (Staples & Nomura, 1976) were found to be unaffected by rate of feeding. In perch (Solomon and Brafield, 1972) and walleye Stizostedion vitreum vitreum
the proportion of energy intake lost as faeces was constant at varying ration sizes. However, this may present a false picture of the proportion of the diet actually assimilated and available for growth, since energy in the soluble excretory substances sometimes exceeds that of the faeces (Huisman, 1976) and may vary with ration size (Savitz et al, 1977). When this is taken into account energy assimilation efficiency has been shown to decline with increase in ration size in perch (Solomon and Brafield, 1972), carp and rainbow trout (Huisman, 1976), rainbow trout (Wurtsbaugh and Davis, 1977a) and guppy (Kinne, 1960).

The apparent digestibility of protein and organic matter by juvenile C. labrosus did not vary with ration size in this experiment. However, it is possible that increased energy loss in soluble excretory substances at high feeding levels may have contributed to the plateauing and depressing of growth under these treatments.

9.3.3. Growth efficiency and protein efficiency ratio

There has been considerable controversy in the literature regarding the relationship between gross growth efficiency and ration size. Paloheimo and Dickie (1966b) reviewed data of various authors and concluded that the relationship could be described as a negative exponential with efficiency declining as ration size increased. The plot of log efficiency against ration size is frequently referred to as the 'K' line.
\[ \frac{W}{R \cdot t} = e^{-a-bR} \]

Where

- \( W \) = weight
- \( R \) = ration
- \( t \) = time
- \( \frac{W}{R \cdot t} \) = gross growth efficiency, \( K \)
- \( a, b \) = constants.

This has since been criticised for several reasons, including:

(i) Gross growth efficiency must be zero at maintenance ration.

(ii) No consideration is given to changes in body composition which may be considerable (Brett, 1969; this work).

This is overcome in later work by the consideration of growth efficiency in terms of energy.

Brett et al (1969) examined growth efficiency of Oncorhynchus nerka and concluded that the 'K' line phenomenon was a special instance observable only at high ration levels, although Le Brasseur (1969) obtained 'K' lines with O. keta growth over a wide range of feeding levels. Kerr (1971) reviewed this work, and reconciled the results in terms of differences in experimental technique and a 'K line' with an initial positive phase at low rations, followed by the negative phase as described by Paloheimo and Dickie. This type of 'K line' has since been confirmed in studies on various fish including trout (Iwata, 1970), carp (Huisman, 1976), tilapia (Pandian and Raghuraman, 1972), cod (Edwards et al 1972) and plaice (Rafail, 1968), and may explain the results of other authors who reported increase in gross growth efficiency with ration size (Wurtsbaugh and Davis, 1977b; Staples and Nomura, 1976; Iwata, 1970; Gerking, 1971; and Rafail, 1968). The maximum efficiency coincides with what
has been described as the 'optimum ration'. The decline in efficiency with further increases in feeding level has been variously attributed to increase of specific dynamic action, principally from increased deamination of amino acids (Wurtsbaugh and Davis, 1977a), increased activity (Kerr, 1971), and increase in musculature rather than length (Edwards et al, 1972).

Wurtsbaugh and Davis (1977b) reported that as ration size increased, gross growth efficiency for large S. gairdneri began to decline while that of small fish continued to increase. Although Paloheimo and Dickie (1966b) predicted that growth efficiency was independent of body size, this was not generally observed in experimental studies (Pandian, 1967b; Gerking, 1971; Menzel, 1960). Staples and Nomura (1976) examined gross efficiency in 3 size groups of rainbow trout and concluded that gross growth efficiency of energy utilisation was similar among fish of all sizes at the same level between maintenance and maximum ration. However, this was not true for efficiency in terms of wet weight which declined with increase in fish size as has been reported for other fish such as mackerel (Hatanaka, et al. 1957). Thus expression of efficiency in various terms may have contributed to the contrasting results reported in the literature, although even when measured in terms of energy, the growth efficiency of Ophiocephalus striatus (Pandian, 1967b) and Epinephelus guttatus (Menzel, 1960), declined with increase in body weight.

Paloheimo and Dickie (1966b) showed that when fish
were fed larger particles, shallower \( k \)-line slopes resulted, reflecting differences in the energy cost of acquiring different foods. Kerr (1971) took this into account in developing a model of gross growth efficiency in which efficiency only remained constant with increasing body size when the energy cost of acquiring a fixed weight of food remained constant. Such conditions may occur in a feeding experiment, but under natural conditions it might be expected that larger fish would expend more energy in obtaining food rations. Kerr (1971) also suggested that spontaneous activity might be of major significance in laboratory examination of efficiency. These factors may be of particular relevance to grey mullet, which normally exhibit considerable tidal feeding movements and consume a high ration of relatively poor quality food. This must be taken into account in considering results from experiments in which normal activity is restricted and rations artificial.

As in Experiment I gross growth efficiency of juvenile *C. labrosus* was higher amongst fish fed the 39.1% protein diet (Section 8.3.3.). In the period 6-12 weeks the effect of ration size on gross growth efficiency was non-significant. In the period 0-6 weeks gross growth efficiency increased to a maximum at a ration of approximately 5% body weight per day and then declined among fish fed the 39.1% protein diet. Assuming that gross growth efficiency increases from the maintenance ration, the small amount of data available for fish fed the 8.5% protein diet suggested that growth efficiency in-
creased to a maximum at approximately the same ration level but remained constant at higher rations. Such conclusions are speculative in view of the number of fish which lost weight, but may lend support to the idea that the decline in gross growth efficiency with increase in ration size in fish fed the 39.1% protein diet was due to increased deamination caused by an increase in the rate of protein turnover. This was also supported by examination of protein efficiency ratios which showed that at lower feeding levels PERs were similar for fish fed both diets but at higher feeding levels those of fish fed the 8.5% protein diet were superior. De Silva & Perera (1976) reported no consistent differences in conversion efficiency between _M. cephalus_ (of similar size to the _C. labrosus_ of this experiment) fed ad lib and 8% body weight per day. Vallet et al. (1970) reported that the gross growth efficiency of _L. aurata_ (10g) varied in a similar manner to that observed in this work, with maximum gross growth efficiency at a ration of 4.5% (dry weight food: wet weight fish) per day. Conversion efficiency of juvenile _C. labrosus_ fed trout pellets also increased to a maximum and then declined as ration increased (Flowerdew & Grove, 1980). The maximum conversion efficiency was approximately the same at all temperatures, but the ration size resulting in maximum conversion efficiency (optimum ration) declined from c 7.7 to c 2.2% dry body weight fish⁻¹ day⁻¹. The lower temperatures prevailing during the period 6-12 weeks may, therefore, have resulted in a reduction of the optimum ration of juvenile _C. labrosus_ in Experiment II.
which, as illustrated in the diagram below, would account for both the lower growth efficiencies observed in the period 6-12 weeks, and the fact that the growth efficiencies of fish fed the 39.1% protein diet declined from a maximum value at the lowest feeding level during this period.
9.3.4. Relative condition and body composition

The effect of feeding level on body composition of the fish is important both economically, because of its effect on market acceptability and storage, and biologically because growth as fat is energetically more costly than growth as lean.

The evidence indicated that the higher relative condition at 6 weeks compared with 12 weeks was genuine, and not due to either technical errors or differential mortality. Examination of the data showed that the decline was due to increase in length rather than any decrease in weight and so it appears that juvenile grey mullet undergo alternate cycles of growth in weight and length as described by Brown (1946b) for trout, and Reay (1973) for sandeels.

The variation of relative condition with dietary protein content was similar to that observed in Experiment I, but appeared to occur only at lower feeding levels. This is consistent with observations on trout, which under temperature stress showed a high rate of change in body constituents on zero rations which declined to insignificant on maximum rations (Elliot, 1976a). Juvenile grey mullet fed 7.5 - 10.0g tank\(^{-1}\) day\(^{-1}\) had the highest mean relative condition. In cod, condition also increased with ration size (Edwards et al 1972) but although the increase became progressively smaller, no decrease of condition was reported at the highest ration used. Possible reasons for the reduction in condition at the highest ration levels in juvenile C. labrosus in Experiment II have already been discussed.
There was a highly significant positive correlation between lipid content and relative condition, which suggested that the increase in relative condition was at least partly attributable to the deposition of fat in the body. Since the energetic cost of laying down fat is greater than that of lean it was possible that some of the decline in the gross growth efficiency (wet weight) with increase of ration size among fish fed the 39.1% protein diet was due to deposition of tissue with a higher fat content. However, the fact that gross growth efficiency continued to decline among fish fed at feeding level V in spite of their reduced fat content and that no decline in efficiency was observed among fish fed the 8.5% protein diet, in spite of the increase of fat content with ration size, suggested that this was not the case.

As recorded previously (Section 8.3.4) there was a highly significant negative correlation between lipid and moisture content. The relationship of both relative condition and moisture content to lipid content was similar to that described in the fish of Experiment I, but in each case, at the same body moisture content or relative condition, the fish from Experiment II contained a larger proportion of fat. This may be partly due to differences in diet and feeding level, but the season of the year may also be important—in the wild, juvenile grey mullet increase fat deposits in the autumn.

The increase in lipid content and decrease in moisture content with increase in ration size has also been re-
corded in Oncorhynchus nerka (Brett et al, 1969) rainbow
tROUT (Huisman, 1976; Wurtsbaugh and Davis 1977a; Staples
and Nomura, 1976), brown trout (Elliot, 1976a), perch
(Solomon and Brafield, 1972), Tilapia mossambica (Pandian
and RaghuRaman, 1972) and bluegill sunfish (Gerking, 1955a).
Cod seems to be the exception, with relative proportions of
protein and lipid remaining constant at all feeding levels
used (Edwards et al, 1972). The slight decline in fat
content of juvenile C. labrosus at the highest feeding levels
was also recorded in Tilapia mossambica (Pandian and
RaghuRaman, 1972). The decline of protein and ash content
with increase in ration size probably reflected the increase
in lipid content. There was no evidence of low protein con-
tent at low feeding levels as reported by Phillips and Brockway,
(1959) in trout. The higher ash content of fish receiving the
8.5% protein diet may have been related to the higher carbohy-
drake level in the diet which was reported to result in
increased ash content in rainbow trout (Austreng, et al., 1977).

9.3.5. Gutted weight, liver weight and histology
As in Experiment I increase in the protein con-
tent of the diet resulted in a significant increase in gutted
weight as a % of total weight, whereas increase in ration size
had no significant effect on gutted weight. The effect of
dietary protein level on liver weight was insignificant except
at the lowest feeding level, but, particularly amongst fish fed
the low protein diet, liver weight increased with ration size.
up to 7.5 g tank$^{-1}$ day$^{-1}$ and then declined. In cod, liver weight as a percentage of total weight was found to increase linearly with ration size and this was attributed to storage of fat in the liver (Edwards et al., 1972). The histological examination of two mullet from each tank suggested that the amount of fat/carbohydrate stored in liver cells increased with ration size. It is possible that, as ration size increased above 7.5 g tank$^{-1}$ day$^{-1}$, a proportionately greater amount of fat was stored in the muscles and gut resulting in the decrease in relative liver weight at high feeding levels. This was not observed in cod - perhaps because cod seems to be unusual amongst fish in storing all excess fat in the liver (Edwards et al., 1972). Examination of guts confirmed the increase in gut mesentery fat deposits with ration size but these observations were not quantitative.

