NUTRITIONAL PHYSIOLOGY OF TURBOT *Scopthalmus maximus* (L.): IMPLICATIONS TO AQUACULTURE

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Facilis descensus Averni;
Noctes atque dies patet atri janua Ditis;
Sed revocare gradum, superasque evadere ad aurus,
Hoc opus, hic labor est!

..... Virgil

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DECLARATION

I declare that the research described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It has not, in part, or in whole been submitted for any other degree.

Isabel Coombs

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SUMMARY

The nutritional physiology of turbot *Scophthalmus maximus* (L.) was investigated in the laboratory under controlled experimental conditions. The study dealt with factors influencing feeding, digestion and absorption in juvenile turbot and explored the partitioning of energy from ingested food. Energy use under varying intrinsic and extrinsic conditions was examined. The results provide an insight into the interrelationships of factors affecting the physiology and hence growth of turbot. The implications of the findings to aquaculture are discussed.

A multi-disciplinary approach was adopted and there were many components to the experimental work: study of the gross morphology, histology and ultrastructure of the alimentary tract of turbot; experimental investigations into the effect of various environmental and intrinsic factors on the relationship between ingestion rate, growth rate and conversion efficiency; the rate of transit of food through the gut and assimilation efficiency; maintenance and feeding respiration; body composition; condition factor and hepato-somatic index. The data obtained were collated and used to construct energy partitioning tables for turbot. A review of infectious diseases of turbot, an investigation into the possible parasitic nature of 'rodlet cells', and a study of the host-parasite relationship between turbot and the tapeworm, *Bothriocephalus scorpii* were also undertaken.

The first part of the research examined aspects of feeding, digestion and absorption in turbot. The gross and fine structure of the alimentary canal were analysed and comparisons made with wild turbot and other teleost species. The alimentary tract of turbot was found to be typical of carnivorous fish and appeared to have a degree of plasticity in its gross morphology. The length of the tract, expressed as a percentage of the body length, was found to be shorter in cultivated fish fed on a highly nutritious, protein-rich, easily digested diet than in wild turbot. Electron microscopy studies revealed the intestinal and rectal microvilli to be exceptionally long (2.5-5.6 μm), with the length decreasing from the intestine to the rectum. Microvilli are known to greatly increase the surface area of the intestine for absorption and the presence of long microvilli may account, in part, for the very high assimilation efficiencies recorded for turbot in this study. Study of the effect of different temperatures and feeding regimes on the ingestion rate, gastric evacuation rate and assimilation efficiency of turbot indicated that a regular, twice daily feeding schedule to satiation resulted in optimal assimilation efficiencies. It was shown that assimilation efficiency, in terms of energy, was lower in fish acclimated to 20°C than for fish at 10°C.

Respiratory and growth experiments were designed to investigate aspects of the energy metabolism in turbot. The energetic costs of maintenance, activity and feeding were calculated by determination of the oxygen consumption of turbot of varying sizes and held under various

conditions. The proportion of energy used for maintenance and activity metabolism was found to be very labile depending on the size of the fish and the temperature of acclimation. The amount of ingested energy lost in feeding metabolism was dependent on size of ration and temperature, whilst the proportion of ingested energy used for growth was very similar under all experimental conditions with only a slight increase seen in the 10°C fish fed twice daily to satiation. On the assumption that the standard metabolic rate of the tapeworm is similar to that of its host, estimates were made of the energetic cost of infection of turbot with the pseudophyllidean tapeworm *Bothriocephalus scorpii* under various conditions. It was estimated that the tapeworm imposed a small, but significant energetic cost to its host of between 1.5-10 % of the host's ingested food energy. The extent of this energy drain was found to be dependent on the size and health of the fish, ration size and quality, and the temperature of acclimation. The most significant costs occurred in small fish where the parasite index was highest and where fish were on a reduced ration and held at a high temperature. These data were collated and used to construct predictive energy budgets for juvenile turbot ranging in weight from 10-100 g.

Direct measurements of ingestion rates, growth rates, and energy content were made to test the precision of the bioenergetics model. Conversion efficiencies were established for fish held under varying experimental conditions. Growth/ration curves were derived to explain the relationship between food intake and growth under different environmental conditions. The condition factor (W/L^3), hepato-somatic index and analyses of body composition were also measured to assess the type of growth occurring under different conditions.

It is widely accepted that stress and disease have an important effect on the growth of fish, although relatively few studies have been conducted on the energetic cost of disease in fish. A major increase in intensive culture of turbot over the last 20-25 years has led to a concomitant rise in the pathological problems of the fish. The infectious diseases of turbot and their importance to intensive culture systems are discussed. A study of the host-parasite relationship between turbot and the tapeworm *B. scorpii* was conducted. Prevalence levels of 40-60 % were found. The intensity of infection in juvenile turbot was quite low, with most fish harbouring one tapeworm. The frequency distribution pattern of parasite numbers per fish was underdispersed which is quite rare for helminth infections and may reflect the unnatural conditions of fish in captivity. The parasite index was greater (1.94) in fish of 0-100 mm than in fish of 101-200 mm (0.45). The energetic cost of infection determined in this study suggests that infection may have little effect on the growth rates of healthy fish fed to satiation but that the parasite will exact a greater energetic drain on nutritionally challenged or immunocompromised fish and consequently would have financial consequences for the aquaculture industry.

The enigmatic nature of rodlet cells, whether they are a parasite or endogenous host tissue, has been debated for over a century. Electron microscopy studies revealed the presence of rodlet cells in the alimentary tract of the turbot. The morphology of the cells in turbot was studied and their distribution within the alimentary tract was determined. Rodlet cells were not discovered in every fish examined. The distribution of rodlet cells within the fish differed between individuals but generally the greatest numbers were found in the stomach. Communication junctions between rodlet cells and neighbouring epithelial cells were frequently observed. Occasionally the limiting membrane of rodlet cells appeared to extend into and encompass part of the surrounding cytoplasm forming a cytoplasmic extension. Intercellular gaps were often observed between rodlet cells and neighbouring epithelial cells. Some disruption of the histological integrity of the mucosal epithelial tissue adjacent to rodlet cells was observed. These observations are consistent with rodlet cells being parasitic in nature and possibly having adverse effects on the host. The distribution of rodlet cells was examined phylogenetically. The findings suggest that they are of very ancient phylogenetic origin and are widespread amongst fish species including those of great economic importance.

In his 'Epistle to the Reader' in John Locke's *Essay on Human Understanding* (1690), Locke refers to the manner in which the essay was written:

……'by catches and many long intervals of interruptions' …… as being ……'apt to cause some repetitions!' …… 'I will not deny' he says 'but possibly it might be reduced to a narrower compass than it is. But to confess the truth, I am now too lazy or too busy to make it shorter'

CHAPTER 1

GENERAL INTRODUCTION

1.1 DEVELOPMENT OF FIN-FISH CULTURE

Fish farming in various forms has been employed by humans as a method of increasing their food resources for over 2000 years. Humans were hunter-gatherers until the mesolithic period (note: most aquaculture books say until neolithic, but strong evidence exists of deep-sea fishing in the mesolithic period in Scotland 4432-4165 BC) when fishing developed as part of a subsistence activity (Coles, 1971; Davis, 1987). Advances in fishing techniques led to an ever increasing use of aquatic organisms as a food source for humans and eventually methods of fish culture were developed, most likely from the need to sustain increasing populations. Evidence of fish ponds in Egypt date back to 2500 B.C and the earliest known document on fish culture comes from China by Fen-Li and dates back to 1400 B.C. Fish culture developed in the Far East and spread to Europe in the Middle Ages. Most of this activity was small scale until relatively recently.

The last 30 years have seen the development of aquaculture in the Western world. The need to adopt a more productive means to feed an expanding world population has been a driving force in the development of fish farming. There is an ever increasing global demand for fish protein and in many developing countries fish is the main source of protein. More recently, access rights in the fishing industry have been curtailed due to overfishing which has proved to be a counter productive method of increasing food source (Pauly and Christensen, 1995; Sharp, 1995; Iversen, 1996; Larkin, 1996; Cook *et al.*, 1997). More than 70 % of the world's fisheries are fully exploited, in decline, seriously depleted or under severe limits to allow recovery of fish stocks (Cook *et al.*, 1997). The turbot *Scophthalmus maximus* (L.) wars of 1994-1995 between Canada and Spain illustrate this problem clearly. Canada fought for legislation to conserve the fragile stocks of turbot (also known as Greenland halibut) in their coastal waters. This problem was brought about by Spain fishing just outside Canada's Exclusive Zone using illegal nets which caught large numbers of immature fish. Spanish fishers claimed a drop in their fishing quota from 60000 to 27000 tons was threatening their livelihood. However, these numbers were misleading as their catch had in fact increased from 4000 to 60000 tons in just five years and the fishing had only started in the first place because stocks of cod *Gadus mohua*, redfish *Sebastes viviparus* and flounder *Platyichthys flesus* had been fished out and because of the previous declines of fish stocks in European waters. Canadian warships seized Spanish fishing vessels and confiscated their illegal nets which had such a small mesh size that turbot too young to spawn were caught. The result of this illegal action by the Canadians and their ensuing negotiations with the European Union was strict legislation to protect the fragile turbot stocks from becoming commercially extinct, as had happened with the northern cod (Cook *et al.*, 1997).

An increasing public demand for certain species such as prawns, salmon and sea bream, especially out of season, accompanied by a decline in natural resources, has been another stimulus in the growth of aquaculture, especially in recent years. The prospect of fewer international conflicts as most forms of aquaculture require to conform only to national laws is also an incentive to increase efforts in the aquaculture business. The pressure on countries to be more self-reliant in food production along with saving and earning in foreign trade is important. Aquaculture can also be significant as a source of rural employment and as a way of improving the nutrition and income of such populations (Pillay, 1993).

However, the growth in aquaculture has not been without setbacks. Due to lack of biotechnological knowledge, many ventures have failed and the industry has often been perceived to be high risk. The problems of aquaculture are much greater than those of agriculture because they prevail in an unfamiliar environment. Problems include dispersal (requiring a need for cages, tanks or ponds), interactions between aquatic species and medium such as temperature, level of oxygen, pH, nutrients in feedstuffs and fertilizers, stocking densities, water turnover and the spread of disease. Also, different species have particular requirements and this means specific knowledge for each fish species is necessary rather than a few general principles. Scientific research in aquaculture is in its infancy; however, the future is hopeful and increases in production are forecast (Iversen, 1996).

Increase in production in aquaculture has risen in the last 15 years from 6 million tonnes in 1976 to 11 million tonnes in 1985 and over to 16 million tonnes in 1992 (Table 1.1). Supplies

Table 1.1 FAO Estimates of total world production in aquaculture (Pillay, 1993; Iversen, 1996)

Total production (tonnes)	Year
6.1 million	1975
8.7 million	1980
10.5 million	1983
10.6 million	1985
13.2 million	1987
16.0 million	1992

from fishing have increased at 0.3 % per annum whilst those from aquaculture have increased at 5.5 % per annum (Nash, 1987). Aquaculture produced 13 % of the production from fisheries in 1994 and by 2000 A.D. it is speculated that this should be 25 % (Iversen, 1996). The fishing industry is very large and has increased to meet the growing demands for protein of an ever

increasing human population. At present the catch per annum is around 80-90 million tonnes, and the most optimistic estimates of total catch of commercial species from the sea is 100 million tonnes. Of this 70 million tonnes would be for human consumption. However, projected population increases suggests 100-140 million tonnes of edible fishery products will be required by 2000 AD. This would mean a deficit in the region of 20-60 million tonnes, thus guaranteeing a major role for aquaculture for the foreseeable future (Pillay, 1993; Iversen, 1996).