Myxosporidian cysts did appear to be more common amongst fish fed at higher feeding levels, but it seemed unlikely that they were so numerous as to interfere with digestion or absorption. In the small number of fish taken from each tank, trematode worms were more common in fish fed the lower protein diet but their contribution to reduced growth, body lipid etc. is unknown.

The thickening of gill epithelium found in fish from most tanks suggested that experimental conditions were not ideal. It is difficult to know if this was due to concentration of waste products or some dietary deficiency (possibly in pantothenic acid or biotin) (Section 8.3.5.). As gill damage was
extreme at the lowest and highest feeding levels it seems likely that both factors were of significance - dietary deficiency at the low feeding level and ammonia concentration at the high feeding level.
CHAPTER 10.

EXPERIMENT IV. AN INVESTIGATION OF THE EFFECTS OF DIETARY ENERGY LEVEL AND SOURCE.

In the experiments described in Chapters 8 and 9 some attempt was made to examine the effect of ration size and dietary protein level on the growth of juvenile C. labrosus. Although dietary protein level has attracted most attention, because this tends to be the most costly part of the diet, dietary energy level and source is also important in determining growth of other fish species (Page & Andrews, 1973; Cowey et al., 1975). The work of Lee & Putnam, (1973) showed that body lipid and protein efficiency ratios of rainbow trout were determined by the protein: energy ratio in the diet regardless of the actual levels of protein or energy.

The first attempt to examine the effect of diets varying in energy source and level was terminated by massive mortality amongst the experimental fish (Appendix 1: Experiment III). The aim of Experiment IV was to investigate improvement of growth in juvenile C. labrosus by the addition of supplementary energy to diets of fixed protein level, and to compare two sources of supplementary energy - corn oil + cod liver oil, and dextrin.
10.1. SPECIAL METHODS

10.1.1. Experimental diets

The composition of the ten experimental diets is shown in Table 10.1 and a scheme of the experimental design on Fig. 10.1. Diets contained 20 or 40% protein and energy at 4 different levels (I, II, III & IV) supplied mainly by either carbohydrate or carbohydrate + lipid. At each protein level diets fell into 2 series of three in which energy increased (i) by increase in lipid content or (ii) by increase in carbohydrate content.

The feeding level of 10% body weight per day (wet weight food : wet weight fish) was chosen on the basis of results of Experiment II. This feeding level resulted in good growth, which was still below maximum, for juvenile _C. labrosus_ fed the higher protein diet.

The protein was supplied as casein supplemented by additional amino acids, and the lipid was a mixture of corn oil and cod liver oil, as in the diets used in Experiment II. The carbohydrate source was dextrin. α-cellulose was used to make up dietary bulk and the assumption was made that it was not assimilated. In the lowest calorie diets, the dietary bulk was not fully made up with cellulose as this would have resulted in diets containing more than 40% cellulose. Such high levels may impair the digestion and absorption of other nutrients. The 80g dry components + lipid were mixed with 80g agar solution, but to compensate for the relative reduction of
dietary bulk, these diets were fed at 8% body weight per day instead of 10%. Thus fish received the same amounts of protein, lipid, carbohydrate and calories as they would have received if dietary bulk had been fully made up, and the diets fed at 10% body weight per day, as to fish in other tanks.

Each diet was fed to fish in two replicate experimental tanks. Diets were allocated at random (Fig. 10.2).

After the conclusion of the experiment the loss of dry weight from the diets over 24 hours was tested by placing a weighed amount of each diet (approximately 10g) in an experimental tank, set up exactly as in the experiment, but which contained no fish. After 24 hours the remaining diet was carefully collected, dried in an oven at 60°C and reweighed. A similar weight of each diet was dried and reweighed as above, to determine the initial water content.

10.1.2. Experimental fish and procedure

The fish were collected during May at St. John's Lake, and placed in a large holding tank at Steer Point. Most fish appeared healthy and readily began to feed on commercial fish pellets.

On 23rd May fish were transferred to the experimental tanks, and fed on a 39.1% protein diet as used in Experiments I and II. The dates of commencement, reweighing and measuring, and ending of the experiment are shown below. As in Experiment II fish were rebranded after 6 weeks.
Tank Nos. 1-4 5-8 9-12 13-16 17-20

Dates of:

(i) initial branding weighing & measuring

30/5 31/5 1/6 2/6 3/6

(ii) weighing, measuring, re-branding

11/7 12/7 13/7 14/7 15/7

(iii) final weighing & measuring

22/8 23/8 24/8 25/8 26/8

Post branding mortality was slight in all tanks except 9 and 10 where it was 36% and 14% respectively on the day after branding, with more fish dying during the next week. Fish in tank 20 were left unmarked to provide some information, by comparison with fish in the replicate tank, on the effect of marking on growth and mortality.

The feeding rates were calculated from the summed initial weights of fish in each tank, and recalculated when fish were reweighed after 6 weeks. When fish died they were identified from their brand and fin mark, and the total wet weight of fish recalculated omitting the initial or 6 week weight of the fish which had died.

The periods of faeces collection were:

(i) 28th June - 5th July (excluding Sunday 3rd and Monday 4th July).

(ii) 9th August - 16th August (excluding Sunday 14th and Monday 15th August).

Method of collection was as previously described.

The tanks were cleaned out and seawater renewed twice a week. In addition to maximum and minimum temperature
records as before, the oxygen concentration and pH were also measured on consecutive days over one water changing cycle. Oxygen concentration was measured using a portable dissolved oxygen meter (Electronic Instruments Limited, model 1520). The light regime was as in Experiment II.

10.2. RESULTS

10.2.1. Temperature, oxygen concentration and pH.

Temperature varied little from tank to tank and the difference between daily maximum and minimum temperature was rarely greater than 2°C. Weekly maximum and minimum temperatures were calculated and plotted in Figs. 10.3.-10.6. Temperature tended to rise during the first six weeks, but remained fairly constant during the second six weeks.

Oxygen concentration and pH were measured over one water changing cycle (Table 10.2.). There was a significant increase in oxygen concentration from 24 to 48 hours after the water change (t = 3.2614, p < .01) and a significant decrease from 48 to 72 hours (t = 2.2615, p < .05). There was no significant difference between oxygen concentration at 24 and 72 hours after the water change (t = .0481, p > .05). The very low oxygen concentration recorded in tank 2 at 72 hours was due to a faulty air line. Analysis of variance of oxygen concentration in the tanks at 72 hours showed that variance due to dietary protein and energy levels were non-significant (Table 10.3.).

The increase in pH from 24 to 48 hours was
highly significant (t = 8.1124, p < .001). On day 2 the mean pH of the water in tanks containing fish fed the 20% protein and 40% protein diets were 7.74 and 7.85 respectively. Variance due to dietary energy level was non-significant but variance due to dietary protein level was significant (p < .05) (Table 10.3).

10.2.2. Experimental diets

All diets were readily consumed. The diets varied in consistency, with the 20% protein diets containing the highest levels of lipid and carbohydrate failing to solidify completely on cooling.

After 24 hours in an experimental tank most of the diets remained in a discrete lump but the 20% protein diets mentioned above, and the 40% protein diet which contained the highest level of carbohydrate showed a tendency to spread out and were difficult to remove from the tank. The % loss of dry weight over 24 hours varied from 14.04 to 28.13%. Variance due to both dietary energy and protein level were non-significant (Table 10.4).

10.2.3. Growth

Brand marks and cut dorsal fin rays on live fish were clearly distinguishable at 6 weeks and at the end of the experiment. Occasionally identification of dead fish proved difficult as these tended to go very dark. In such cases
the mean initial weight for fish in that tank was subtracted from the total in order to recalculate the daily ration.

For each fish the following were calculated:

(i) Change in weight during periods 0-6 weeks and 6-12 weeks.
(ii) Total change in weight.
(iii) Change in log weight in periods 0-6 weeks and 6-12 weeks.

For each fish from each tank the following were calculated:

(i) Mean and standard deviation of length, weight and condition at the beginning of the experiment and after 6 and 12 weeks.
(ii) Mean and standard deviation of change in weight from 0-6 and 6-12 weeks and over the whole experiment.
(iii) The mean instantaneous growth coefficient of fish during 0-6 weeks and 6-12 weeks was calculated as the mean change in log weight divided by time in months.

All the above calculations and statistical analysis were limited to fish which survived for the whole experiment.

Mean length, weight and condition of fish from each tank are shown in Tables 10.5 - 10.7. Means of standard length and weight are plotted in Figs. 10.3 and 10.4 for fish fed diets increasing in lipid energy content, and in Figs. 10.5 and 10.6 for fish fed diets increasing in carbohydrate energy content.
The mean standard length and weight of fish receiving diets increasing in lipid energy increased fairly steadily throughout the experiment, with fish fed the higher energy diets tending to be longer and heavier after 12 weeks. Increase in dietary protein from 20% to 40% appeared to have relatively little effect.

The mean standard length and weight of fish fed diets increasing in carbohydrate energy increased only very slowly. Fish receiving the 40% protein diet at the lowest energy level lost weight in the period 0-6 weeks although in most tanks there was a slight increase in mean fish weight in both 0-6 and 6-12 weeks.

Instantaneous growth coefficients are shown in Table 10.8 and Figs. 10.7 and 10.8. In most tanks growth from 0-6 weeks was faster than growth from 6-12 weeks. The 40% protein diets resulted in a higher instantaneous growth coefficient only amongst fish fed diets containing energy, supplied as carbohydrate + lipid, at levels III and IV. Amongst fish fed the lowest energy diet the instantaneous growth coefficient of fish fed the lower protein diet was greater. Increase in dietary lipid energy resulted in an increase in instantaneous growth coefficient at both dietary protein levels in the period 6-12 weeks. In the period 0-6 weeks increase in dietary lipid energy from level II to III had little effect on the instantaneous growth coefficient of fish fed the 20% protein diet, but resulted in a marked increase among fish fed the 40% protein diet. At the highest lipid level there was a slight decline in instantaneous growth coefficient among fish fed both the 20 and 40% protein
diets. Increase in dietary carbohydrate energy from level I to II resulted in an increase in instantaneous growth coefficient among fish fed the 40% protein diet, and the 20% protein diet in the period 0-6 weeks. Further increase of carbohydrate energy resulted in either no further increase or, particularly amongst fish fed the 40% protein diet, a slight decline in instantaneous growth coefficient.

Growth was investigated further by the analysis of change in weight of individual fish. Some authors (e.g. Cowey et al, 1970a) have found that change of weight is proportional to initial weight and taken this into account. Regressions of total change of weight on initial weight of juvenile C. labrosus were calculated for each tank, and are summarised in Table 10.9. In 8 out of 19 cases variance due to regression was marginally significant ($p < .05$) and in two it was highly significant ($p < .001$). The regression coefficients varied from -.9385 to +.3586 although most fell in the range .22 to .32. Variance of initial weight was investigated (Table 10.10). Amongst fish fed diets increasing in lipid content variance due to protein level was non-significant, but variance due to energy level was highly significant. However, examination of initial weights showed that they did not vary in a pattern consistent with observed growth. Amongst fish fed diets increasing in carbohydrate energy, variance in initial weight due to energy level was highly significant. This appears to be due to the fact that fish fed the lowest energy diet were considerably larger at the beginning of the experiment. How-
ever, these fish tended to show least growth. Thus although there were significant correlations between initial weight and change of weight within some tanks, change in weight of fish fed on different diets appeared to be more strongly influenced by other factors.

Weight of fish in tank 20, in which fish were not branded, was compared with weight of fish in tank 18 which were fed the same diet at the same feeding level to assess the effect of branding. Fish in tank 18 were slightly heavier, but the difference was non-significant at 0, 6 and 12 weeks (t = .2959, .9404, .8393). There was, therefore, no evidence to suggest that marking the fish impaired their growth.

Change in weight over the course of the experiment is illustrated in Figs. 10.9 and 10.10. The mean and standard deviation of change of weight of fish in each tank are shown on Table 10.11 and plotted against dietary energy level in Figs. 10.11 to 10.13. Weight change tended to increase with dietary energy level and the variance of weight change due to dietary energy level was highly significant (p < .001) (Table 10.12).

In the period 6-12 weeks weight gain was similar, and increased with increase in dietary lipid level, at both protein levels. Amongst the same fish in the period 0-6 weeks and over the whole experiment, change in weight tended to be greater among fish fed the 40% protein diets, and although it increased with dietary energy level from 3.08/3.18 kcal g to 3.80/3.90 kcal g⁻¹, it declined slightly in
fish fed diets containing 4.70/4.80 kcal g\textsuperscript{-1}. Variance of weight change due to increase in dietary lipid energy was highly significant among fish fed at both protein levels ($p < .001$) (Table 10.13.).

Fish receiving diets containing 40% protein showed increased weight change with increase in dietary carbohydrate energy from 2.38 to 3.18 kcal g\textsuperscript{-1}, and a decline in weight change from 3.18 to 3.88 kcal g\textsuperscript{-1} in the periods 0-6 weeks, 6-12 weeks and over the whole experiment. Variance of weight change due to increase in dietary carbohydrate energy was highly significant among fish fed the 40% protein diet ($p < .001$). Amongst fish fed 20% protein diets increase in carbohydrate energy level appeared to have little effect on weight change. Although variance of weight change due to increase in dietary carbohydrate energy level was highly significant in the period 6-12 weeks ($p < .001$), it was only marginally significant in the period 0-6 weeks ($p < .05$) and non-significant over the whole experiment (Table 10.14.).

At the dietary energy level of 3.80 - 3.98 kcal g\textsuperscript{-1} fish were fed approximately isocaloric diets containing 20 and 40% protein in which energy was supplied by (i) lipid + carbohydrate and (ii) carbohydrate only. The mean change of weight of these fish is contrasted in Fig. 10.14. At both protein levels weight gains were superior when energy was supplied by lipid + carbohydrate rather than carbohydrate alone. Weight gains of fish were compared between replicate tanks and between tanks in which fish were fed diets of the same protein content but differing in energy source. The results are summarised on Table 10.15.
Differences between the weight change of fish in replicate tanks were non significant, whereas in all comparisons between groups of fish fed diets differing in energy source, weight gain was significantly greater when energy was supplied as lipid + carbohydrate (p < .01).

10.2.4. **Mortality**

Mortality is shown in Table 10.16 and Fig. 10.15. The high mortality in tanks 9 and 10 in the period 0-6 weeks was largely due to an abnormally high immediate post branding mortality. Excluding these, mortality tended to be slightly greater in the period 0-6 weeks than 6-12 weeks, but did not vary consistently with dietary protein or energy level. Variance of mortality due to energy and protein level was non significant amongst fish fed diets increasing in lipid energy and amongst fish fed diets increasing in carbohydrate energy (Table 10.16.).

In Fig. 10.16 mean total change of weight was plotted against mortality and replicate tanks connected by a straight line. Although there was no significant correlation between change of weight and mortality, there was a tendency among replicate tanks for weight change of fish to be greater in the tank in which mortality was greatest.

10.2.5. **Relative condition**

Relative condition was calculated for each fish individually at 0, 6, and 12 weeks using the length-weight relationship of captured fish of the same age group (Chapter 4). The mean and standard deviation of relative

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condition of fish in each tank are shown on Tables 10.5 to 10.7 and plotted against dietary energy level in Figs. 10.17 and 10.18. Analysis of variance of relative condition showed that the interaction between energy and protein level was significant (p < .01). The relative condition of fish fed the 20 and 40% protein diets were analysed separately and the results summarised in Tables 10.17 to 10.19.

At the beginning of the experiment mean relative condition was, as expected, similar in all tanks. Variance due to energy level was non significant except among fish fed the 40% protein diet (p < .05) and variance due to dietary lipid energy was non significant except among fish fed the 20% protein diet (p < .001). However, reference to Fig. 10.17 indicates that there was no consistent variation of relative condition with dietary energy level at the beginning of the experiment.

After 6 and 12 weeks the relative condition of fish fed all diets containing 3.08 to 4.80 kcal g⁻¹ varied little with dietary energy level or dietary protein level. At energy level III fish fed the 40% protein diet with energy supplied by carbohydrate + lipid had a slightly higher relative condition than fish fed an approximately isocaloric diet in which energy was supplied by carbohydrate. This was not observed amongst fish fed the 20% protein diet.

After 6 and 12 weeks the relative condition of fish receiving the 40% protein diet at the lowest energy level was considerably lower than that of other fish. This difference was slight or non existent amongst fish fed the 20%
protein diet at the lowest energy level.

Variance of condition due to energy level at 6 and 12 weeks was significant (p < .01) amongst fish fed diets increasing in carbohydrate level, and the 20% protein diets increasing in lipid level. Variance due to energy level was non significant for fish receiving the 40% protein diets increasing in lipid level.

Mean relative condition of fish in each tank over the course of the experiment is plotted in Fig. 10.19. Relative condition tended to increase during the experiment, particularly amongst fish fed higher energy diets. The relative condition of fish fed the lowest energy 40% protein diet declined markedly in the period 0-6 weeks, but increased slowly from 6-12 weeks.

10.2.6. **Apparent digestibility**

Apparent digestibility of dry matter and protein was estimated as described in section 8.2.7. Apparent digestibility of lipid was estimated in the same way, but this was incomplete due to an insufficient sample of faeces being collected from some tanks. Apparent digestibility of organic matter excluding cellulose was also estimated, assuming cellulose was not digested by *C. labrosus*.

The apparent digestibility of dry matter by the fish in different tanks during the first and second periods of faeces collection is shown in Table 10.20, and plotted against dietary energy level in Fig. 10.20. It showed little variation with dietary protein level, but tended to increase with dietary
energy content at least up to 3.80-3.98 kcal g\(^{-1}\). However, the proportion in the diet of cellulose, which was assumed to be non digestible, decreased with increasing energy level, which would account for at least part of the observed increase in apparent digestibility. Apparent digestibility of organic matter excluding cellulose was, therefore, calculated and is shown in Table 10.21, and plotted against dietary energy level in Fig. 10.21. The apparent digestibility of organic matter excluding cellulose increased from energy level 2.28/2.38 to 3.08/3.18 kcal g\(^{-1}\) but declined with further increase in dietary energy supplied either as lipid or carbohydrate. Analyses of variance are summarised on Table 10.22. Variance of apparent digestibility of organic matter excluding cellulose due to energy level, protein level, or faeces collection during periods 0-6 or 6-12 weeks, was non significant among fish fed diets increasing in lipid content and increasing in carbohydrate content.

The apparent digestibility of protein is shown in Table 10.23 and Fig. 10.22. Analyses of variance are summarised on Table 10.24. Among fish fed diets increasing in lipid content both energy level and protein level appeared to have little effect on apparent digestibility of protein, even though variance due to both was statistically significant (\(p < .05\)). Among fish fed diets increasing in carbohydrate content, variance due to period of faeces collection was significant (\(p < .05\)) and there was a slight tendency for apparent digestibility of protein to be lower in the 6-12 week period. Variance due to energy level was non significant for fish fed
40% protein diets increasing in carbohydrate level, but significant (\(P < .01\)) for fish fed 20% protein diets increasing in carbohydrate level. This was mainly due to a marked reduction in apparent digestibility of protein at the highest carbohydrate level, particularly in the 6-12 week period.

Incomplete data on the apparent digestibility of lipid is presented on Table 10.25. and Fig. 10.23. It did not appear to vary consistently with dietary energy or protein level. Variance due to dietary energy level was non significant.

The digestibility of dextrin was calculated by assuming that organic matter excluding cellulose consisted of protein, dextrin, and lipid only. Since the apparent digestibility of organic matter excluding cellulose, protein and lipid were known, the proportion of dextrin which was absorbed could be calculated. Estimated digestibilities of dextrin are shown on Table 10.26, and plotted against dietary energy and dietary dextrin content on Fig. 10.24. Digestibility of dextrin tended to increase with dietary energy level up to 3 kcal g\(^{-1}\), and to be greater amongst fish fed the 20% protein diet. It was highly correlated with dietary dextrin content (\(r = .8284, p < .001\)) irrespective of dietary protein level, increasing markedly with increase in the dextrin content of the diet up to 50%. Further increase in dextrin content caused no further increase in dextrin digestibility.
10.2.7. Growth efficiency and Protein Efficiency Ratio

Gross growth efficiency was calculated as described in section 8.2.4. Estimates apply only to fish which survived to the end of the experiment. It was assumed that fish which died did not consume food from the time of their last weighing. This was probably a fair assumption since some mortality occurred immediately after weighing, probably as a direct result of handling stress, and fish found dead in the tanks at other times were often thin and emaciated. Where fish survived from 0-6 weeks but died during the period 6-12 weeks, they were assumed to have eaten the mean food consumption for fish in that tank during 0-6 weeks, and this quantity was subtracted before calculation of gross growth efficiency. Similar adjustments were made in the calculation of protein efficiency ratio.

Gross growth efficiencies are shown in Table 10.27 and plotted against dietary energy content in Fig. 10.25. With a few exceptions gross growth efficiency of fish in replicate tanks was similar. Gross growth efficiency tended to increase with dietary energy content. This was as expected since, as energy content increased, the quantity of cellulose in the diet, assumed to contribute little or nothing to the nutrition of the fish, decreased. At dietary energy level III the gross growth efficiencies of fish fed diets containing carbohydrate as energy source were markedly lower than those of fish receiving approximately isocaloric diets in which energy was supplied as lipid + carbohydrate. This cannot be attributed to the effect of cellulose, since the lipid and carbohydrate

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diet contained more cellulose than the carbohydrate diet.

Gross growth efficiencies were recalculated excluding dietary cellulose i.e. making the assumption that cellulose made no contribution to the nutrition of the fish. Growth efficiencies (excluding cellulose) are shown in Table 10.28 and plotted against dietary energy level in Fig. 10.26. With increasing lipid in the diet growth efficiency (excluding cellulose) tended to increase with dietary energy level from 3.08/3.18 to 3.80/3.90 kcal g\(^{-1}\) and either remain constant (fish fed 40% protein diets) or decline slightly (fish fed 20% protein diets) at the highest energy level. Analyses of variance are summarised in Table 10.29. Variance of gross growth efficiency (excluding cellulose) due to dietary energy level was significant (p < .05) in the period 6-12 weeks and for the whole experiment. Variance due to protein level was non significant over all periods.