1.2 DEVELOPMENT OF TURBOT CULTURE

Turbot are an important food source in British and European waters not because of the size of the fishery but because of the extremely delicate and highly regarded flavour of the flesh and consequently their high market value. Evidence of humans eating turbot in Scotland dates back to the Mesolithic period (4432-4165 BC) at Morton Tayport in Fife (Coles, 1971). This is a site situated near the sea that has yielded abundant remains of marine molluscs, crabs and various fish, including turbot. Many people consider turbot to have the finest flavour of all sea fish and it was even regarded as a luxury fish in Ancient Rome where the Roman poet, Martial (89 AD, cited in Ker, 1968) made allusions to turbot as the food of the rich in one of his famous epigrams:

> "You get the choicest mushrooms; I get fungus pigs won't touch.
> You toy with turbot; I'm down here with the catfish"

Natural production of turbot in Europe is around 10000 tonnes and approximately 1000 tonnes in Great Britain; but these amounts cannot meet the public demand (Pillay, 1993). Early research by Purdom *et al.* (1972) on the rearing of turbot at high densities proved the fish to be hardy and that, although carnivorous, they could be fed on various feedstuffs and prepared foods. This knowledge helped to renew interest in the farming of turbot.

One of the early problems in turbot culture was the short supply of seed stock and as a consequence the early culture of turbot involved rearing wild juveniles caught at sea. The small size of the larvae meant new techniques had to be developed to culture the fish through their first year. Also, in the early seventies the basic biology of the species was poorly understood.

Turbot were farmed in Scotland and France in the early 1970s and as a consequence of research developments in these countries and the biotechnological advances made subsequently, the production of farmed fish has increased in recent years. Success has been achieved in the rearing cycle making reproduction in captivity now possible. However, high death rates of turbot

larvae of approximately 10 days old means a low output from hatcheries, although turbot are fairly robust after this stage. Different on-growing methods achieve varying standards of success but generally the young turbot are reared in tanks and, if sites are suitable, the final stages of on-growing can be carried out in surface cages. Research has shown that a slight drop in salinity below full strength sea water improves growth with the optimum temperature for rearing being around 18°C (Pillay, 1993). Availability of warm water is therefore important to any commercial venture in turbot culture.

The production of cultured turbot in Britain reached 10 tonnes in 1976 (Person-Le-Ruyet, 1990). Relatively slow progress was made over the next few years but gradually on-growing farms were supplied with hatchery-reared juveniles. In 1987, Scotland was the greatest producer of farmed turbot with Golden Sea Produce (a Norsk Hydro Subsidiary) producing 100 tonnes and 200000 juveniles per annum (Person-Le-Ruyet, 1990). The use of warm waste water from the cooling station at Hunterston combined with the use of high quality waste fish for food meant that fish could be held close to their optimum temperature of 18°C and fast growth rates achieved.

More recently, Spain, Portugal, Germany, Denmark and Norway have also begun to culture turbot whilst research effort has declined in Scotland and France over the last fifteen years. Over recent years Spain has shown most enthusiasm for turbot farming. This is most likely correlated with a healthy market and highly favourable climatic conditions, especially in Galicia in north-west Spain where production of turbot reached 1500 tonnes in 1993 (Toranzo *et al.*, 1993a).

It is not surprising therefore that turbot are one of the success stories of modern fish culture. This is partly due to their high market value because they are classed as a luxury fish, partly because they are a robust fish and are relatively easy to feed (Purdom *et al.*, 1972), and partly due to the decline in natural production (Pillay, 1993). However, despite the relative success in turbot culture and the biotechnological advances made, there still remain large gaps in scientific knowledge in the areas of nutrition, pathology of diseases of turbot and in the physiology of the fish (Person-Le-Ruyet, 1990). At the time when the research detailed in this thesis was carried out (1976-1979) the most basic biological knowledge on turbot was lacking and the main aim of the study was to use laboratory investigations to explore factors affecting the nutritional physiology of turbot *Scophthalmus maximus* (L.) and investigate their implications to aquaculture.

1.3 BIOLOGY OF THE TURBOT *SCOPHTHALMUS MAXIMUS* (L.)

1.3.1 Systematics

The following system of classification for the turbot is based on that advised by Wheeler (1992a) and will be used throughout this thesis unless otherwise specified.

ORDER : PLEURONECTIFORMES

FAMILY : SCOPHTHALMIDAE

Scophthalmus maximus (Linnaeus, 1758)

Wheeler (1992a) noted that Nielsen (1973, 1986) used the binomen, *Psetta maxima* for the turbot, thus separating the turbot and the brill, *Scophthalmus rhombus,* into two separate genera. This system of classification has been used by many fish biologists. However, Wheeler states that this is both unnecessary and still needs confirmation by detailed study and until this is carried out the recommended name for the turbot should be *Scophthalmus maximus* (L.) after Norman (1934).

1.3.2 General morphology of *Scophthalmus maximus* (L.)

Excluding the caudal rays, the body of the turbot is broad and round in shape and lies left hand side uppermost. The large mouth lies to the left of the eyes and bears small numerous teeth in both of the strongly curved jaws. The dorsal fin begins between the upper eye and mouth and extends to the fleshy part of the tail. The operculum is directed posteriorly and ends at an angle near the base of the small pectoral fin. The broad based pelvic fins are placed quite far towards the anterior of the body and are separated from the anal fin by a narrow space. The anal fin ends near the fleshy part of the tail. The caudal rays are quite long and rounded. The body of the turbot lacks scales and is studded with numerous bony tubercules, mostly occurring on the coloured upper side. The lateral line is arched steeply over the pectoral fin and then runs in a straight line to the tail. The colour of the upper-side varies according to the sea bed and the need for protective camouflage but is usually a dull, sandy brown with brown specks all over the body and fins and with dense spots over the tail (Wheeler, 1992b). Turbot may reach lengths of approximately 600 mm when adult with a maximum recorded length of 1000 mm (Pillay, 1993).

1.3.3 Life history of *Scophthalmus maximus* (L.)

In the North Sea and Irish Sea, turbot spawn from April to August and in the Western Channel from May to September. Spawning takes place over gravel sea beds between 10-80 m. The eggs measure approximately 1 mm in diameter and are very numerous, each female producing from 5 to 10 million eggs, depending on her size. The eggs and larvae are planktonic

and this phase lasts roughly two months. The larvae metamorphose into flatfish at between 20 and 30 mm in length (Jones, 1973). Metamorphosis appears to correspond with the arrival of the young flatfish at sandy beaches (from July to October), where the fish spend their first year of life after which the fish move offshore to deeper water (Jones, 1972). Turbot are fished commercially from 3-4 years of age.

1. 4 FISH ENERGERTICS: THE ENERGY BUDGET

Fish farming is essentially dealing with a resource (fish) which the fish farmer wishes to manage in the most efficient manner possible. This involves an understanding of the factors which influence the growth and condition of the fish. Early growth studies often looked only at quantitative changes in weight or in weight and length and neglected any qualitative changes in body composition. Ivlev (1939), Winberg (1956), Brett (1970), Beamish *et al.* (1975) and Warren and Davies (1967) all developed schemes of assessing growth in terms of a total energy concept (energetics). They introduced and developed the idea of a thermodynamically balanced 'energy equation' where the energy consumed in the food could be balanced against energy expenditure (loss in faeces, urine and through gills, mucous and sloughed cells, energy used for metabolic processes and the component used in the production of fish tissue). In this way all the energy in the system should be accounted for. This equation is often referred to as the energy budget and has been investigated in great detail over the years (Brett, 1979; Cho *et al.*, 1982; Brett, 1983; Calow, 1985; Weatherley and Gill, 1987; Cui and Liu, 1990; Xie and Sun, 1993; Cho and Bureau, 1995; Hartman and Brandt, 1995; Cui *et al.*, 1996). The concept of fish energetics and the energy budget is discussed in detail in Chapter 4.1.1.

The useable/net energy gained from the digested and assimilated food is available for maintenance, activity, growth and reproduction. However, growth and reproduction have the lowest priority out of these components. When food is restricted or, is of poor quality, or other environmental and intrinsic factors are less than optimal, growth and reproduction are the first factors to be compromised.

The present study was concerned with investigating the importance of various components of this equation for juvenile turbot held under controlled experimental conditions in an attempt to define the parameters which resulted in the optimum utilization of the food energy consumed and hence the optimum conditions for growth (whilst maintaining desirable body composition). The bioenergetics model (see Chapter 4) was constructed using data obtained on: a) the energy value of the food consumed, ingestion rates and assimilation efficiencies (Chapter 3); b) the energy used for respiratory metabolism (Chapter 4); and c) from data obtained on the

energy requirements of the tapeworm, *Bothriocephalus scorpii* which was found to infect 50 % of the turbot (Chapter 6).

1. 5 FEEDING, DIGESTION AND ABSORPTION IN FISH

The first requirement of fish culture is an adequate supply of food containing sufficient energy and the appropriate nutrients for routine maintenance, the process of assimilation, any activity and for growth and reproduction of the fish (Pitcher and Hart, 1982; Cho and Bureau, 1995). In intensive fish culture a fish does not have to seek and capture food as it is presented to the fish on a regular basis. A knowledge of how much food the fish requires, how often this should be presented, the optimal proportion of nutrients and energy in the food, how efficiently the food is digested and absorbed, and how quickly the food passes through the alimentary tract, are essential if the optimum conditions for the maximum utilization of the food are to be achieved.

The morphological structure of the mouthparts of a fish to a large extent determine the type of food eaten (Al-Hussaini, 1949a,b; de Groot, 1971). The morphological, physiological and biochemical properties of the rest of the alimentary tract determine the extent to which the food eaten is digested and absorbed (de Groot, 1971). There is considerable difference in the structure and length of the alimentary canal of herbivorous, omnivorous and carnivorous fish. Generally speaking, carnivorous fish have the shortest intestines, whereas herbivorous fish have highly coiled, lengthy intestines (Fänge and Grove, 1979). The length of the gut tends to increase with the amount of indigestible food or roughage. This is possibly to compensate for the lack of folding found in these guts which allows a relatively unimpeded passage of roughage through the gut. In contrast to these herbivorous fish, multiple branched folds are often found in the intestines of highly specialized predatory fish (Kapoor, 1975). However, some fish are known to change their diets under certain conditions such as seasonal changes, during spawning and due to some parasitic infections. The adaptability of the length and structure of the alimentary tract to changes in diet has been demonstrated by various authors (Angelescu and Gneri, 1949; Kapoor *et al.*, 1975; Stroband, 1977; Kuperman and Kuz'mina, 1994). It has been demonstrated that the size of the intestinal microvilli varies between fish and it is known that the microvilli greatly increase the surface area of the digestive and transportive area of the intestine and are also involved in membrane digestion (Kuperman and Kuz'mina, 1994). It would therefore seem probable that the differences in the growth properties of various fish species may be correlated with the morphological characteristics and the digestive and absorptive properties of their alimentary tract (Weatherley and Gill, 1987).

Fish in intensive fish culture tend to be fed a highly nutritious, easily digested, protein-rich diet and thus it was decided to compare the gross structure and length of the alimentary tract of farmed turbot fed on a pelleted diet with that of wild turbot fed on natural prey items in order to discover if there was a shortening of the alimentary tract in farmed fish. Observations were also made to discover if any changes in the length of the gut occurred as a response to changes in feeding levels, frequency of feeding or acclimation temperature.