With increasing carbohydrate in the diet growth efficiency (excluding cellulose) tended to increase with dietary energy level from 2.28/2.38 to 3.08/3.18 kcal g\(^{-1}\), and decrease with further increase of energy level to 3.88/3.98 kcal g\(^{-1}\). Variance of growth efficiency (excluding cellulose) due to energy level was significant in the periods 0-6 and 6-12 weeks, and, for fish fed the 40% protein diets, over the whole experiment (p < .05) (Table 10.30). Dietary protein level appeared to have little effect on growth efficiency (excluding cellulose).
Net growth efficiency was calculated for fish in each tank as

\[
\text{Net growth efficiency} = \frac{\text{summed weight gain}}{\frac{\text{dry weight food consumed}}{\text{digestibility of consumed food}}} \times 100\% \\
\]

Net growth efficiencies are shown in Table 10.31 and plotted against dietary energy level on Fig. 10.27. Analyses of variance of net growth efficiency are summarised on Tables 10.32 and 10.33.

The pattern of variation of net growth efficiency with dietary energy level was similar to that of gross growth efficiency (excluding cellulose) suggesting that differences in digestibility of the diets were not a major factor affecting efficiency of utilisation. Among fish fed diets increasing in lipid energy, net efficiency tended to increase with energy level and protein level. Variance due to both were significant over the whole experiment. Among fish fed diets increasing in carbohydrate energy net growth efficiency did not appear to be consistently related to dietary energy or protein level. Variance due to energy level was nonsignificant except for fish fed the 40% protein diet, among which there was a loss of weight in fish fed the lowest energy diet in the period 0-6 weeks.

Protein efficiency ratios are shown in Table 10.34 and plotted against dietary energy level in Fig. 10.28. Analyses of variance are summarised in Tables 10.35 and 10.36.

Among fish fed diets increasing in lipid energy PER tended to be higher among fish fed the lower protein diet, and to increase with increase in dietary energy level, with the exception of a decline in PER of fish receiving the 20% protein
diet at the highest energy level in the period 0-6 weeks. Variance of PER of fish fed diets increasing in lipid energy due to energy level was significant in the period 6-12 weeks (p < .05). Over the whole experiment variance of PER due to dietary protein and energy level was highly significant (p < .01) (Table 10.35.).

Among fish fed 20% protein diets increasing in carbohydrate energy PER, remained fairly constant with increase in dietary energy in the period 6-12 weeks and over the whole experiment. The PER of fish fed the 20% protein diet from 0-6 weeks, and the 40% protein diet from 6-12 weeks and over the whole experiment increased as dietary energy, supplied as carbohydrate, increased from level I to II, but decreased with further increase of dietary energy to level III. PER of fish fed the 20% protein diet tended to be higher than that of fish fed the 40% protein diet. Variance of PER of fish fed diets increasing in carbohydrate energy due to dietary energy level was non significant except in the period 0-6 weeks, but variance due to protein level was significant (p < .05) in periods 0-6 and 6-12 weeks, and over the whole experiment.

PER was plotted against the protein:energy ratio in the diet (mg protein : kcal energy) in Fig. 10.29. PER was significantly negatively correlated with the protein:energy ratio in the diet, both in fish fed the 20% protein diet (r = .6372, p < .05) and in fish fed the 40% protein diet (r = .8543, p < .01). The regressions of PER on protein:energy ratio in the diet were compared by covariance analysis.
and there was found to be no significant difference in slope or elevation. One regression was, therefore, calculated to describe the relationship between PER and dietary protein: energy ratio.

\[ \text{PER} = -0.0027 \text{(protein:energy ratio)} + 0.4094 \quad (p < 0.001) \]

10.2.8. **Body composition**

The results of proximate analysis of fish are shown in Table 10.37. The protein content was estimated by multiplying the nitrogen content by 6.25, a factor which takes into account the mean nitrogen content of proteins. The composition of pooled dried fish from each tank is shown in Figs. 10.30 and 10.31. In most cases the composition of fish from replicate tanks was similar. Moisture, protein, lipid and ash content are plotted against dietary energy level in Figs. 10.32 and 10.33. Analyses of variance are summarised in Table 10.38.

The most marked effect of increasing dietary energy among fish fed diets increasing in lipid content at both protein levels was the increase in body lipid content and the decrease in moisture content. The percent ash and protein also decreased, probably largely due to the increase in lipid content. Variance of all 4 body constituents due to energy level among fish fed diets increasing in lipid content was significant \((p < 0.01)\). The body composition of fish fed the 20 and 40% protein diets was similar, and variance of ash, lipid, moisture and protein content due to dietary protein level were non significant.
In contrast, the body composition of fish fed diets increasing in carbohydrate energy content altered relatively little with increasing dietary energy content among fish fed at either protein level. Variance of protein, lipid and moisture content due to dietary energy level was non-significant. Variance of ash content due to energy level was non-significant among fish fed the 20% protein diets but significant among fish fed the 40% protein diets, apparently due to a decline in ash content with increase in dietary energy level from 2.38 to 3.98 kcal g\(^{-1}\). Moisture and ash content tended to be slightly greater amongst fish fed the 40% protein diet at energy levels I and III but dietary protein level appeared to have little effect on body composition.

There was a significant negative correlation between lipid and moisture content (p < .001) (Fig. 10.34.) The regression equation calculated by the method of least squares was

\[
\text{lipid content} = -2.15 \times \text{moisture content} + 174.22 \\
(\% \text{ dry weight}) \quad (\% \text{ wet weight})
\]

There was a significant positive correlation between lipid content and relative condition (p < .01) (Fig. 10.34). The best straight line was estimated as above, and the regression equation calculated as

\[
\text{Lipid content} = 64.63 \times \text{relative condition} - 45.63 \\
(\% \text{ dry weight})
\]

Fish lipid content was plotted against protein : energy ratio in the diet in Fig. 10.35. Lipid content was sig-
nificantly negatively correlated with dietary protein : energy ratio among fish fed both the 20% protein diet ($r = -0.7330$, $p < 0.05$) and the 40% protein diet ($r = -0.7409$, $p < 0.05$). The regressions of lipid content on protein : energy ratio were compared using covariance analysis and found to be not significantly different in slope, but significantly different in elevation ($p < 0.01$) i.e. one regression did not adequately describe the relationship for fish fed both experimental diets.

10.2.9. Variation of gutted weight, gut weight, and liver weight.

As described in Section 8.2.9, gutted weight, gut weight and liver weight were expressed as a percentage of total weight of each fish in the sample from each tank so as to enable comparisons among fish of different sizes.

Mean and standard deviations of gutted weight and gut weight are shown in Table 10.29 and plotted against dietary energy level on Figs. 10.36 and 10.37. Gutted fish weight tended to increase with dietary energy level, irrespective of dietary protein content or energy source, up to 4 kcal g$^{-1}$ but declined slightly in fish fed diets containing 4.7/4.8 kcal g$^{-1}$. Variation in the proportion of gut weight was approximately opposite to variation in the proportion of gutted fish weight. There was a highly significant negative correlation between them ($r = -0.9575$, $p = <0.001$). Analyses of variance of gut weight and gutted fish weight are summarised on Tables 10.40 and 10.41. Variance due to dietary energy level was highly significant.
(p < .001) except among fish receiving the 40% protein diets increasing in lipid content. Variance due to dietary protein level was significant (p < .05) in comparisons among fish fed diets containing 4.70/4.80 and 3.08/3.18 kcal g\textsuperscript{-1}. Protein level in the diet did not appear to have a consistent effect on gut or gutted fish weight.

Mean and standard deviation of liver weight are shown on Table 10.42 and plotted against dietary energy level on Fig. 10.38. Dietary protein level had little effect in liver weight, but increase in dietary energy level, particularly from 2.28/2.38 to 3.08/3.18 kcal g\textsuperscript{-1}, tended to cause an increase in the proportion of liver weight. There was no significant difference between the liver weights of fish fed diets containing 3.8 to 3.98 kcal g\textsuperscript{-1} in which energy was supplied as carbohydrate or lipid + carbohydrate. Analyses of variance of liver weight are summarised on Table 10.43. Variance due to dietary protein level was non-significant at all energy levels. Variance due to energy level was significant (p < .05) among fish fed diets containing 20% protein increasing in lipid content, and 40% protein increasing in carbohydrate content. There was a highly significant positive correlation between liver weight and fat content of the body (r = .6966, p < .001). The regression equation was calculated by the method of least squares

\[
\text{Lipid content} = \text{liver weight} \times 24.1704 - 25.0203 \\
(\% \text{dry body weight}) - (\% \text{wet body weight})
\]
Liver weight was significantly negatively correlated with dietary protein : energy ratio among fish fed both the 20% protein diet \((r = .8114, p \ll .01)\) and the 40% protein diet \((r = .7784, p \ll .01)\) (Fig. 10.35). When the regressions of liver weight on protein : energy ratios were compared using covariance analysis, they were found to be not significantly different in slope, but significantly different in elevation \((p \ll .01)\) i.e. one regression did not adequately describe the relationship for fish fed both experimental diets.

10.2.10. Histology

The gills, gut and liver of 3 fish from each tank were examined histologically.

(i) Gills: The gill sections of fish from Experiment IV generally showed considerably less damage than had been observed in fish from Experiments I and II. The exception was fish fed diets containing 2.28/2.38 kcal g\(^{-1}\) at both protein levels, in which gill sections showed thickening of epithelium, disintegration of lamellae, and plaque cells (Plate 10.1). Myxosporidian cysts were also relatively common on the gill lamellae of these fish. As energy in the diet increased, thickening of epithelium and distortion of lamellae became less common, but there was a marked contrast between the gills of fish fed diets containing 3.80–3.98 kcal g\(^{-1}\) which differed in energy source (Plates 10.2 and 10.3). Apart from slight lifting of epithelium, gill damage was not characteristic of fish fed the highest energy diets (Plate 10.4).
(ii) Guts: Fat around the pyloric caeca increased markedly with increase in dietary lipid content. Myxosporidia cysts were found on the gut and pyloric caeca of some fish but their frequency did not seem to relate to dietary protein or energy level. No particular abnormalities were noted.

(iii) Liver: Dietary energy level had relatively little effect on the appearance of the liver.

10.3. DISCUSSION

10.3.1. Environmental factors

Some of the information collected in Experiments I and II suggested that conditions in the tanks were not ideal, and that concentration of waste products, particularly ammonia, might be inhibiting growth. In the absence of a continuous record of temperature, oxygen concentration and pH, which would have been preferable, these variables were recorded daily over one water changing cycle. With the exception of one tank in which the air line was faulty, oxygen concentration was in excess of the 6.5 mg l⁻¹ at which growth and food consumption of juvenile coho salmon was impaired at 20°C (Herrman et al., 1962). Grey mullet are active fish with relatively high rates of standard metabolism (Belokopytin, 1968; Nordlie and Leffler, 1975), but they are also particularly well adapted to conditions of low oxygen concentration, and Rhinomugil corsula at least, is able to respire anaerobically without accumulating an oxygen debt. It seems unlikely that oxygen concentration was a limiting factor on growth in the experiment described in this chapter.

The significant increase in pH may have been associated
with the ionisation of ammonia, but ionic equilibria in seawater are very complex. Hampson (1976) reported a progressive increase in pH in a freshwater tank system, but a decline in a seawater system. The higher the pH the greater the tendency for ammonia to remain in the toxic form. The higher pH in tanks where fish were fed the more protein rich diet supported the suggestion that the increase of pH was associated with ammonia excretion. The effect of low levels of ammonia on growth is not clearly known, and different species differ markedly in sensitivity (Hampson 1976). The effect of the increased environmental pH may be ameliorated by lowering of pH caused by excretion of carbon dioxide in the thin layer of water immediately adjacent to the gill membrane.

10.3.2. Growth

The mean weight of all fish increased from 3.12 to 3.82 g over the course of the experiment, an increase of only 22.31%, although between tanks weight gain varied from -13.86% to +49.71%. In a 4 week period during the first 6 weeks of the experiment the mean weight of fish of the same species and age group in St. John's Lake increased by 69.38%. This is considerably in excess of the 31.98% maximum increase in mean weight of fish in the first 6 weeks of Experiment IV. Growth which is slow in comparison with wild fish is not uncommon in laboratory experiments, and some possible reasons for this have already been discussed (Section 8.3.1).

In most tanks growth proceeded at a relatively steady
rate and there was no evidence for alternate cycles in growth of length and weight as had been observed in previous experiments. Growth in weight was slightly greater during the first half of the experiment when temperatures were lower, which was as expected when fish were maintained on a fixed feeding level, but which contrasts with previous experiments in which fastest growth occurred in the period of highest temperature. The room could not be completely blacked out and so it is possible that the declining day length in the second half of the experiment affected the rate of weight increase.

In some of the tanks there was a significant correlation between the initial weight and change in weight of individual fish. In most cases the regression coefficient was positive, which may indicate a hierarchical effect in which larger fish had an advantage. Particularly in the case of lower energy diets, the ration was immediately surrounded by fish and consumed voraciously, but observation of feeding behaviour did not reveal any aggression between fish, or that larger fish had any advantage over smaller ones. The occurrence of a significant initial weight-change of weight regression did not appear to be related to dietary protein or energy level. Weight change-frequency histograms drawn up for fish in several of the tanks where the regression of weight change on initial weight was significant, did not indicate that the distribution of weight change differed from normality.

Any hierarchical effects, if they existed, were weak in comparison with those observed in other species e.g. trout.
(Brown, 1946a) and were not taken into account in the analysis.

The lack of influence of dietary energy level on mortality was consistent with observations on rainbow trout (Edwards et al, 1977; Higashi et al, 1964), brook trout (Ringrose, 1971), channel catfish (Tiemeier et al, 1965) and plaice (Cowey et al, 1975), although Austrøng (1979) reported lower mortality in salmonids when dietary lipid level was raised from 8 to 16%. Creach and Murat (1974) and McLaren et al (1946) reported that increase in carbohydrate content of the diet resulted in increased mortality in carp and rainbow trout respectively. However, in both these cases increase in dietary carbohydrate was accompanied by a decline in dietary protein. Comparing replicate tanks in Experiment IV, the tendency for growth of surviving C. labrosus to be greater where mortality was higher was not due to an increase in food availability since the ration was reduced proportionately as fish died. It may have been due to an effect of space per se, or may have been mediated via factors such as concentration of waste products (Section 8.3.1.).

There is some controversy regarding the energy equivalents most suitable for estimating the metabolisable energy content (gross energy-energy of faeces-energy excreted) of fish diets. This has been discussed previously (Section 8.3.1.). The energy equivalents used in this work are compared with those used by other authors, whose work on dietary energy level and fish growth is cited, in the Table below.
### Metabolisable energy (kcal g⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>This work</td>
<td>4.5</td>
<td>4.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Edwards et al (1977)</td>
<td>3.9</td>
<td>1.6-2.9</td>
<td>8.0</td>
</tr>
<tr>
<td>Lee and Putnam (1973)</td>
<td>4.0</td>
<td>4.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Phillips &amp; Brockway (1959)</td>
<td>3.9</td>
<td>1.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Dupree and Sneed (1966)</td>
<td>4.0</td>
<td>4.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Ogino et al (1976)</td>
<td>4.0</td>
<td>3.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Adron et al (1976)</td>
<td>4.5</td>
<td>4.0</td>
<td>9.5</td>
</tr>
</tbody>
</table>

The use of 4 kcal g⁻¹ for carbohydrate in this thesis is probably justified because the lower valued generally apply to more complex carbohydrates such as starch. The value of 4.5 kcal g⁻¹ for protein was determined by Smith (1971) taking into account the proportions of ammonia and urea excreted by fish. However, in more recent work (Smith et al, 1978) the net energy available from 1g protein was calculated as 4.24 and 3.27 kcal for protein metabolism resulting in the excretion of ammonia and urea respectively. Allowing for the heat increment, this does suggest that 4.5 kcal g⁻¹ is a maximum estimate of the metabolisable energy available from protein.

Other aspects of experimental procedure (Section 8.3.1.) may have affected growth, but ration size was of particular importance because there is some evidence to suggest that fish fed to satisfy an energy requirement (Rozin and Mayer, 1961). Some experimentors have fed diets ad libitum (Ogino et al, 1976;
Austreng et al., 1977; Atherton, 1975; Higashi et al., 1964) in which case there was a tendency for caloric intake to regulate food consumption and depression of appetite to occur among fish fed high lipid diets (Atherton, 1975). Thus, when an ad libitum feeding regime was used, ration size was introduced as an additional experimental factor. Other authors have utilised a fixed restricted ration, as in Experiment IV (Dupree and Sneed, 1966; Tiemeier et al., 1965; Adron et al., 1976). This has some advantages with regard to interpretation of results, although it must be recognised that a fixed % body weight ration will vary from perhaps 100% of ad libitum intake for high energy diets to a considerably lower percentage of the ad lib. intake for low energy diets. Also, in the context of fish culture, the combined, integrated effects of dietary energy level and ration size on protein conversion and growth are highly relevant.

The increase in weight gain with increase in dietary energy level suggested that carbohydrate (at least at lower energy levels) and lipid 'spare' protein in the diet of juvenile grey mullet. This protein sparing effect of dietary calories is well documented among higher animals (Munro, 1951) although the mechanism is not clearly understood. The additional calories are used to synthesise ATP which 'spares' amino acids, which would otherwise be metabolised for energy, for protein synthesis. However, it seems likely that in addition some metabolites common to carbohydrate and fat have a direct effect on protein metabolism, perhaps by participating in amino acid

Although increased growth or increased PER is considered indicative of a protein sparing action an increase in dietary energy generally results in an increase in deposition of body fat (Section 10.3.5.). However, experiments such as those of Smith (1971) in which increase in dietary energy resulted in an increase in nitrogen retention, have established the existence of a true protein sparing effect in fish.

Bearing in mind the difficulties of drawing comparisons among experiments differing in design e.g. feeding regime, the protein (mg) : energy (kcal) ratios which supported maximum growth of juvenile C. labrosus were 42.5-52.5% for fish fed 20% protein diets and 83.5-102.5 for fish fed 40% protein diets, which tend to be lower than figures reported for channel catfish (104-120; Page & Andrews, 1973), bass (133 - Alliot et al, 1979) and salmonids (105-135, Garling & Wilson, 1976). This suggests that there may be more scope for using supplementary dietary energy to spare protein for growth in juvenile C. labrosus than in some other fish species.

In addition to dietary energy level, energy source is an important factor affecting the protein sparing action of additional dietary calories. Comparison of growth on diets of energy level 3.80-3.98 kcal g⁻¹ indicated very clearly that for 324
juveniles of *C. labrosus* fed diets containing a minimum of 2% lipid and 30% carbohydrate, additional energy supplied as lipid resulted in greater protein sparing for growth than energy supplied as carbohydrate. Similarly Edwards et al (1977) reported that among rainbow trout fed isocaloric diets growth was depressed when carbohydrate was increased at the expense of fat. Experiments with turbot (Adron et al, 1976) also suggested that lipid had a greater protein sparing action than carbohydrate. However, it does appear that carbohydrate has an essential role in fish growth, since growth of chinook salmon (Buhler and Halver, 1961) and plaice (Cowey et al, 1975) were reduced when all dietary carbohydrate was replaced isocalorically by lipid.

The increase in growth with increase in lipid calories indicated that juvenile grey mullet were able to utilise lipid in the form of a mixture of corn oil and cod liver oil to spare protein for growth. Amongst other species of fish, lipids have usually been found to have some protein sparing action, but their degree of effectiveness varied markedly. Dupree (1969) found that, in channel catfish, solid corn oil had a greater protein sparing effect than either liquid corn oil or beef tallow. However, in other experiments, addition of 9% animal tallow or 9% menhaden oil to the diet were found to have similar effects on growth of channel catfish (Murray et al, 1977). In rainbow trout protein was spared by addition to the diet of cod liver oil (Atherton, 1975), fin whale oil and pollack oil (Higashi et al, 1964), corn oil (Phillips and Brockway, 1959), soyabean oil and cod liver oil (Ogino et al. 1976; Austreng, 1976).
olive oil (Higuera, et al. 1977) and herring oil (Lee & Wales, 1973), but lard was reported to have no protein sparing action (Atherton, 1975). An increase in the melting point of the capelin oil included in diets for rainbow trout and salmon parr resulted in reduced growth (Austreng, 1979). This may be due to poor absorption of high melting point fats (Tunison and McCay, 1935) but in experiments performed by Yu et al (1977) up to 50% of the herring oil in diets of rainbow trout could be replaced by lard without affecting growth. In experiments involving chinook salmon fingerlings, peanut oil was reported to have a protein sparing effect (Fowler et al, 1966), whereas corn oil was not (Buhler and Halver, 1961). Different protein sparing effects of different lipids may be partly due to differences in digestibility, but may also depend on specific characteristics of their fatty acids such as degree of saturation.

Growth (0-6 weeks) of juvenile C. labrosus fed 40% protein diets increasing in lipid content was greater than that of fish fed corresponding 20% protein diets, when dietary energy was $\geq 3.80/3.90 \text{ kcal g}^{-1}$. When the diet contained $3.08/3.18 \text{ kcal g}^{-1}$ the growth of fish fed the 20 and 40% protein diets were similar. The ration level was chosen on the basis of results from Experiment II but the environmental temperature was considerably higher during Experiment IV, and this would be expected to result in an increase in energy requirement for maintenance. The lack of effect of dietary protein level on growth among fish fed diets containing 3.08/3.18 kcal g$^{-1}$ would occur if the metabolic energy requirement was such that at least half the protein in the 40% protein diet
was metabolised for energy, so that a similar amount of protein was available for growth from both diets. Increase in dietary energy resulted in a slight increase in growth of fish fed the 20% protein diet, but among fish fed the 40% protein diet there was more protein in the diet to be 'spared', and therefore a greater potential for increased growth with increase in dietary energy level. In the second half of the experiment (6-12 weeks) the temperature was higher, which would be expected to result in an increase in maintenance energy requirement. Dietary protein level had no effect on growth, which was as expected if the maintenance energy requirement had been increased to the level at which more than half the protein in the 40% protein diets, even those containing most lipid, was required to be metabolised for energy. Under these circumstances, as dietary energy level increased protein would be spared for growth, but since protein available for growth never exceeded 20% dry diet the fish receiving the 40% protein diet would not be at any advantage.