Turbot are a highly carnivorous fish with a very fast growth rate in comparison to other species of flatfish (Jones, 1972), and therefore a study of the general histology and fine structure of the absorptive epithelium of the alimentary tract was carried out and comparisons made with that reported for other fish species of different feeding habits. The ultrastructure of fed and fasted turbot was also compared to study the adaptability of the turbot alimentary tract. The pH of the contents of the different regions of the alimentary tract was determined to give an indication of the micro-environment of the digestive enzymes present in the turbot gut. The effects of different feeding regimes and different temperatures on the ingestion rates, the rate of passage of food through the gut and on the assimilation efficiency of turbot were also examined to give an insight into the conditions for optimum feeding, digestion and absorption in turbot.

1.6 RESPIRATORY METABOLISM IN FISH

A large proportion of the energy assimilated by fish is used for the various components of metabolism. These components are generally referred to as 'standard metabolism' (rate of oxygen consumption of a fish at rest in the post absorptive state and thermally acclimated, *i.e.* maintenance metabolism); 'routine metabolism' (rate of oxygen consumption of a fish during normal spontaneous activity); 'feeding metabolism' (oxygen consumption of a fish associated with the digestion, absorption and processing of food, often referred to as the specific dynamic action or SDA); and finally, the 'active metabolism' (the rate of oxygen consumption of a fish swimming steadily against a current). These are based on the definitions given by Brett and Groves (1979) and are explained in greater detail in Chapter 4. A knowledge of how this metabolic energy expenditure is affected by different intrinsic and extrinsic factors such as age, sex, reproduction, nutritional state, the ability to acclimatize to stress, temperature changes, salinity changes, availability and type of food, stress and disease is very important. The effect of these factors on each of the different components of metabolism can give an insight into the relationship between the food consumed and how the assimilated energy from this ingested food is utilized and hence, how much energy is left over for growth.

With fish, metabolic rate is usually determined by measuring oxygen consumption. Various methods have been devised to quantify oxygen consumption and these are reviewed in Chapter 4.2.1. An oxycalorific coefficient is used to convert the amount of oxygen consumed to an energy equivalent and in this way the amount of energy required for the maintenance of bodily functions, for routine activity, for feeding activity and for active metabolism can be calculated. A figure of 19.4 kJ per l of oxygen is recommended for fish and this is based on the assumption that the primary respiratory substrates are protein and lipid (Jobling, 1994).

In this study, preliminary experiments were conducted to find out if a diurnal activity in oxygen consumption was present. A standard procedure was then developed to determine the oxygen consumption of juvenile turbot at rest. These methods are discussed in detail in Chapter 4. The relationship between standard metabolic rate and body size was examined to find out the maintenance energy requirements of fish of various sizes. The effect of acute temperature changes on the standard metabolic rate was examined as well as short and long term acclimation of standard metabolic rate to temperature to determine the maintenance requirements of fish at different temperatures. Fish were fed meals of varying sizes to determine the extra energy required to process food and the length of time taken for the respiration rate to return to its former pre-feeding level. Long term adaptation of the standard metabolic rate to different levels and frequencies of feeding was also examined. Investigations were also made into the energy requirements for active fish. The assimilation efficiency of fish of varying sizes, on different feeding levels and frequencies and held at different temperatures was also determined and therefore a picture of how much of the food consumed was assimilated and then used for maintenance, routine activity and for feeding at different temperatures was calculated. From this, an estimate of how much energy was left over for growth could be calculated. These estimates could then be tested against the growth experiments conducted during the present study, in which the initial and final weights of the fish and the exact energy consumed in food was also known.

1.7 GROWTH AND PRODUCTION IN FISH

Only a portion of the food energy consumed by fish is assimilated, the rest is lost in excretion. The assimilated energy is used for the maintenance of bodily functions, the processing of food, for routine activity and swimming and, if the fish is infected, energy is possibly used by intestinal helminths. The remainder of the assimilated energy is available for growth (both somatic and reproductive) (Brett and Groves, 1979; Weatherley and Gill, 1987; Jobling, 1994). Although growth may appear to have the lowest immediate priority, reproductive growth it is ultimately the feature that dictates the survival of a species.

Many factors affect the growth rate of fish but in general there is a decrease in specific growth rate with increase in size and age of the fish. Feeding, digestion and absorption are all influenced by a number of factors and in turn these affect the growth rate of fish. In fish farming the growth of healthy, palatable fish tissue at as fast a rate as possible with minimum cost is the main priority and consequently the factors that affect the growth of fish are of great interest.

Growth in fish may be measured in terms of temporal changes in weight, length, energy content or the relative proportions of lipid, protein, carbohydrate and water. The composition of the fish body varies with changes in quantity and quality of food, and also with fish age and reproductive state. In some cases, an index of growth such as the condition factor (CF, the relationship between the weight and length (expressed as W/L^3), or the hepato-somatic index (HSI, weight liver/weight fish x100) may be used to express the 'well-being' or energy status of fish (Chapter 4.2.2.4; 4.2.2.5). In fish farming, however, it is valuable to measure growth in terms of the fate of the assimilated food in respect to the efficiency of utilization (conversion efficiency) or, in terms of the fate of assimilated food in respect to the relative growth of the body tissues and the composition of these tissues. The latter method gives a deeper insight into the efficiency with which the ingested food energy is deposited as body tissue. For example, a fish fed maximally will tend to have a higher lipid content than one fed on a reduced ration and older fish tend to lay down more lipid than younger fish (Weatherley and Gill, 1987). However, the conversion efficiency (100 x growth/ration) is perhaps the quickest and simplest indicator of the nutritional adequacy of a particular diet or ration level, the suitability of the environment in which the fish is held, and the state of health of the fish (Brett and Groves, 1979).

The relationship between the growth rate and ingestion rate was examined by Paloheimo and Dickie (1966) for several species of fish and they concluded there was a tendency for gross conversion efficiency to fall with increasing ration size. Brett *et al.* (1969) developed a simple method of using growth-ration curves, where they plotted the growth rate against rations (ingestion rate) and from this the ration levels approximating to starvation, maintenance, optimal and maximal growth could be determined. The use of the gross conversion efficiency equation and the effect of various environmental factors (temperature, light, salinity, oxygen tension) and biotic factors (ration, size competition) on the growth-rations curve has been investigated for various species over the years (Ursin, 1967; Warren and Davies, 1967; Brett *et al.*, 1969; Kerr, 1971a,b,c,d; Ware, 1975; Elliott, 1976a,b,c; Brett and Groves, 1979; Allen and Wooten, 1982; Weatherley and Gill, 1987). This has enabled some of the factors which control and limit growth rate to be determined for certain species. Methods for measuring growth rate and the concept behind growth-ration curves are discussed in detail in Chapter 4.

In the present study, feeding experiments were carried out which provided data on the amount of food consumed per day by fish held under different temperature and feeding conditions. Changes in weight and length for each group during a ten week period were noted, providing information on growth rates and the condition factor of the fish. Growth-ration curves were produced for each group of fish. Conversion efficiencies were also obtained and used to investigate the relationship between certain biotic and environmental factors and growth. The body composition of each group was analysed for protein, lipid, moisture and energy content and the hepato-somatic index obtained to give further insight into the type of growth achieved by each group.

It was proposed to use these direct measurements of growth rates, using weight and length measurements and total energy content, to test the bioenergetics model constructed for the energy partitioning of food consumed by turbot under different conditions. The bioenergetics model was constructed from data obtained on the ingestion rates, assimilation efficiencies, respiratory metabolism and on the energy requirements of the tapeworm, *Bothriocephalus scorpii* in turbot held under varying experimental conditions.

1.8 STRESS AND DISEASE IN FISH

1.8.1 Stress in fish

It was considered that an understanding of the effect of stress and disease in fish was essential to any study on the factors affecting the energy metabolism of fish and therefore a short review is included here.

Stress responses in fish have evolved as adaptive mechanisms enabling fish to maintain homeostasis when confronted with changing external or internal forces (stress factors). For example, thermal acclimation involves physiological changes in response to temperature change that allow the fish to adapt and survive at a new temperature. Also, behavioural responses such as the fight or flight reaction involve adaptive reactions enabling the fish to either escape or deal with an external stress factor. However, it follows that the performance capacity of a fish must be reduced due to the energy costs associated with trying to maintain homeostasis. Further problems may be encountered due to stress under certain conditions, notably intensive fish culture or experimental procedures where the stress factors may become chronic and where the stress response becomes ineffective and possibly damaging to the fish. It is widely accepted that stress factors appear to play a significant role in fish disease (as well as disease being a stress factor itself), on the growth and condition of fish, on the reproductive capacity of fish and

ultimately on fish populations (Wedemeyer and MacLeay, 1981; Kent and Fournie, 1993; Waring *et al.*, 1996).

Various interpretations of the word stress exist, with some authors describing stress as the stimulus which provokes a stress response in animals (Pickering, 1981; Ulanowicz, 1978) whilst others have defined stress as the response of the system to a stimulus (Esch and Hazen, 1978). All are based on the premise that a stimulus, or number of stimuli, act on a biological system and result in a response by the system. Although the difference is fundamentally one of semantics (Pickering, 1981), confusion can occur unless scientists are careful to specify clearly their understanding and usage of stress concepts (Lazarus, 1971).

Further difficulties are encountered when attempts are made to define the parameters that constitute a stress response in an animal in precise terms. Seyle (1950) defined stress as "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force". Problems arise over what is defined as normal and therefore the distinction between normal and stressed. These have been partly overcome by attempting to quantify the immediate physiological responses of an animal to a stress factor. Seyle described a common response to a number of stress factors and the components of this response are known as the General Adaptation Syndrome (GAS). These consist of non-specific physiological and biochemical changes and they occur in temporal order of: 1) the alarm reaction; 2) the stage of resistance, or compensation, (during which adaptation to achieve homeostasis under the changed circumstances is taking place); and 3) the stage of exhaustion (when adaptation has ceased to be adequate and homeostasis is not achieved). However, the GAS is not easy to define or measure as the response is highly polymorphic and may vary depending on the stress factor(s) involved and may be species-specific. This concept implies that the health and condition of the fish are impaired, at least momentarily, by stress and might be viewed in functional terms. For example, Brett (1958) quantified an animal's response to stress by measuring changes in its performance capacity with respect to its survival capacity. According to Brett, stress may be defined as "a stage produced by an environmental or other factor which extends the adaptive responses of an animal beyond the adaptive range, or which disturbs the normal functioning to such an extent that the chances for survival are significantly reduced."

Stress responses are generally classified as primary (neural and neuro-endocrine responses including increased production of corticosteroids and catecholamines), secondary (the biochemical, physiological and immunological consequences of the primary responses (Mazeaud *et al.* 1977) and tertiary (behavioural changes, reduced growth rate and increased susceptibility to disease (Wedemeyer and MacLeay, 1981). The parameters that are often assessed, because of

their ease of measurement, are plasma cortisol, plasma glucose and plasma electrolytes. Again, caution should be used in interpreting the stress response by looking at one particular level to the exclusion of the other two as the stress response is really an integrated response of the fish at all three levels involving many reactions (Pickering, 1981). Although most of these stress responses can be viewed as adaptive, certain energy costs are exacted and in many situations, particularly those encountered in intensive fish cultivation and in experimental fish biology, chronic stress conditions may occur which can depress the growth rate and increase the susceptibility to disease.