The protein sparing effect of additional calories must have a limit at which growth is limited by the efficiency of conversion of food protein to fish protein rather than the availability of energy to spare protein for growth. This may explain why increase in dietary energy, by addition of lipid, from 3.08/3.18 to 3.80/3.90 kcal g⁻¹ resulted in a greater increase of growth than increase in dietary energy from 3.80/3.90 to 4.70/4.80 kcal g⁻¹.
Several authors report reduced weight gain of fish fed high energy diets, as was also observed in juvenile
_C. labrosus_ fed diets containing 4.70/4.80 kcal g<sup>-1</sup> (10% lipid in the wet diet) in the first 6 weeks of Experiment IV. In experiments such as those of Page & Andrews (1973) and Garling & Wilson (1976) in which fish were fed ad libitum, this may have been due to the high dietary energy suppressing feed intake to the extent that growth was limited by the amount of protein consumed. However, the reduction in growth at high levels of dietary lipid energy was also observed when ration was restricted in juvenile grey mullet, (this thesis) turbot (Bromley, 1974), brook trout (Ringrose, 1971) and channel catfish (Dupree and Sneed, 1966). Atherton (1975) suggested that lipid in excess of 15% of the wet weight diet had a specific toxic effect on rainbow trout, but Higashi et al (1964) found that if dietary fat was protected from oxidation, levels up to 25% could be tolerated with no ill effects. Both experiments utilised fish oils as the energy source. In considerably smaller channel catfish (c. 1g) corn oil in excess of 5% of the wet diet resulted in reduced weight gain (Dupree and Sneed, 1966). Similarly, in small turbot (≤ 2.5g), dietary lipid (1:1 mixture of corn oil and cod liver oil) in excess of 4% resulted in depression of growth (Bromley, 1974). From the data available it is impossible to determine whether these differences reflect differences in species, lipid source, size and age of the fish, or experimental conditions such as temperature. The decline in growth observed at high lipid levels may be due to reduced dig-
estive efficiency although there is evidence to suggest that high levels of at least some dietary fats are well digested (Section 10.3.3.). Solomon and Brafield (1972), in an energetics study on perch (Perca fluviatilis), found that both growth and respiration increased with increase in energy intake. It is possible that, in fish fed the highest energy diets, metabolic rate was increased to such an extent that energy was diverted from growth.

In juvenile grey mullet the reduction in weight gain in the period 6-12 weeks compared with 0-6 weeks can be attributed to the effect of increase in temperature on fish fed a fixed ration. When fish are fed ad libitum there is evidence to suggest that temperature and dietary lipid level interact in their effect on growth. When channel catfish were fed 35% protein diets an increase in dietary lipid from 5 to 12% resulted in increased gain at 28°C, but had no effect at 23°C. Similarly, in experiments with rainbow trout, Higashi et al (1964) attributed an increase in the optimum fat content of the diet from 15 to 25% in two trials to an increase in environmental temperature. Ikehara and Nagahara (1978) reported an interaction between the protein sparing action of different lipids and temperature in young rockfish Sebastes thompsoni. Pollack oil was most effective at 15-21°C but corn oil was most effective at 23-24°C.

Very little information is available on the effect of age or body size on the protein sparing effect, but results reported by Page and Andrews (1973) for channel catfish suggest
that it may be of importance. On a 25% protein diet increase in lipid from 6 to 12% resulted in reduced weight gain for fish of 14g, but increased gain in fish of 114g. Body size has not been found to exert a large influence on absorption and so it seems likely that this is a reflection of differences in growth efficiency and protein metabolism, which vary markedly with age.

Thus protein sparing effect of lipid observed under one set of experimental conditions may alter with variations in fish size, temperature, dietary protein level and lipid source.

Growth of young grey mullet on the 20% protein diet in which energy had been increased, by addition of lipid, to 3.80 kcal g\(^{-1}\) was greater than the growth of fish fed the 40% protein diet containing 3.18 kcal g\(^{-1}\). However in Experiment IV, the difference in growth due to dietary protein level was small, even at similar energy levels, suggesting that it might have been better to investigate the protein sparing effect with lower protein or higher energy diets. Nevertheless it appears that in juvenile grey mullet there is considerable scope for reducing dietary protein intake and supplementing the diet with additional energy in the form of lipid without any reduction of growth.

Since carbohydrate is often the cheapest component of fish diets, its protein sparing effect is of particular interest. In juvenile *C. labrosus*, increasing dietary energy from 2.28/2.38 kcal g\(^{-1}\) by increasing dietary carbohydrate at constant protein level, resulted in increased gain, but further increase of
carbohydrate energy resulted in reduced gain. Phillips et al (1948) reported that in trout, carbohydrate in excess of 12% in the diet was toxic. McLaren et al (1946) fed more nutritionally balanced diets and reported that 45% carbohydrate could be tolerated without deleterious effects. Similarly chinook salmon fingerlings could tolerate carbohydrate levels as high as 48%, although carbohydrate level had no appreciable effect on growth and no protein sparing effect was demonstrated (Buhler & Halver, 1961). However in experiments with rainbow trout, there was no effect on growth when protein level in the diet was reduced from 65 to 55% and sucrose increased from 0 to 15%, and on a 35% protein diet growth was improved when sucrose content was raised from 0 to 41% (Luquet et al, 1975). Low levels of carbohydrate had a protein sparing effect in bass, but high levels of carbohydrate resulted in poor growth (Alliot et al, 1979). In channel catfish a slight protein sparing effect was demonstrated with increase in dietary dextrin (Dupree and Sneed, 1966; Nail, 1962). Tiemeier et al, (1965) also working with channel catfish, reported that the protein sparing effect of carbohydrate was evident at a dietary protein level of 25%, but not 35%. Although Koops et al (1974) found that the addition of maize starch to the diet of rainbow trout resulted in improved growth, Buhler and Halver (1961) reported that growth of chinook salmon fingerlings increased as the molecular weight of dietary carbohydrate decreased. Nitrogen retention in trout was greater when lower molecular weight
carbohydrates were used as the energy source, which indicates that carbohydrate complexity may be an important factor in determining their protein sparing effectiveness (Smith 1971).

The depression of growth at high dietary carbohydrate levels observed in juvenile $C. \text{labrosus}$ was also reported in channel catfish fed dextrin in excess of 10% wet diet (Dupree and Sneed, 1966), although in another experiment increased growth was reported when carbohydrates were increased from 9.3 to 18.6% (Nail, 1962). In rainbow trout growth was depressed when more than 25% metabolisable energy was supplied as carbohydrate (Edwards et al, 1977). The reasons for this growth depression are unknown. In Experiment IV lack of essential fatty acids was an unlikely cause, since the high carbohydrate diet contained the same quantity of lipid as the 3.08/3.18 kcal g$^{-1}$ diet on which growth was superior. Poor digestibility of high levels of carbohydrate may be of importance, although this does not appear to be the cause in juvenile $C. \text{labrosus}$. There was some evidence to suggest depression of protein digestibility at high levels of dietary carbohydrate. This is discussed more fully in Section 10.3.3.

The low growth of $C. \text{labrosus}$ on the lowest energy diet may have been the result of the low energy intake, or it may have been partially due to the $\alpha$-cellulose content of the diet, which increased as energy content decreased. In many experiments involving varying dietary energy levels, $\alpha$-cellulose has been used to make up dietary bulk (Ringrose, 1971; Lee and Wales, 1973; Page and Andrews, 1973; Ogino et al, 1976; Nail,
1972; Adron et al, 1976; Cowey et al, 1975). The $\alpha$-cellulose was assumed to be neither digested nor absorbed, nor have any effect on digestion of other nutrients. However, Vallet et al (1970) reported decreased growth of $L$. aurata with increase in dietary $\alpha$-cellulose from 0 to 60%, and addition of high levels of $\alpha$-cellulose to the diets of chinook salmon decreased growth, because the increased dietary bulk prevented adequate protein or calorie consumption (Buhler and Halver, 1961). However, in studies on channel catfish Dupree & Sneed (1966) concluded that reduced weight gain when dietary cellulose was increased from 21 to 51% was not due to reduced intake, but to a depression of digestibility of non cellulose organic matter. When cellulose was increased from 0 to 21% growth improved, which was consistent with the observations of Buhler and Halver (1961) that small amounts of cellulose had a growth stimulating effect, possibly as a result of slowing the passage of food through the gut. Cellulase activity, probably associated with gut microflora, has been identified in $C$. labrosus, but the low growth of fish fed the higher cellulose diets indicated that the contribution of cellulose to metabolisable energy was probably non significant. In Experiment IV dietary cellulose was limited to 23%, at the expense of a reduction of ration size of lowest energy diets, in order to keep the cellulose level below that likely to cause significant growth depression (Buhler and Halver, 1961; Dupree and Sneed, 1966).

Among fish fed diets increasing in carbohydrate con-
tent the effect of dietary protein level on growth was negligible at energy levels 3.08/3.18 and 3.88/3.98 kcals g\(^{-1}\), but at the lowest energy level weight change was markedly lower amongst fish fed the 40% protein diets. The ration for fish fed the lowest energy diets were at or below maintenance i.e. almost all the diet would be metabolised for energy. Under these circumstances the fish receiving the 40% protein diet would be metabolising more protein and, therefore, excreting more ammonia than those receiving the 20% protein diet. The effect of ammonia on growth was discussed in Section 8.3.1. The significantly greater pH in tanks of fish fed the higher protein diet suggested that they may have been at a slight disadvantage, although pH did not decrease significantly with increase in dietary energy level. Histological studies on gills of fish fed the lowest energy diets indicated damage which may have been caused by ammonia, but ideally ammonia concentration in the experimental tanks should have been measured at regular intervals.

10.3.3. Apparent digestibility

The apparent digestibility of the experimental diets was of particular interest since in estimating metabolisable energy, digestibility of dietary components was assumed to be the same in all diets. Some of the variation in the digestibility of dry matter with dietary energy content must be attributable to
the variation in α-cellulose content. Comparison of dietary cellulose content with dry matter digestibility did not suggest that dietary cellulose was absorbed.

The apparent digestibility of organic matter (excluding cellulose) did not vary significantly with energy level. Solomon and Brafield (1972) in energetic studies of perch, reported that, as energy content of the diet increased, the proportion digested remained approximately constant. Since the 2.28/2.38 and 3.08/3.18 kcal g⁻¹ diets in Experiment IV had the same cellulose content, and the lowest and highest digestibilities respectively, there was no evidence to suggest that cellulose had a depressive effect on the digestibility of non cellulose organic matter as was reported by Dupree & Sneed (1966) in channel catfish and Smith (1971) in trout.

The lack of variation of digestibility of protein with increase in dietary lipid energy was consistent with the observations of Higuera et al (1977) on rainbow trout fed diets increasing in olive oil content. However in juvenile C. labrosus there was evidence for a reduction in digestibility of protein from the diet containing the highest level of dextrin. The digestibility of protein by rainbow trout was also depressed by an increase in dietary carbohydrate level (Kitamikado et al, 1964; Austreng et al, 1977). The interference of carbohydrate with protein absorption may account, at least partly, for depression of growth of fish fed high carbohydrate diets (this work, Dupree and Sneed, 1966; Edwards et al, 1977).

Data on lipid absorption was incomplete, but there
was no evidence to suggest that it was affected by dietary lipid level. Similar conclusions were drawn from studies of rainbow trout fed diets increasing in olive oil content (Higuera et al, 1977). The high digestibilities reported here for juvenile grey mullet (82.6 to 94.2%) are typical of low melting point fats. Digestibility seems to increase with temperature (Atherton and Aitken, 1970) and decrease with increase in melting point of the fat (Takeuchi et al, 1978).

Digestibility of dextrin was not measured directly but estimated from the digestibilities of organic matter, protein and lipid. Inaba et al (1963) and Singh and Nose (1967) measured the digestibility of carbohydrates by rainbow trout, and reported that digestibility decreased from 77 to 45% as dietary level increased from 20 to 60%. However in juvenile grey mullet the reverse occurred, with digestibility of dextrin increasing markedly with dietary dextrin content up to 50% of the dry diet, and remaining constant at higher dietary levels.

The importance of possible solution of dextrin from the faeces was difficult to assess. The percentage loss of dry weight from all diets over 24 hours was found to be somewhat similar despite widely differing contents. If approximately the same amount of dextrin was lost by solution from all faeces samples, then apparent digestibility would tend to rise with increase in dietary dextrin content as was observed. However, Moitra and Bhattacharya (1975) found that amylase activity in all parts of the alimentary tract of *Channa punctatus* increased on a high carbohydrate diet. A real variation in the digestibility of
dextrin may partly account for the small effect of dietary protein level on growth of juvenile grey mullet in Experiment IV. In diets designed to be of the same energy density, the 20% protein diet contained more dextrin, a higher proportion of which would, therefore, be digested, thus giving fish fed the 20% protein diet an advantage in terms of metabolisable energy over those receiving the 40% protein diet. This effect would be particularly great at the lowest energy level, because dextrin digestibility declined most steeply when dietary dextrin level declined from 30 to 10%.

10.3.4. Growth efficiency and Protein Efficiency Ratio

The observed increase of gross growth efficiency with increase in dietary energy level was due in part to the decrease in proportion of the diet as non nutritive bulk (β-cellulose), and does not necessarily imply an increase in the efficiency of conversion of the nutritive part of the diet into growth. However, the gross growth efficiency of fish fed diets with a lipid + carbohydrate energy source was markedly greater than that of fish fed approximately isocaloric diets containing carbohydrate only as an energy source, and lower levels of cellulose, which suggested that genuine differences in efficiency of conversion existed.

These were further investigated by calculating gross growth efficiency (excluding cellulose) and net efficiency in which dry weight digestibilities of the diets were taken into
account. Net growth efficiency was slightly higher than gross growth efficiency (excluding cellulose) and varied similarly with dietary energy level and energy source, which suggested that cellulose did not contribute significantly to the nutrition of juvenile C. labrosus, and also, that differences in growth efficiency could not be attributed to differences in digestibility of the experimental diets.

The increase in growth efficiency with increase in lipid energy in the diet was also observed in rainbow trout (Lee and Putnam, 1973; Lee and Wales, 1973; Koops et al 1974), channel catfish (Page and Andrews, 1973) and turbot (Adron et al, 1976). Particularly amongst fish fed the 20% protein diet, growth efficiency declined when fish were fed the highest energy diet. This was also observed in turbot fed diets exceeding 4% lipid (Bromley, 1974) and in brook trout where growth efficiency was low in fish fed the lowest and highest energy diets (1.6 and 2.9 kcal g⁻¹) (Ringrose, 1971). It may be that, at the highest energy level, the conversion of food protein to fish protein was already proceeding at a maximum rate, resulting in 'wastage' of excess calories, or even a reduction of growth efficiency due to the stimulating effect of increased energy intake on metabolic rate and maintenance energy requirement. In brook trout (Ringrose, 1971) and channel catfish (Page and Andrews, 1973), although body lipid increased with dietary energy to a certain level, on higher energy diets supplementary energy was not accumulated in the body as fat but dissipated in some way. The fact that the depression of
growth efficiency at the highest energy level was more pronounced among *C. labrosus* fed the 20% protein diet which contained the highest proportion of non protein calories was consistent with these observations.

Any sparing effect of supplementary dietary energy on dietary protein would be expected to be reflected in the PER. The negative correlation between PER and dietary protein:energy ratio suggested that dietary energy 'spared' protein in juvenile *C. labrosus*. A similar relationship was observed in rainbow trout (Reinitz et al., 1978; Lee & Putnam, 1973). Since there was no significant difference between the regressions calculated for *C. labrosus* fed the 20 and 40% protein diets, the improvement of PER in *C. labrosus* appeared to depend more on the protein:energy ratio than the actual dietary protein or energy level, as was also observed for rainbow trout (Lee & Putnam, 1973). However, at the same protein:energy ratio, the PER of *C. labrosus* fed diets containing energy as lipid + carbohydrate was higher than that of those fed diets where energy was supplied mainly by carbohydrate. Juvenile *C. labrosus* appeared to be able to assimilate high levels of dietary carbohydrate, but the observed PER suggested that the energy available to the fish from the carbohydrate may have been less than calculated i.e. the protein:energy ratios of high carbohydrate diets were underestimated.

An increase in PER with increase in dietary lipid energy has been recorded in several species of fish including channel catfish (Murray et al., 1977), rainbow trout (Ogino et al., 1976; Atherton and Aitken, 1970), yellow tail (Takeda et
al, 1975), turbot (Adron et al., 1976) and plaice (Cowey et al., 1975.). The PER of C. labrosus fed the 40% protein diet increased with increase in lipid energy, suggesting that the maximum protein sparing effect had not been reached. The PER of fish fed the 20% protein diet was a maximum at a dietary energy level of 3.80 kcal g⁻¹ in the first half of the experiment and over the whole experiment. In the period 6-12 weeks, the fact that PER was constant or even rose slightly at the highest energy level, whereas net efficiency declined, suggested that dietary constituents other than protein were being less well utilised in the high energy diet. The decline in PER at high dietary energy level was also recorded by Fowler et al. (1966) in chinook salmon fingerlings fed diets increasing in peanut oil content, where maximum PER occurred at 2.35 kcal g⁻¹ with lower values at 1.63 and 3.05 kcal g⁻¹. Similarly, the PER of turbot was greater when the diet contained 4% lipid rather than 1 or 7% lipid (Bromley, 1974). However, Lee and Putnam (1973) in experiments with rainbow trout fed diets containing similar levels of protein and 2.96 to 4.40 kcal g⁻¹, found that PER increased with decrease in the protein:calorie ratio even at the highest energy level. Factors limiting the sparing action of lipids clearly require further investigation.

The growth efficiency of juvenile C. labrosus fed diets increasing in carbohydrate energy also declined at the highest energy level, but comparison with fish fed isocaloric diets in which energy was supplied as lipid + carbohydrate indicated that this was not due to the maximum rate of conver-
sion of food protein to fish protein having been reached. In experiments on rainbow trout in which the carbohydrate con-
tribution to a fixed total metabolisable energy was increased from 25 to 38%, the observed decline in growth efficiency was attributed to reduction in digestibility of carbohydrates at high dietary levels (Edwards et al., 1977), which was not shown to be true in juvenile *C. labrosus*.

Over the whole experiment variance of PER due to increasing carbohydrate in the diet was non significant, which indicated that dextrin in the diet of juvenile *C. labrosus* did not exert a significant protein sparing action. Work on rainbow trout (Lee and Putnam, 1973), chinook salmon (Buhler and Halver, 1961) and channel catfish (Tiemeier et al., 1965) suggested that PER improved when corn starch, dextrin and wheat corn respectively were added to the diet. However Buhler and Halver (1961) reported maximum PER at 20% dextrin in the diet, and for turbot, Adron et al. (1976) found that the PER was greatest amongst fish fed a diet containing 9% carbohydrate than 18% carbohydrate. When plaice were fed approximately isocaloric diets Cowey et al. (1975) reported that the PER of fish fed lipid only as an energy source was lower than that of fish fed lipid + carbohydrate. However, where some carbohydrate is included in the diet it appears that increase in lipid energy has a greater effect on PER than increase in carbohydrate energy (this work, Adron et al., 1976).

10.3.5. **Body composition and relative condition**

Body composition is of importance in the study of
the effect of dietary energy level on fish growth since increased weight gain achieved at the expense of an increasingly fatty carcass, as was observed when plaice were fed diets increasing in energy content (Cowey et al, 1975) is of dubious practical advantage. Experiments in which nitrogen balance was measured indicated that increase in dietary energy does result in increased nitrogen retention i.e. a true protein sparing effect (Atherton and Aitken, 1970). However, body lipid content has been found to be positively correlated with dietary lipid in chinook salmon (Fowler et al, 1966; Buhler and Halver, 1961), rainbow trout (Ogino et al. 1976; Austreng, 1976; Lee and Putnam, 1973; Phillips and Brockway, 1959), channel catfish (Garling and Wilson, 1976), turbot (Adron et al, 1976) and bass (Alliot et al, 1979), and in this study on juvenile grey mullet. This suggests that some of the increased growth of fish fed higher energy diets, and of fish fed isocaloric diets in which energy was supplied as lipid + carbohydrate rather than carbohydrate, was due to increased fat decomposition. There was no significant increase in lipid content of C. labrosus when dietary carbohydrate energy was increased. This was consistent with the observations of Buhler and Halver (1961) in which increase in dietary dextrin had no effect on body lipid or protein of chinook salmon, and of Austreng et al (1977) in which rainbow trout fed diets high in carbohydrate contained less fat than fish fed isocaloric diets containing a higher proportion of lipid energy. However, the lipid content of carp was found to be positively correlated with dietary carbohydrate (Ogino and Saito, 1976).
The decline in body lipid of juvenile \textit{C. labrosus} with increase of protein:energy ratio, and the difference in the relationship for fish fed diets differing in protein level, contrasted with the results of Lee & Putnam (1973) who reported that in rainbow trout protein:calorie ratios were positively correlated with percentage body fat regardless of the dietary levels of protein and lipid. It also contrasts with Experiment I in which body lipid rose with increase in protein:energy ratios in the diet. However, the lipid content of channel catfish was negatively correlated with protein:energy ratios, as was observed in juvenile mullet (Page and Andrews, 1973). Both the dietary energy source and protein level, as well as the protein:energy ratio, have an effect on the percentage body fat of juvenile grey mullet. As was noted in Experiments I and II there was a high negative correlation between lipid and moisture content in juvenile \textit{C. labrosus}.

Most authors have not found a strong relationship between ash and protein content of fish and dietary energy level (Adron et al, 1976; Phillips and Brockway, 1959; Ringrose, 1971; Buhler and Halver, 1961), although the ash and protein content of rainbow trout increased with increase in the proportion of dietary energy as carbohydrate (Austreng et al, 1977), and the ash content of channel catfish declined when they were fed low energy diets (Page and Andrews, 1973). In juvenile \textit{C. labrosus}, by far the greatest variation with dietary energy level was in lipid content, and variations in the proportion of protein and ash seemed to be largely a reflection of changes in the proportion of body lipid.
Relative condition was positively correlated with body lipid content although they were not as highly correlated as in previous experiments. The main effect of dietary energy level on condition was a reduction of mean relative condition of fish fed the 40% protein diet at the lowest energy level. Apart from this, although mean relative condition of fish in most tanks tended to increase, the influence of dietary energy level on condition at the conclusion of the experiment was negligible. This contrasts with results of experiments with rainbow trout in which condition was found to increase linearly with increase in dietary lipid content (Lee & Putnam, 1973). Amongst C. labrosus fed diets containing 3.80 to 9.98 kcal g⁻¹ relative condition of fish fed diets containing carbohydrate and lipid + carbohydrate as energy source were similar. This also contrasts with the results of experiments on rainbow trout in which condition of fish increased when some carbohydrate in the diet was isocalorically replaced by lipid (Edwards et al, 1977). Both studies on rainbow trout were of longer duration than Experiment IV - 18 and 24 weeks respectively, which may be a relevant factor.