External stress factors which occur or may arise in fish cultivation or during experimental procedures include transport, handling, crowding, anaesthesia, changes in water quality and temperature changes. Internal stress factors such as disease or nutritional imbalance are often problems in fish culture.

1.8.1.1 Stress factors involved during transportation

Transportation can often cause fish to become stressed and may even lead to fatalities on occasion. This may be due to a lack of oxygen if no extra source is supplied to replace that used by the fish in transit, or possibly due to an excess of oxygen if too much oxygen is bubbled through the water (Edwards, 1995). Elevated levels of oxygen are known to reduce the respiration rate of fish and this may increase the level of carbon dioxide in the blood. Higher levels of carbon dioxide in the blood may also occur due to higher levels of carbon dioxide in the water. This can result in a lower haemoglobin oxygen carrying capacity, lower blood pH and consequently may stress the oxygen transport system (Randall *et al.*, 1982). Ideally, the water should be constantly aerated as well as being supplied with extra oxygen, as the aeration serves to induce a greater exchange of the gases contained in the enclosed body of water. This is especially important for long journeys where levels of carbon dioxide would tend to accumulate in the absence of adequate agitation (Pooley, 1995) and also in warm weather conditions under which oxygen saturation decreases.

In the present study, measures were taken to reduce stress to a minimum during transportation of the juvenile turbot to Glasgow. Details are discussed in Chapter 2.

1.8.1.2 Stress responses to crowding

The majority of fish living in groups tend to be organized into dominance hierarchies. Scott and Currie (1980) demonstrated that the subordinate individuals in a hierarchy of *Xiphophorus helleri* (swordtail) had more active inter-renal tissue than dominant ones and they suggested that the raised levels of corticosteroids in the subordinate individuals may lead to stress-induced suppression of immunity in these fish. Under crowded conditions with a limited

food source, hierarchical feeding relationships may be exacerbated thus preventing equal food distribution and may result in differences in the growth rate of fish. Pfuderer *et al.* (1974) discovered a substance (stress pheromone) which occurred in both the flesh and the water of carp, *Cyprinius carpio* held in crowded conditions. This substance when extracted and administered to fish held under uncrowded conditions caused depression of heart and growth rates of uncrowded carp. Perlmutter *et al.* (1973) also found a 'stress pheromone' was released in *Trichogaster trichopterus* (gourami) when these fish were held under crowded conditions. This led to a suppression of immunity in the fish to infectious pancreatic necrosis virus (IPNV). Crowding may also cause stress by reducing the water quality and/or by increasing the probability of disease by providing conditions favourable for the survival and transmission of disease organisms. Stress-induced immunosuppression may then aggravate the situation.

Ellis (1981) suggested that stress may cause disturbance in the defence systems of fish as these are under regulatory mechanisms involving hormonal control (for example, corticosteroids) which are altered during stress. This may suppress or exaggerate a number of responses which may result in an imbalance in other physiological systems and eventually cause or be a contributing factor to disease. Wedemeyer and McLeay (1981) listed a number of stress-mediated diseases including furunculosis caused by *Aeromonas* species, bacterial gill disease caused by *Pseudomonas*, vibriosis caused by *Vibrio anguillarum*, columnaris due to *Flexibacter columnaris*, some parasitic infestations including *Trichodina* species, *Ichthyobodo necatrix* (costiasis) and fungal infestations such as *Saprolegnia* species. They suggested these may be used as indicators of crowding and other stress factors.

In the present study, particular attention was paid to the aquarium conditions and maintenance of the turbot in Glasgow (Chapter 2). However, in the early stages of the work, when the fish were obtained from Hunterston, outbreaks of vibriosis were common as was infection with a possible cytophagan bacterium causing fin-rot.

1.8.1.3 Stress responses to poor water quality

According to Smart (1981), a reduction in appetite, growth and food conversion efficiency may be a sensitive indicator of stress due to poor water quality or to fluctuations in water quality. Stress factors such as reduction in, or fluctuations of, dissolved oxygen, increased levels of ammonia and elevated carbon dioxide concentrations may all contribute to this depression as well as decreasing the ability of the fish to withstand handling procedures.

Stress response due to fluctuations in dissolved oxygen are discussed in Chapter 2.1.2. Levels of ammonia may increase in intensive fish-rearing units and under laboratory conditions, due to the ammonia excreted by the fish as the end product of nitrogen metabolism. This is a

particular problem when fish are fed on high protein diets. Care must be taken to ensure an adequate flow of water and to check the pH and ammonia content of the water. Unionized ammonia (UIA) concentrations of between 0.016 and 0.021 mg NH_3 $N1^{-1}$ are recommended as the maximum concentrations of unionized ammonia that can be tolerated by fish for long periods. However, Alderson (1979) found the growth of turbot was unaffected by UIA levels of 0.1 mg NH_3 $N1^{-1}$ in sea water at 16°C. Nevertheless, it is prudent to monitor the levels of UIA and keep them within the recommended levels and to be aware that the toxicity effects are decreased by increased salinity (Bower and Bidwell, 1978) and increased by low oxygen concentrations (Lloyd, 1961).

Elevated levels of carbon dioxide are often encountered in intensive fish cultivation units. Smart (1981) found the growth and food conversion efficiencies were similar for groups of fish held at carbon dioxide concentrations of 12 and 24 mg l^{-1} for 275 days, but with fish held at 55 mg l^{-1} for this time there was an initial loss of appetite and growth and food conversion efficiencies were poor for the first 28 days. There was some improvement after this period, suggesting a certain degree of acclimation.

It is important that all parameters of water quality should be considered together as they have a complexity of interactions. Other factors such as disease and the nutritional state of the fish can also affect the fish's ability to cope with fluctuations in water quality. Maintenance procedures for the present study are discussed in Chapter 2.

1.8.1.4 Stress responses to handling procedures

Handling procedures such as capture, anaesthesia, antibiotics administration, weighing, measuring and confinement are known to cause various stress responses in fish. Pickering and Pottinger (1989) demonstrated a rise in plasma cortisol levels in salmonids from a basal level of under 5 ng ml^{-1} to a level of 40-200 ng ml^{-1}, due to handling. If the duration of the exposure to the stress factor is short (acute), then the plasma cortisol levels return to basal levels after 24 hours. However, if the exposure to the stress factor is chronic, then plasma cortisol levels may remain elevated for days or even weeks. They eventually return to basal levels, although the kinetics of cortisol secretion and plasma clearance are probably different from those of unstressed individuals.

Anaesthetics are often used during handling procedures and Hunn *et al.* (1968) demonstrated that MS222 (tricaine methanosulphate) and its derivatives are cleared from the blood of rainbow trout within 8 hours and from the urine within 24 hours of administration of the compound at a concentration of 100 mg l^{-1}. Houston *et al.* (1971a,b) noted an increase in blood

Na^+ for 1-2 hours in brook trout treated with MS222 at 100 mg litre^{-1}, along with elevated levels of tissue K^+, decreased levels of tissue Ca^{++} and hyperglycaemia over a period of several days. Soivio *et al.* (1977), showed that blood oxygen tension and pH were severely depressed after use of MS222 but recovered after 20 minutes. Eddy (1981) referred to the profound effect of capture, handling and anaesthesia on the salt and water balance of fish and emphasized the importance of adopting procedures which minimize stress when designing experiments. Capture and handling methods used in the present study are discussed in Chapter 2.1.5.

It is clear that many external and internal stress factors invoke an integrated stress response involving the primary, secondary and tertiary levels of response in fish and that these involve many reactions. The more severe and prolonged the stress factor, the more numerous and far reaching effects this has on the fish with for example, depression of growth rate, increased susceptibility to disease and decrease in reproductive capacity occurring. Ultimately, under severe stressful conditions, death may occur.

An awareness of the physiological stress responses of fish to the often unavoidable stress factors involved in fish culture and in experimental fish biology is of paramount importance in order to employ adequate recovery times before experimental procedures begin.

1.8.2 Disease in fish

It was noted above (1.8.1) that many external and internal stress factors invoke an integrated stress response in fish and that certain conditions, notably intensive fish culture and experimental laboratory conditions may exacerbate the stress. These stress factors may cause a depression in growth rate as well as increasing the susceptibility of the fish to disease. Disease itself is a stress factor and therefore any work investigating the energetics of fish should also be aware of any affect disease may have on the condition and growth rate of the fish. It is known that many diseases depress the appetite of the host (Ash *et al.*, 1984).

In the early stages of the present study many of the fish obtained were infected with vibriosis, caused by the facultative bacterium *Vibriosis anguillarium,* and also with a possible cytophagan bacterium causing fin-rot. These fish had reduced appetites and heavy fatalities occurred. In view of the increase in turbot culture in recent years and the concomitant rise in the number of pathological problems of the species causing high, economic losses (Novoa *et al.*, 1992; Toranzo, 1993a,b); a review on the importance of infectious diseases of fish and diseases of turbot in particular was carried out in Chapter 5.

A prevalence of 47 % infection with the pseudophyllidean tapeworm *Bothriocephalus scorpii* was found in turbot in the present study. These parasites take up a large proportion of the gut and are known to derive all their energy and nutrients from the host's intestine and must

therefore effect a nutritional drain on their host. It is possible that this may have very little effect on a healthy, well-fed host with unlimited food supply, but if the infection is combined with other stressors (physical, chemical, behavioural, dietary or other disease) which have a limiting effect on the host's food intake, then the proportion of the host's assimilated food used by the parasite will increase (Ash *et al.*, 1984). As the present study was mainly concerned with the factors affecting the energy partitioning of the food consumed by turbot, knowledge of the amount of damage caused and the energy consumed by the tapeworm seemed essential. This problem was addressed in Chapter 6.

Investigations into the fine structure of the alimentary tract of the turbot in the present work revealed an enigmatic cell in the epithelial layer. A literature search showed the nature of this cell to be very elusive, with some authors believing the cell to be an endogenous fish cell and attributing various functions to it including immune system and secretory activity (Duthie, 1939; Catton, 1951, Leino, 1974, 1982; Morrison and Odense, 1978; Leknes, 1986; Balabanova and Matey, 1987; Smith *et al.*, 1995), whilst other authors believed it to be parasitic in nature (Thélohan, 1892a,b; Laguessé, 1895; Dawe *et al.*, 1964; Hale, 1965; Bannister, 1965; 1966; Anderson *et al.*, 1976; Mayberry *et al.*, 1979; Viehberger and Bielek, 1982; Barber and Westermann, 1983; Richards *et al.*, 1994; Wayne and Mayberry, 1994). The role of parasites in adversely affecting the condition and growth of fish has been stressed (Toranzo *et al.*, 1993b). Since the present work was concerned with the energetics of turbot, it seemed important to carry out investigations into the nature of this cell and these are reported in Chapter 7.

1.9 RESEARCH OBJECTIVES

This study had wide ranging and numerous aims, all relevant to the understanding of the biology and farming of turbot.

1 To study the effects of changes in temperature, diet, feeding level and feeding frequency on feeding, digestion and absorption in farmed juvenile turbot. This involved investigation of ingestion rates, assimilation efficiencies and rates of gastric evacuation (Chapter 3).

2 To examine the gross structure, the general histology and fine structure of the alimentary tract and relate these to the function of the gut and compare them with those of other fish with a slower rate of growth. Also to investigate the adaptability of these features (Chapter 3).

3 To examine the effects of changes in temperature, feeding levels and feeding frequencies

on the standard, routine, feeding and active energy metabolism of farmed juvenile turbot by investigations on the respiratory physiology (Chapter 4).

4 To devise a standardized technique to carry out investigations on respiratory physiology (Chapter 4).

5 To study the effect of changes in temperature, feeding level and feeding frequency on the growth rate, production and gross conversion efficiencies of farmed juvenile turbot (Chapter 4).

6 To examine the host-parasite relationship between the pseudophyllidean tapeworm, *Bothriocephalus scorpii*, and the turbot, *Scophthalmus maximus* (L)., and estimate the amount of the host's assimilated energy the parasite consumes (Chapter 6).

7 To investigate the importance of infectious diseases in turbot culture (Chapter 5).

8 To investigate the nature of the rodlet cell in turbot (Chapter 7).

9 To construct an energy budget predicting the amount of energy available for growth under different feeding conditions and temperatures and for parasitized and non-parasitized fish, based on the information gained on the assimilation efficiency and energy metabolism of turbot under varying laboratory conditions. Also to use the more conventional data obtained from the growth studies to test the predictions of the energy budget.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 MAINTENANCE OF TURBOT IN THE LABORATORY

2.1.1 Introduction

A reliable supply of healthy fish stock is essential for carrying out experiments on the nutrition, growth and respiratory metabolism of any species of fish. It is important to transport fish under conditions that cause minimal stress to the holding facilities and to maintain the fish in optimal, standardized conditions for use in experiments. A thorough and painstaking maintenance regime is then required to keep fish healthy. Careful handling techniques and appropriate acclimation periods should be employed in all experimental procedures.

This research was carried out in the earlier days of modern fish farming when studies of the effects of stress, the causative agents of disease and the characteristics of diseases of fish in intensive culture were in their infancy. Nevertheless, the effects of stress and disease on the well being and growth of the fish was considered and attempts were made to maintain the turbot used in as stress-free an environment as possible. Particular attention was given to thorough, regular cleaning of experimental and holding tanks with minimum disturbance to the fish. Water quality was checked regularly, usually most days.

2.1.2 Fish source and collection

The source, collection, transport and fate of the experimental fish, turbot *Scophthalmus maximus* (L.) is depicted in a flow chart (Figure 2.1). Wild stock 0-group turbot were collected from Borth beach, Dyfed, near Aberystwyth, Wales during September, by the White Fish Authority (WFA). The procedures for capture and handling of the stock are described in the WFA Field Reports number 110 (1973/1974) and number 129 (1974).

After capture, the turbot were transferred to a holding tank where they were held for up to four days before transportation to the Marine Farming Unit at Hunterston, situated in Ayrshire, Scotland. The fish were held at Hunterston until weaned onto the WFA7 pelleted diet and then were transported to the Marine Farming Unit at Ardtoe, Acharacle, Argyllshire, Scotland. Fish from the small size grade were selected and maintained in an enclosed building at ambient temperature by WFA staff until required for research at Glasgow. During the first year of the study, fish were obtained from Hunterston where the sea water was warmed by the neighbouring power station. There were, however, major problems of disease and consequently large numbers of fatalities at both Hunterston and Glasgow. The disease was found to be vibriosis (diagnosis made by researchers at the Aquatic Pathobiology Unit at Stirling University), caused by infection with the facultative bacterium *Vibrio anguillarum*, although many fish also displayed symptoms similar to those described for the 'hepato-renal' syndrome (Anderson *et al.*,

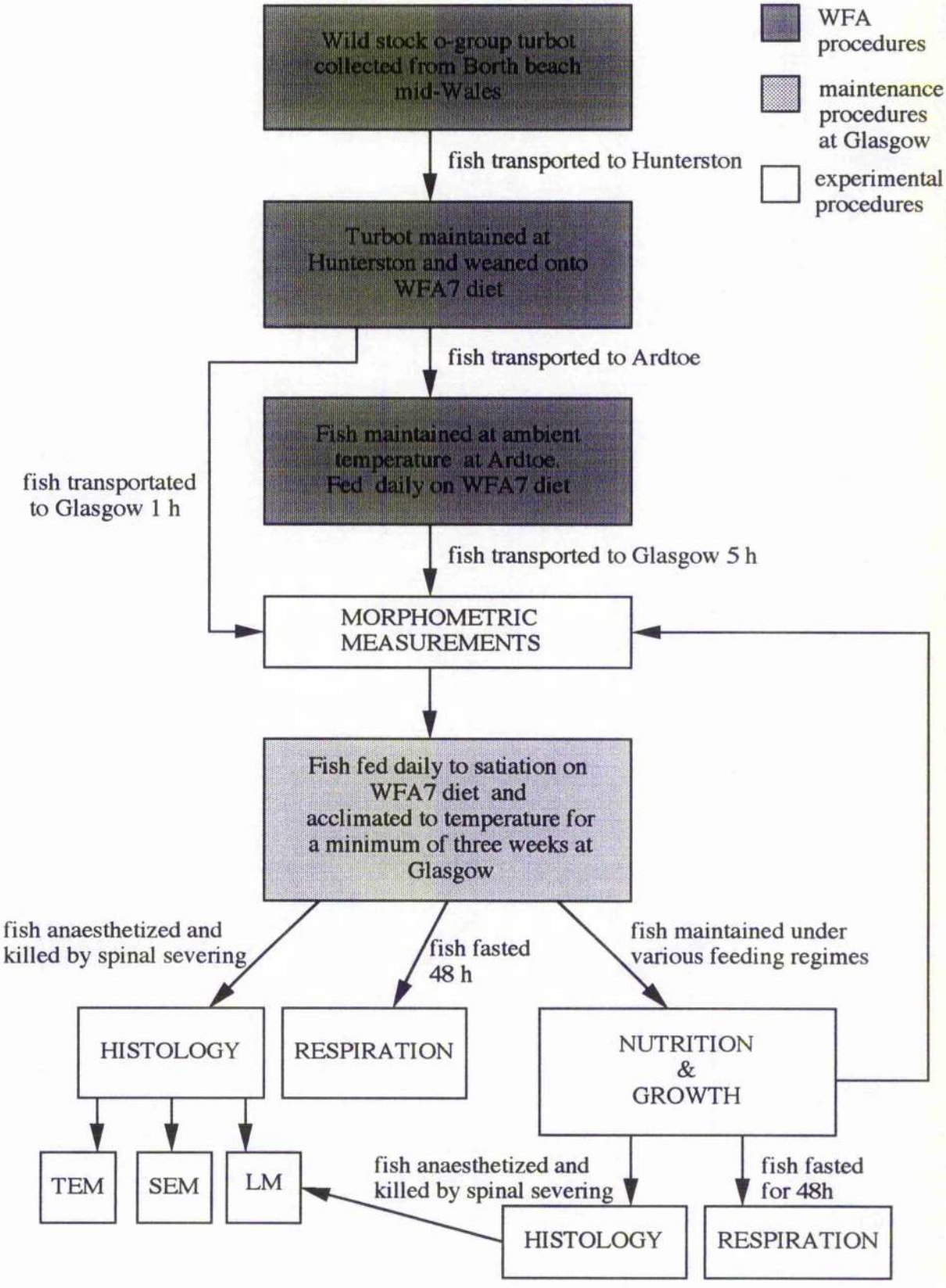


Figure 2.1 History of fish from collection at Borth to laboratory in Glasgow

1976; Dick *et al.*, 1976). Subsequently, stock fish were held at Ardtoe whereupon there were fewer disease problems. The majority of the results presented were obtained using fish from Ardtoe.

2.1.3 Transportation of fish to Glasgow

Specimens of stock animals were collected from Ardtoe and transported to Glasgow by road. Large, heavy duty polythene bags (80 l) were placed inside large plastic dustbins (120 l). Sea water (50 l) was transferred from the holding tanks to the polythene bags and fish of selected size were gently caught by net and placed in the bags. The water in the bags was well aerated, then the bags were filled with oxygen from a cylinder and sealed with polytape. The fish were transported at an approximate density of forty 20 g fish per bin. The journey lasted approximately five hours and a minimum of two stops was made in order to maintain the condition of the fish. At these times, the water was agitated for a couple of minutes to encourage gas exchange, then the bags were refilled with oxygen and resealed. This method, whereby the oxygen at the top of the bag was allowed to diffuse slowly into the water, was found to be effective for the transfer of healthy fish stock. Recent literature indicates that in some cases too much oxygen (where extra oxygen is bubbled through the water) and in others a lack of oxygen (where no extra oxygen is supplied to replace that lost in transit) may be responsible for serious stress-related problems including fatalities (Edwards, 1995).

On arrival, the containers were moved into a 10°C constant temperature aquarium, air stones were introduced to each container and air was gently bubbled through the water. The fish were then allowed to settle and come to temperature over a two to three hour period. The animals were then placed in holding tanks and two to three days were allowed to pass before feeding of the fish commenced. Generally, a three-week acclimation period was adhered to before experiments were undertaken.

2.1.4 Aquarium conditions and maintenance of fish in Glasgow

Juvenile turbot were held in glass fibre tanks, measuring 400 mm x 560 mm x 240 mm, (total volume, 54 l) in a 10°C constant temperature aquarium under a 12 h light - 12 h dark regime. The level of sea-water in the tanks was maintained at 36 l. Larger holding tanks, measuring 1200 mm x 560 mm x 240 mm (total volume, 160 l) were available for maintenance of turbot prior to experiments. Stocking densities were dependent on the size of the fish and the size of the holding tanks, but generally no more than twelve 50 g fish were held in the smaller experimental tanks.

As a re-circulating sea-water system was not available, it was necessary to have a strict and careful regime for the maintenance of the fish. Each tank was moderately aerated with an airstone and the sea-water was filtered through an Eheim pump. Fish were generally fed once per day with White Fish Authority pellets (WFA7) unless experimental procedures called for an alteration in diet and pattern of feeding. The following day's food was removed from the -20 °C freezer each evening and allowed to thaw overnight in a refrigerator. Food was offered to the fish until they began to refuse, whereupon all uneaten food was siphoned from the tank. Faeces and any other particulate matter were also removed daily.

The water quality of each tank was monitored for changes in salinity and topped up with freshwater or with clean sea-water from the reservoir tank when necessary (sea-water was delivered periodically from Millport, Isle of Cumbrae, and held in a large reservoir tank at environmental temperature). Each tank was thoroughly cleaned once per week and filled with fresh sea-water which had been allowed to come to desired temperature. The fish were transferred to another tank during cleaning. Generally, these maintenance procedures, including cleaning, took between two to three hours per day.

Various maintenance arrangements were tested but the one described above was found to be the most satisfactory in maintaining reasonably good water quality whilst minimizing the stress caused by handling the fish. A high death rate of 50 % resulting from other arrangements (fish held at Hunterston) was reduced to almost zero by adopting these procedures and fish were held in the aquarium at Glasgow for as long as 9 months. Occasionally, it was necessary to administer antibiotics to the fish, especially during the early stages of the work when fish were obtained from Hunterston and outbreaks of vibriosis were common as was infection with a possible cytophagan bacterium causing fin-rot. At first signs of any infection, Terramycin (Pfizers) was administered at a dose of 20 mg l^{-1}. Alterations in maintenance procedures were sometimes necessary due to experimental protocol. Any such changes are described in the relevant sections.

2.1.5 Capture and handling procedures

To reduce the stress arising from capture and handling during weighing and measuring of the fish, the anaesthetic MS-222 (tricaine methane sulphonate) was introduced into a tank at a dose of 100 mg l^{-1}. Fish were removed from the tank and carried to the laboratory in a bucket, weighed and measured there and then quickly returned to a tank of clean, aerated sea water in the aquarium unless further experimental procedures were to follow.