10.3.6. Gut, liver and gutted fish weight and histology

Lee and Putnam (1973) reported that the relative weight of the gastro-intestinal tract increased with dietary fat level due to an increase in size of pyloric caeca and intestines. In this study on C. labrosus there was a slight tendency for the relative gut weight to increase with increase in dietary lipid energy, and decrease with increase in dietary carbohydrate energy. When guts were examined histologically
there was an increase in the amount of fat deposited around the pyloric caeca and intestine of fish fed diets increasing in lipid content, but no other abnormalities were noted.

The relative liver weight of *C. labrosus* tended to increase with dietary energy level and was highly correlated with total body lipid although this does not necessarily imply that the increased liver weight was due to an increase in liver lipid. In turbot (Adron et al., 1976), yellowtail (Takeda et al., 1975) and rainbow trout (Austreng, 1976) liver lipid was found to increase with increase in dietary energy level, but in other studies liver size was unaffected by diet (rainbow trout - Yu et al., 1977; channel catfish - Garling and Wilson, 1976). The decline in liver weight of *C. labrosus* with increase in protein:calorie ratio at each dietary protein level, and the significant difference in elevation between the regressions calculated for fish fed the 20 and 40% protein diets, contrasted with the work of Lee and Putnam (1973) on rainbow trout who found that the protein:calorie ratio was positively correlated with liver size regardless of the dietary level of protein or lipid.

When dietary energy level was increased from 2.28/2.38 to 3.08/3.18 kcal g⁻¹ by increasing carbohydrate energy the relative liver size of *C. labrosus* increased, although further increase did not occur at higher dietary carbohydrate energy levels. Buhler and Halver (1961) reported that liver size of chinook salmon increased with increasing dextrin in the diet. The relative liver weights of *C. labrosus*
fed diets containing 3.80-3.98 kcal/g were similar, irrespective of whether energy was supplied as carbohydrate or carbohydrate + lipid. This contrasts with the results of experiments with rainbow trout in which liver size of fish fed isocaloric diets rose as the proportion of carbohydrate calories increased (Edwards et al., 1977).

No marked changes of liver structure with diet were noted in juvenile C. labrosus. Early work on trout suggested that high levels of dietary carbohydrate were toxic, causing damage to the livers (Phillips et al., 1948). However, more recent work has shown that high levels of dietary carbohydrate can be tolerated without significant pathological alteration to the liver (Austreng et al., 1977; Buhler and Halver, 1961; Lee and Putnam, 1973; Garling and Wilson, 1976). Similarly, increase in dietary lipid was reported to cause no abnormalities in the liver in most cases (Austreng, 1976; Ogino and Saito, 1970; Cowey et al., 1975), although Lee and Wales (1973) reported that rainbow trout fed diets containing 8 & 16% but not 24% lipid showed abnormal nuclei and bile duct proliferation.

Dietary protein level appeared to have little effect on gill structure of fish in Experiment IV, but gill damage typical of nutritional gill disease, although the aetiology of such gill damage is difficult to determine, was characteristic of fish fed the lowest energy diets and diets containing high levels of carbohydrate as the energy source. There was no evidence to suggest that this gill damage was related to ammonia concentration in the tank, and it seems more likely that it reflected some nutritional inadequacy in the...
diet, perhaps associated with the low levels of lipid, particularly lipid of fish origin, in these diets.
Although grey mullet are successfully farmed in many regions of the world very little attention has been paid to their aquacultural potential in Britain. There is a record of monks near Plymouth impounding mullet in ponds and growing them for future consumption (Hickling, pers. comm.) but this practice has long been discontinued. Webber & Riordan (1976) emphasised the importance of prior knowledge of growth rate, nutritional requirements and conversion efficiency in the assessment of aquacultural feasibility. It was in the study of these factors for the mullet species occurring in South-west England that this thesis aimed to make a contribution. Some of the most relevant results are summarised in this section.

The growth rates reported by Hickling (1970b) and Kennedy & Fitzmaurice (1969) for juvenile C. labrosus were largely confirmed. In the estuaries examined, juvenile C. labrosus and L. ramada appeared to grow faster than L. aurata, but this is not necessarily so in other regions of the world, which suggests that environmental factors, among which salinity seems likely to be of particular importance, have a differential effect on growth in the three species.

In all species there was a marked seasonal variation in growth with periods of fast growth alternating with periods of no growth. Comparisons with growth rates reported from other parts of the world showed that the growth of the same species was slower in South-west England than elsewhere. This suggests that there would be considerable scope for improvement of growth by manipulation of the environment. Since the Mugilidae are principally warm water fish, increasing water temperature, perhaps
by using industrial waste heat, would undoubtedly be advantageous, although Experiment I clearly illustrated the interaction between ration size and temperature. Thus improved growth would only occur at higher temperatures in fish also given a high ration; on low rations the reverse would occur. Being a shallow water estuarine fish, juvenile mullet would be particularly suited to such an environment - able to take advantage of higher water temperature and yet tolerate considerable temperature fluctuation. The marked seasonal variation in growth observed in the natural environment was also reflected in the feeding experiments under an artificial lighting regime. It seems likely that temperature played an important part in this respect, but since the juvenile mullet appear to feed actively throughout the winter, further elucidation of the controls on the commencement and cessation of the growing period, with a view to possible extension, is necessary.

The particularly fast growth of \textit{C. labrosus} in the fish ponds at Arcachon (Arné, 1938), and the fact that St. John's Lake seemed to be a favourable environment for the growth of \textit{O} group \textit{C. labrosus} and \textit{L. aurata}, suggested that growth may be improved in other ways. For example, in both the ponds at Arcachon and St. John's Lake, access to the feeding grounds was not restricted by the tide as to other localities. The considerably faster growth of juvenile \textit{M. cephalus} in Chilka Lake compared to the nearby Mahanadi Estuary in India is perhaps a further example of a similar phenomenon (Jhingran & Mishra, 1962; Thakur, 1967).
The salinity of St. John's Lake varied from almost freshwater at low tide to almost full strength seawater at high tide. Its shallowness resulted in large fluctuations of temperature and this, coupled with the high organic input, would be expected to result in at least periodic depletion of oxygen levels. Due to the limited facilities available, the conditions imposed on the fish during the feeding experiments were also sub-optimal, particularly in terms of water quality. In surviving and growing in both these environments, juvenile mullet displayed a tolerance of environmental fluctuation which not only would be advantageous in reducing financial risk, but which would also permit simple husbandry techniques. The experimental fish underwent considerable stress in terms of net capture, transport and handling but the only time this resulted in significant mortality was when it coincided with low temperature in the middle of winter. In general, juvenile *M. labrosus* reacted well to confinement in the experimental tanks with no evidence of aggression or a strong hierarchy, and readily consumed the wide variety of diets offered to them.

At maturity much food energy is diverted from growth to gametogenesis. The fact that mullet are late maturing in England may be of advantage in an aquafarming venture in which size of individuals at harvesting is independent of the need to sustain a wild population. The inflexion point of the growth curve appears to occur at a larger size in grey mullet than in many other fish and so, provided current legal minimum lengths as applied to captured fish can be bypassed by the fish farmer, the crop can be
harvested before growth rate and conversion efficiency decline drastically. However, size at harvest of most farmed species is determined more by market demand and processing considerations than biological criteria such as decline in growth efficiency. This is a field which requires much further study, but recent advances in the production of a sausage-like product from grey mullet, in which size of individual fish is relatively unimportant, may be relevant.

The feeding behaviour of the grey mullet is one of the main factors which recommends it for future culture. Most of the fish currently involved in culture in Britain were chosen on the basis of fast growth and high market value. However, the cost of complete pelleted rations is very high and, in addition to supply and storage problems, the aquaculturist must increasingly compete with, for example, cattle feed manufacturers, for sources of cheap high quality protein such as trash fish. The economic and energetic advantages of an omnivorous facultative feeder such as the grey mullet, which can exploit the lower trophic food levels, are considerable. Not only can a portion of the nutrition be provided from the pond itself, but organic wastes such as domestic sewage, brewing and food processing industry wastes can be used when available, both directly as nutrients, and indirectly via stimulation of increased natural productivity. However to design such a system with success it is necessary to have more information on mullet nutrition and the way in which it differs from that of salmonids and other cultured fish.

The high optimum dietary protein level indicated for
juvenile *C. labrosus* in Experiment I, similar to that of carnivorous fish, was initially surprising in view of their poor natural diet. However, the highly significant interaction with feeding level is important, since the 'optimum' dietary protein level decreased markedly with increase in ration size. Similarly the ration at which conversion efficiency was a maximum increased as protein level in the diet decreased. Grey mullet differed markedly from carnivorous fish such as plaice (Cowey et al., 1970a) in the relationship between protein efficiency ratio and % protein in the diet. In plaice PER was a maximum at 40% protein in the diet whereas in mullet, like carp (Ogino & Saito, 1970), PER increased with decrease in % protein in the diet down to the lowest level used (8.5%), although below this there must be a decline in PER to zero on diets supporting zero growth. In field studies in St. John's Lake juvenile grey mullet were characterised by a rapid passage of food through the gut and a high daily food consumption of a diet which Hickling (1970b) showed contained only 5-15% organic matter. Thus it appears that mullet exploit their ability to utilise protein very efficiently from diets so low in protein content that they would not support growth of carnivorous species.

The high PER of fish fed low protein diets suggested that mullet were able to utilise high levels of dietary energy to spare protein for growth, and stimulated further study of this important phenomenon in the third experiment. Increase in dietary lipid energy resulted in improved growth and protein efficiency.
ratios. The protein : energy ratio in the diet resulting in optimum conversion efficiency was lower than for salmonids, and lower on the lower protein diet. Increase in dietary carbohydrate energy resulted in a moderate increase in growth at low and intermediate levels, but the highest carbohydrate level for fish fed both protein level diets resulted in some growth depression. One of the most striking results of this experiment was the significant negative correlation between PER and protein : energy ratio over all diets. This suggested that, even at the lowest protein : energy ratios used, maximum protein sparing had not been achieved and there was further scope for reduction.

Although in all the experiments described in this thesis, growth and conversion efficiencies of mullet were low, there was ample evidence that they could be improved by environmental manipulation. For example, there was histological evidence that water quality was a limiting factor in some cases. A special feature of grey mullet nutrition was the ability to consume and convert diets of low protein : energy ratio into high quality edible protein. Such diets must be consumed in larger quantities to achieve maximum conversion efficiencies than would diets richer in proteins, but, since such food materials are likely to be waste products, low conversion efficiencies are relatively unimportant. In view of this it appears that the future of mullet culture may lie, not as an industry in itself, but in association with, for example, trout culture. The feeding habits of mullet would make them ideally suited to
utilising trout faeces and excess food to reduce waste, and the adaptability of mullet nutrition is such that use could also be made of gluts of higher protein food, such as trash fish, if these became available. It has been suggested (Odum, 1968a) that mullet obtain an appreciable portion of their nutrition from bacteria on the particles taken into the gut. This may make them especially suitable for using single cell proteins, brewing sludge and sewage sludge as a source of nutrients although problems such as heavy metal accumulation and other health hazards would have to be overcome. There is also a danger that fish feeding on such unusual foods may acquire odd flavours. A significant effect of diet and feeding level on body composition was illustrated in the feeding experiments described in this thesis, and this may have important implications for storage and processing. However, one of the major problems facing the potential mullet farmer is undoubtedly consumer acceptability. However, if mullet culture is viewed as a low investment enterprise, making use of waste products as the major nutrient source, the costs of production and risk should be relatively low. As costs of other protein sources and fishing increase, the necessary investment in marketing the product may become a more attractive possibility.