2.2 MEASUREMENTS OF TURBOT

The following standard techniques were used to obtain the length and weight of juvenile turbot. The conditions prior to weighing and measuring (*e.g.* the length of time since last meal and the temperature of acclimation) are given in the relevant chapters but generally measurements were taken 48 h after the last meal was offered.

i) **Total body length:** the body length was measured from the tip of the lower mandible to the posterior edge of the caudal fin. Measurements were made to the nearest millimetre.

ii) **Total body weight:** the fish were blotted and weighed to the nearest milligram. Fish were killed by lethal exposure to anaesthesia (MS222 at 1g l^{-1}), followed by spinal severing. The carcass was placed over crushed ice and dissected to remove the internal organs for measurement.

iii) **Liver weight:** the liver was dissected out, blotted and weighed to the nearest milligram.

iv) **Gut length and weight:** the gut was dissected out from the oesophagus to the rectum inclusive and its length measured to the nearest millimetre. The gut was then blotted and weighed to the nearest milligram.

v) **Carcass weight:** all visceral organs were removed, the fish carcass was blotted and then weighed to the nearest milligram.

2.3 MEASUREMENTS OF THE TAPEWORM *BOTHRIOCEPHALUS SCORPII*

Some juvenile turbot were found to be infected with the diphyllobothriid tapeworm, *Bothriocephalus scorpii*. Identification of the tapeworm was made by microscopic observation (see Chapter 6) of both fresh and fixed and stained preparations. Prevalence and intensity data were gathered from samples of fish randomly selected from each batch collected from the Marine Farming Units. The number of worms per fish was counted and the location of the scolex in the intestine noted. The tapeworms were then removed and the fresh length and weight of each was measured.

i) **Length of tapeworm:** the worms were carefully removed from the gut, untangled and

their length measured to the nearest millimetre.

ii) **Weight of tapeworm:** the worms were weighed to the nearest milligram.

iii) **Prevalence** = $\frac{\text{number of infected hosts}}{\text{number of hosts sampled}} \times 100$

iv) **Intensity** = number of parasites per host

v) **Parasite index** = $\frac{\text{weight of tapeworms}}{\text{weight of fish}} \times 100$

2.4 DIET FORMULATIONS

Turbot were fed WFA7 pellets during maintenance and throughout experiments unless experimental procedures called for a variation in diet.

(i) Composition of WFA 7 diet by weight:

Cooper's vitamin binder mix	20 %
Sprats	20 %
Queen offal	20 %
Iceatlantic pure white fish meal	40 %
Energy value	12.38 kJ (g wet weight)$^{-1}$

(ii) Composition of Coopers vitamin binder mix (Coopers Nutrition Products Ltd.)

Moisture	10.8 %
Protein	18.8%
Ash	5.8 %
Oil	10.8 %
Carbohydrate (inert)	

The vitamin mix provided the following vitamins and minerals Kg^{-1}:

Vitamin A, 36000 i.u.; Vitamin D_3, 4000 i.u.; Vitamin E, 225 i.u.; Vitamin B_2, 100 mg; Vitamin K, 30 mg; Nicotinic acid, 300 mg; Pantothenic acid, 150 mg; Folic acid, 20 mg; Vitamin B_1, 400 mg; Vitamin B_6, 40 mg; Iodine, 9 mg; Salt, 1%; Inositol, 800 mg; Biotin 2 mg; Vitamin C,

800 mg; Vitamin B_{12}, 60 µg; Choline chloride, 400 mg; Magnesium, 900 mg; Iron, 400 mg; Cobalt 5, mg; Manganese, 33 mg; Copper, 5 mg; Binding agent 98.85 %.

(iii) Composition of Iceatlantic pure white fish meal:

Oil	2.45 %
Protein	71.5 %
Moisture	9.4 %

(iv) Composition of chromic oxide diet :

Sprats	68 %
Cooper's vitamin binder	10 %
Iceatlantic fish meal	20 %
Chromic oxide	2 %

(v) Composition of sprats:

Dry matter	28.5 %
Of dry matter:	
Protein	57 %
Oil	34 %
Free fatty acids (oleic)	8.3 %
Fibre	Nil
Ash	8.6 %

(vi) **Composition of Pruteen** (supplied by ICI):

Moisture	10 %
Protein	72 %
Total lipids	8.2 %
Ash	7.7 %

Analysis has shown that Pruteen contained the following amino acids:

Amino acids; Lysine 4.7 g, Methionine 1.8 g, Cystine 0.5 g, Alanine 5.0 g, Arginine 3.6 g, Aspartic acid 6.5 g, Glutamic acid 7.6 g, Glycine 3.8 g, Histidine 1.4 g, Isoleucine 3.4 g, Leucine 5.2 g, Phenylalanine 2.6 g, Proline 2.4 g, Serine 2.4 g, Threonine 3.3 g, Tryptotophan 0.7 g, Tyrosine 2.3 g, Valine 4.1 g.

Pruteen is the name of the material resulting from the fermentation of methanol by the micro-organism *Methylophilus methylotrophus.* The organism grows rapidly and efficiently on

methanol as its only carbon and energy source and gives a product which is rich in protein and in the essential amino acids lysine and methionine. The micro-organism is harvested and dried to yield granules or powder containing over 70 % protein. Digestibility trials were conducted for ICI comparing the assimilation efficiencies of juvenile turbot fed on diets containing Pruteen as the major protein source with those fed on diets containing protein from other sources (see Chapter 3 for experimental design and results).

2. 5 DETERMINATION OF DRY WEIGHT AND ASH CONTENT

The dry weight of foodstuffs and body tissues was determined by drying samples to a constant weight in an oven set at 100°C. The ash content was determined by heating samples of pre-dried material for six hours in a blast furnace set at 600°C.

2.6 DETERMINATION OF ENERGY CONTENT USING THE GALLENKAMP BOMB CALORIMETER

The energy content of dried foodstuffs was determined by combusting 10 x 1 g samples in an atmosphere of pure oxygen at 25 atmospheres of pressure. The heat of combustion is taken up by a large heavy stainless steel shell. The temperature rise in the shell is measured by a thermocouple. The instrument was calibrated by measuring the temperature rise produced by the combustion of a standard quantity of benzoic acid and results expressed in kJoules.

2.7 DETERMINATION OF LIPID CONTENT USING THE STANDARD SOXHLET METHOD

The standard Soxhlet technique was used to extract the crude lipid components from pre-dried material (Grodzinski and Klekowski, 1975). A known weight of pre-dried material was wrapped in a paper towel and fastened with staples to form a small, secure parcel. Controls using pre-weighed parcels consisting of paper and staples only were used. All parcels were weighed and placed in the Soxhlet apparatus where extraction of lipids with a hot solvent mixture of chloroform:methanol (2:1) for 10 cycles (5 hours) was undertaken. The samples were removed from the apparatus and the excess solvent driven off by drying the samples in an oven at 100°C overnight. The samples were then re-weighed to the nearest mg and any weight loss was considered to be due to the extraction of the crude lipid component from the dry sample.

2.8 ASSIMILATION EFFICIENCY DETERMINATION USING THE INDIRECT CHROMIC OXIDE METHOD

2.8.1 Introduction

The nutrient assimilation efficiencies of turbot maintained under various conditions were determined indirectly by incorporating known quantities of chromic oxide (Cr_2O_3), a physiologically inert reference material, into the diet. The concentration of chromic oxide could then be determined chemically in the food and faeces and from the ratio of concentrations of chromic oxide to that of a nutrient in the food and faecal samples, the assimilation efficiency could be calculated without direct measurement of either food intake or faeces output (Maynard and Loosli, 1969). The following equation was used:

$$\text{Assimilation efficiency } \% = 100 \times \left(1 - \frac{Cr_2O_3 : \text{Nutrient ratio in food}}{Cr_2O_3 : \text{Nutrient ratio in faeces}}\right)$$

A wet mix of the chromic oxide diet (see above) was prepared by diluting the diet 1:2 with deionized water (for variations in the composition of this diet and in experimental procedures, see relevant chapters). Generally, food was withheld from fish for 48 h prior to voluntary or force feeding with the chromic oxide diet. The fish were then fed 2 % of their body weight of the diet twice per day and at the same time each day, using a 5 ml plastipak syringe for force feeding procedures. Tanks were regularly checked for appearance of faecal material and these were promptly removed. Beamish *et al.* (1975), suggested that faecal material should be removed from tanks as soon as possible as the protein nitrogen content of faecal material allowed to remain in a tank of bass *Dicentrarchus labrax*, kept at 25 °C was found to decrease by approximately 18 % over twenty four hours. Faecal samples from each group of experimental fish (n = 10-12) were collected by syphoning out the faeces from the bottom of the respective tanks. Samples from each group were washed with deionized water, filtered and dried in an oven at 100 °C to constant weight. All samples for micro-chemical analysis were ground using a mortar and pestle, thoroughly mixed and diluted 1:10 w/w with silica gel. The dilution with silica gel enabled small samples to be measured out accurately for analysis. For most experiments, a second and third series of force feeding with the chromic oxide diet followed by collection of faecal samples was undertaken giving three samples for each experimental condition.

2.8.2 Chemical analyses:

Chemical analyses were carried out on pre-dried material in accordance with the following methods:

2.8.2.1 Chromic oxide determination

The quantity of chromic oxide in each sample was determined using the method described by McGinnis and Kasting (1964). The chromic oxide was oxidised to dichromate and analysed spectrophotometrically by the diphenylcarbazide reaction.

Reagents:

Wet oxidation mix (WOM): 2 g sodium molybdate dissolved in 30 ml distilled water; add 30 ml concentrated sulphuric acid; 40 ml 70 % perchloric acid added finally.

Diphenylcarbazide reagent: 0.25 % w/v diphenylcarbazide in 50 % aqueous acetone (freshly made).

Standards:

Standards were prepared by mixing chromic oxide with silica gel such that 10mg aliquots contained 600, 300, 150 and 100 μg chromic oxide.

Method:

1 10 mg sample heated with 1 ml WOM for 30 min at 220°C

2 Resulting mixture diluted to 50 ml with distilled water

3 To 10 ml of this diluted mix, 1 ml of diphenylcarbazide reagent was added (with syringe to ensure rapid mixing).

4 Mixture centrifuged for 5 minutes to remove silica gel particles and absorbance at 540 nm determined.

5 Blank prepared from 10 mg silica gel and treated in the same way as faecal sample.

2.8.2.2 Protein determination using standard micro-Kjeldahl technique.

Samples were digested in sulphuric acid and the resulting ammonium nitrogen determined spectophotometrically by the phenol-hypochlorite reaction. Ammonium sulphate was used as a standard.

Reagents:

Digestion mix:

300 mg selenium oxide dissolved in 15 ml distilled water; 85 ml N-free sulphuric acid added to above and made up to 250 ml with distilled water. (Can be stored).

Phenol reagent:

5 ml 80 % aqueous phenol; 20 mg sodium nitroprusside; made up to 400 ml with distilled water. (Used fresh).

Hypochlorite reagent:

2 ml sodium hypochlorite (1N in 0.1N NaOH); 80 ml 2.5 % w/v sodium hydroxide made up to 200 ml with distilled water. (Freshly made).

Method:

1 10 mg sample heated to 350°C with either 1 or 2 ml digestion mix (depending on whether food or faecal sample) until digestion products become clear and then for 1 hour after this.

2 Mixture allowed to cool and made up to 20 ml with distilled water

3 To 1 ml (2 ml) of diluted digestion product

add a) 2 ml 2.5 % Na OH

b) 4 ml phenol reagent

c) 2 ml hypochlorite reagent

Make up to 10 ml with distilled water, mixed well and centrifuged to remove Silica gel particles.

4 Allow colour to develop for 20 - 30 minutes at room temperature and determine absorbance at 635 nm.

5 Blank prepared from: 2 ml digestion mix, 98 ml distilled water.

Most proteins contain approximately 16 % by weight of nitrogen and therefore the protein content of each sample can be determined from its nitrogen content by multiplying by a factor of 6.25.

2.8.2.3 Energy value determination

The energy value of foodstuffs and fish tissues was commonly determined using a Gallenkamp Ballistic Bomb calorimeter using benzoic acid as the standard (see above). However, in some cases, and particularly when only a limited amount of material was available for analysis, the energy value was determined using the wet oxidation technique.

Wet oxidation method:

Using this technique, organic material is oxidised with potassium dichromate in concentrated sulphuric acid and the unreduced dichromate determined spectrophotometrically (O'Shea and McGuire, 1962).

Reagents:

Wet oxidation mix:

2.5 g $K_2Cr_2O_7$ mixed with 10 ml distilled water and made up to 500 ml with concentrated sulphuric acid.

Standard:

Solution of 0.5 % potassium dichromate in concentrated sulphuric acid diluted 25 fold with distilled water and absorbance at 347 nm determined (solution contains 10 mg $K_2Cr_2O_7$ per 10 ml sample used).

Method:

1 10 mg samples mixed with either 2 ml or 4 ml digestion mix (for faecal and food samples, respectively) and heated at 80-100°C for 1 h.

2 Mixture cooled and diluted to 50 ml with distilled water and centrifuged to remove silica gel particles.

3 Absorbance read at 347 nm.

4 A blank containing 0.5 ml distilled water and 2 ml digestion mix was similarly treated.

Calculation of total energy:

Mean absorbance of unknown = E_1

Mean absorbance of standard = E_2

If $E_1 = E_2$, then no reduction of $K_2Cr_2O_7$ has occurred

$\Delta E = E_2 - E_1 = E_2\text{-}E_1/E_2 \times 10$ mg $K_2Cr_2O_7$

Since 3 mg $K_2Cr_2O_7$ = 0.489 mg O_2 and taking mean oxycalorific coefficient of fat/carbohydrate/protein of 3.38 calories, then 1mg $K_2Cr_2O_7$ = 0.551 calories

(10 mg $K_2Cr_2O_7$ = 5.51calories = 23.1joules).

From the difference between the absorbance value of the standard and the unknowns the amount of reduced dichromate and the energy value of the samples may be determined.

Hence ΔE = E2-E1/E2 x 5.52 calories

However, oxidation of protein tends to be incomplete during wet oxidation procedures and hence a correction factor must be applied. According to Forster and Gabbot (1971) a value of 60% for oxidized protein may be generally applicable and therefore a correction for unoxidized protein may be carried out as follows:

If the amount of protein in sample (N x 6.25) from micro Kjeldal is X mg

Then unoxidised protein is 0.4X μg

Since calorific coefficient of protein is 5.65 calories/mg and 40 % of protein of tissue sample is unoxidized then the correction factor for unoxidized protein in the sample is : 0.4 x 5.65 x protein content in sample (X) = 2.26X calories.

Then total energy in sample:

TOTAL ENERGY = $(5.52\Delta E/E_2 + 2.26X) \times 4.184$ joules.

2.9 DETERMINATION OF OXYGEN CONSUMPTION USING A CONSTANT-FLOW RESPIROMETER

A constant-flow respirometer was used to measure the oxygen consumption of fish in the present study. The theory behind the measurement of respiration rate and the design of the apparatus used are described in Chapter 4.

2.10 MICROSCOPY TECHNIQUES

2.10.1 Preparation of fish tissue for light microscopy

Generally, fish were prepared for light microscopy in the following manner.

i) Sampling procedure and fixation:

The alimentary tract was dissected out and placed in neutral buffered formalin solution. The inside of the gut was infused with fixative and the gut was then cut into the following sections: a) oesophagus and stomach; b) pyloric caeca and anterior intestine; c) posterior intestine and d) rectum. The sections were left overnight in fixative.

ii) Tissue processing:

1 Samples rinsed in tap water, washed twice in 70 % alcohol and left overnight in 70 % alcohol.

2 Wash in 70 % alcohol for 3.5 h

3 Change and wash in 70 % alcohol for further 4.5 h

4 Wash in 90 % alcohol for 2 h

5 Wash in absolute alcohol for 1.5 h

6 Change and wash in absolute alcohol for further 1.5 h

7 Absolute alcohol and xylene (50:50) for 1 h

8 Xylene for 1 h

9 Xylene and wax (50:50) for 1 h

10 Paraffin wax 1) for 2 h

11 Paraffin wax 2) for 3 h

iii) Embedding:

Metal blocks were filled with molten paraffin wax and tissue specimens with labels were placed in the wax. The blocks were then placed on a tray with ice and water and the tops of the blocks flamed every few seconds and extra molten wax added. This was carried out to prevent air bubbles forming due to shrinkage of the paraffin wax. The blocks were then left on ice until hardened and ready for sectioning and staining.

iv) Sectioning:

The wax blocks were mounted on wooden blocks and trimmed ready for sectioning. Serial sections were cut at 5 μm intervals on a rotary microtome. It was decided to cut 20 sections (=100 μm) and miss 200 sections (=1000 μm =1 mm) throughout the length of the gut.

Microscope slides were cleaned with alcohol and labelled using a diamond cutter. A mixture of albumen and water was placed on the slide, then the sections were carefully laid on the slide and then left on a hotplate overnight.

v) Staining:

Sections were stained with Ehrlich's Haematoxylin and Eosin for general staining.

Fixatives:

Neutral buffered formalin solution (pH 7.0): Luna (1968)

Formalin (40 % w/v formaldehyde)	100 ml
Distilled water	900 ml
Na_2HPO_4	6.5g
$NaH_2PO_4\ H_2O$	4 g

Fish saline for turbot:

Ingredients expressed in g l^{-1}: NaCl, 9.28; KCl, 0.37; $NaH_2PO_4.2H_2O$, 0.06; $CaCl_2$, 0.22; $MgCl_2$, 0.095; Glucose, 0.45; Hepes buffer, 1.09. Dissolve each component separately (except $CaCl_2$) in 750 ml distilled water and then dissolve $CaCl_2$ in 250 ml distilled water. Autoclave both solutions, cool and mix.

2.10.2 Preparation of fish tissue for Transmission Electron Microscopy

Generally, fish were anaesthetized and then killed by spinal severing and guts dissected out in fish saline. The procedure was carried out over a container of crushed ice to keep the tissues cool and delay lysis of tissue. Small sections of gut measuring less than 1 mm were cut from each of the following regions: oesophagus, stomach, anterior intestine, posterior intestine

and rectum (3-4 sections from each region). The tissues were then fixed and stained using the following procedures.

i) Fixation:

1 1 mm sections of tissue immersed in gluteraldehyde fixative for 2 h

2 Rinsed twice with phosphate buffer and left overnight

3 Fixed in osmium fixative and left for 2 h in a cool dark place.

4 Rapidly rinsed twice with buffer

5 Dehydrate in alcohols :

30 % - 10 min

50 % - 10 min

70 % - 15 min

90 % - 15 min

Absolute alcohol - 15 min (twice)

Absolute alcohol (anhydrous) - 15 min (twice)

1,2 Epoxypropane - 15 min (twice)

ii) Embedding:

1 For embedding, a 1:1 mix of Araldite mix plus accelerator with 1,2 epoxypropane was prepared and left for at least for 5 h

2 Araldite mix poured over labels in foil dishes. Specimens placed at bottom of oven (45 °C) and left for at least 5 h.

3 Place in 60°C oven for 18 h then remove.

iii) Sectioning and staining:

Thin and thick sections were cut on an LKB Ultramicrotome Type 1. Thick sections were prepared using a dry knife and lifted with a glass splinter then placed on a microscope slide with albumen and stained with methyl blue. Sections were examined using a Leitz microscope.

Thin sections were cut with a glass knife filled with 10 % alcohol then stretched with a waft of chloroform and placed on the dull side of a copper grid. The shiny side of the copper grid was then placed down on filter paper in a Petri dish and stained with saturated uranyl acetate in 50 % ethanol (15 min) followed by aqueous lead citrate solution (15 min). Sections were examined using an AE1 EM801 electron microscope at an accelerating voltage of 60KV and photographs were recorded on Ilford EM4 plates.

iv) Reagents:

Buffer:

A 2.26 % sodium dihydrogen orthophosphate (11.3 g in 500 ml distilled water)

B 2.52 % sodium hydroxide (6.3 g in 250 ml distilled water)

C 5.4 % glucose

D 83 ml of A added to 17 ml of B and checked for pH of between 7.2-7.4

Fixative:

Gluteraldehyde fixative: 9 ml of 25 % gluteraldehyde added to 50 ml of solution D

Buffer rinse: 9 ml of distilled water added to 50 ml of solution D

Osmium fixative: 0.5 g phial of osmium tetroxide added to 45 ml solution D and 5 ml of solution C

Embedding resin:

Araldite mix: To 27 ml hardener and 23 ml resin (already mixed) added 1 ml accelerator (2 % DMP 30). Made in 100 ml plastic disposable beaker and mixed thoroughly before use.

2.10.3 Preparation of fish tissue for Scanning Electron Microscopy

Generally, fish were prepared for scanning electron microscopy in the following manner.

i) Sampling procedure and fixation:

The alimentary tract was dissected out in fish saline and suitable sized sections to fit the mounting stubs were then cut from the anterior intestine, posterior intestine and rectum. These sections were then opened out and placed in gluteraldehyde fixative and processed as follows.

1 Sections of tissue immersed in gluteraldehyde fixative for 2 h.

2 Gently washed with buffer rinse to remove surface blood and mucous.

3 Placed in 1% osmium tetroxide fixative for 2 h.

4 Tissues trimmed to expose surfaces to be scanned.

5 Washed in buffer rinse for 2 h.

6 Tissues dehydrated in acetone:

30 % acetone	10 min
50 % acetone	10 min
70 % acetone	10 min
90 % acetone	10 min
Analar acetone	10 min
Analar acetone	overnight

7 Change acetone and critical point dry

8 Attach tissues to aluminium stubs using silver paint. Allow to dry 30 minutes in an oven at 37°C

9 Paint with gold

ii) Procedure for critical point drying:

The tissues are dried over CO_2 to obtain a distortion-free surface followed by gold coating to enhance resolution.

1 Dehydrated tissues (in 100 % acetone) transferred to basket filled with acetone. Care taken not to allow drying.

2 Basket placed in pressure drier (handle out) and door closed

3 Inlet valve opened to allow liquid CO_2 in to replace acetone. (This allows tissues to be dried in CO_2 as it changes from liquid to gaseous form thus preventing disfiguration of tissues.)

It is important to make sure liquid level does not fall below level of basket.

4 Flush system 2-3 times over a period of 1-2 h to remove acetone.

Open vent valve anticlockwise then drain valve - watch liquid level.

Close drain and then vent valve.

Turn hot water system on and allow pressure to rise slowly to 1400 psi (liquid CO_2 turns to gas at approximately 1100 psi)

5 Vent off gas slowly, remove basket and mount specimens on stubs as described.

The dried sections were glued to the stubs using Acheson's Dag 915 cement and were then coated with gold in a Polaron SEM coating unit E500 and finally examined in a Philips Scanning Electron Microscope at 25KV.

2.11 DATA ANALYSES

All statistical analyses were performed using Minitab version 10.0 for Windows. Parametric tests include t-tests, regression analysis and analysis of covariance. Mann-Whitney and Kruskal-Wallis non-parametric tests were also used. Statistical significance has been accepted at a probability level of less than 0.05. Means are presented ± their standard deviation unless otherwise stated.

CHAPTER 3

FEEDING, DIGESTION AND ABSORPTION IN TURBOT

3.1 INTRODUCTION

Fish require an adequate supply of food containing the necessary nutrients required in appropriate amounts. Their food must contain sufficient energy and nutrients for routine maintenance, the process of assimilation, any activity, growth and reproduction. In intensive culture conditions, a fish does not have to seek and capture food; it is presented with food on a regular basis. Knowledge of how much food a fish requires, how efficiently this food is digested and absorbed, and how quickly the food passes through the gut under different environmental and intrinsic conditions is essential in determining how often this food should be presented, the optimal proportion of nutrients and energy content of the food and the overall conditions for maximum efficiency of utilization of the food.

3.1.1 Feeding mechanisms and the alimentary tract

The morphological structure of the mouthparts of a fish determine to a large extent the type of food eaten (Chapter 1.4). Some fish experience changes in diet under certain conditions (for example, during winter, spawning, specific food shortages, parasitic infection or intensive fish farming). Various authors have shown the adaptability of the alimentary canal to changes in the diet (Angelescu and Gneri, 1949 cited in Kapoor *et al.*, 1975; Lange, 1962 cited in Kapoor *et al.*, 1975; Stroband, 1977; Kuperman and Kuz'mina, 1994) although others have been more sceptical of the relationship between feeding habits and gut structure (Bishop and Odense, 1966). It would seem most likely that the phylogeny of a fish to a large extent, but not exclusively, determines its feeding characteristics and also its ability to adapt to a variety of feeding conditions, although it might be expected that some fish are more flexible in this respect than others.

Fish under intensive culture tend to be fed a highly nutritive, easily digested, protein-rich diet and it has been suggested that this may lead to a shortening of the alimentary canal (Crompton, 1973; Jobling, 1994). Wild turbot (>110 mm in length) feed on prey such as fish that move very quickly whereas very young fish (60-110 mm in length) feed predominately on mysids and young shrimps (Braber and de Groot, 1973a). The mouth of the turbot is almost symmetrical with an apical aperture enabling the fish to snap at fast moving prey in front of it (Van Dobben, 1937, cited in de Groot, 1971). They are visual predators and do not rely on chemical stimuli to any discernible degree, a system which correlates with the presence in the brain of a relatively small olfactory lobe and a large optical lobe (Evans, 1937). However, chemoreception may play an integrated part in the feeding strategy of larval turbot (Knutsen, 1992). According to de Groot (1971) digestion is slow (96 hours to clear all traces of food from the alimentary tract at 10°C) which he maintains is in accordance with the feeding behaviour of

devouring whole fish and the presence of a large stomach and short intestine. The buccal and pharyngeal cavities, oesophagus and stomach represent about 50% of the turbot's alimentary canal, the rest consists of a short, simple intestinal loop with two small intestinal caeca (often referred to as pyloric caeca) and rectum. Braber and de Groot, (1973b) found the length of the alimentary tract of turbot to be approximately equal to the body length throughout the life cycle, although there is a gradual change in the relative lengths of the buccal-pharyngeal cavity and oesophagus-stomach lengths. They postulated that the gradual increase in the relative size of the buccal-pharyngeal cavity was probably correlated with the increase in the size of the prey although the length of the intestine and rectum remain the same.

In this study, comparisons were made between the gross structure and length of the alimentary tract of farmed turbot fed on a pelleted diet with that of wild turbot feeding on natural prey items. Observations were also made to find out if any changes in the length of the gut were caused by differences in feeding level, frequency of feeding or by acclimation temperature.

The extent to which food is digested and absorbed is largely dependent on the structure and function of the alimentary tract. Various studies have been carried out on the basic structural organization and ultrastucture of the intestinal epithelium of teleost fish in relation to their feeding habits (Al-Hussaini, 1949a,b; Moshin, 1962; Iwai, 1968a,b, 1969; Western, 1969; Noaillac-Depeyre and Gas, 1973; Anderson, 1986; Cousin *et al.*, 1987; Kuperman and Kuz'mina, 1994; Murray *et al.*, 1994a,b, 1996). One aim of the present study was to compare the ultrastructure of the intestinal epithelium of turbot, a carnivorous fish with a very fast growth rate, with that reported for other fish to see if the turbot's life style is reflected in its intestinal composition. In addition, the adaptability of the structure of the alimentary tract of fed and fasted fish were compared.

3. 1. 2 Factors affecting food intake, digestion and absorption in fish

The quantity of food consumed, digestion and absorption of food may be influenced by a number of different factors including size of fish, nutritional history, the size of meal, availability of certain food items, size of food items and consistency, frequency of feeding, digestibility of nutrients, light regime, temperature, water quality and condition of fish (Tyler, 1970; Edwards, 1971; Elliot, 1972, 1975a, b; Jobling *et al.*, 1977; Thorpe, 1977; Elliot and Persson, 1978; Windell, 1978; Fänge and Grove, 1979; Flowerdew and Grove, 1979; Jobling, 1980, 1981a,b; Grove *et al.*, 1985; Pandian and Vivekanandan, 1985; Talbot, 1985; Bromley, 1987, 1994; Cousin *et al.*, 1987; Munilla-Moran and Stark, 1989, 1990) (see Figure 3.1). Stress induced by the presence of dominant individuals (Abbot and Dill, 1989; Huntingford *et al.*, 1993), crowding (Jørgensen *et al.*, 1993) or handling (Waring *et al.*, 1996) may also influence food intake and

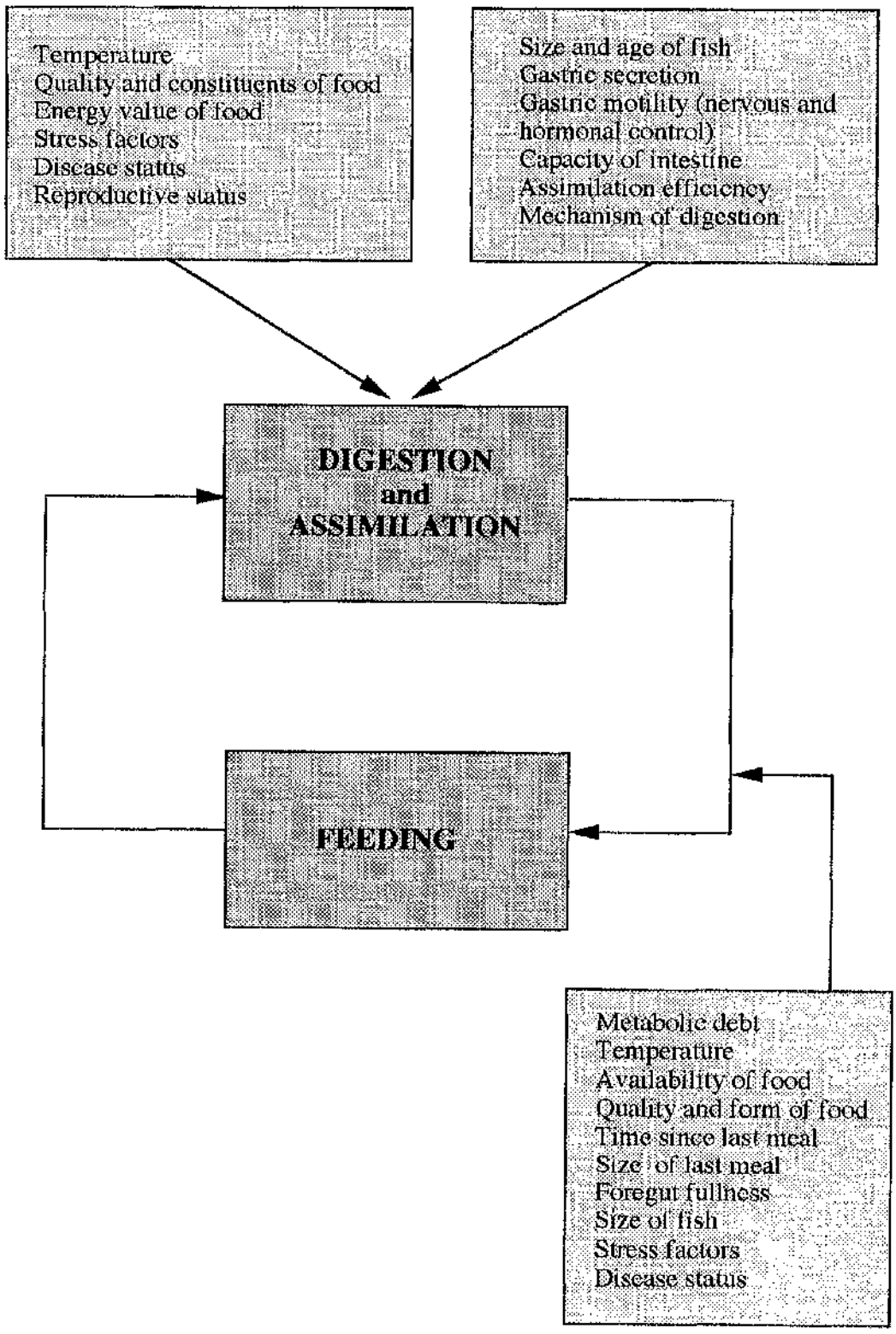


Figure 3. 1 Flow chart showing factors influencing feeding and digestion in fish

growth. This may be mediated by adrenaline or adrenergic releases stimulated by stress, which are known to reduce the blood supply to the digestive system. Disease status may also be an important factor in the nutrition and growth of fish. For example, *Schistocephalus solidus* infection in the three-spined stickleback, *Gasterosteus aculeatus*, is known to have an adverse affect on the nutrition, growth and metabolism of the infected fish (Arme and Owen, 1967; Walkey and Meakins, 1970; Lester, 1971; Pennycuick, 1971a,b,c,d; Pascoe and Mattey, 1977; Tierney, 1994). Infection with the tapeworm *Bothriocephalus acheilognathi* has been reported as causing sluggishness and emaciation in farmed fish (Hoole, 1994). Low level infection of various salmonid fish with the tapeworm *Eubothrium salvelini* has been shown to have a deleterious effect on growth and survival of the fish (Boyce, 1979; Hoffmann *et al.*, 1986; Bristow and Berland, 1991). In addition, increasing levels of severity of infection of Atlantic salmon with the tapeworm *Eubothrium crassum* has been shown to have a negative correlation with the condition factor and gonadal development in the fish (Dorucu, 1996).

Feeding, digestion and absorption in fish are interrelated and this may obscure to some extent the role of each in influencing the way in which energy is distributed between assimilation, maintenance, activity and growth. However, by altering experimental conditions it is possible to determine the feeding rate, digestion rate and assimilation efficiency under specific conditions. In this way, judgement can be made on the conditions which give the maximum efficiency of utilization of food.

3. 1. 3 Research aims

A number of feeding experiments were carried out to gain insight into aspects of feeding and digestion in turbot and to explore the adaptability of these features under different conditions. In particular, the study had the following objectives:

1 To examine the gross structure of the alimentary tract of juvenile turbot and relate this to the function. To compare the structures of fish fed on a natural diet and those fed on an artificial diet and assess whether changes in feeding rates, feeding patterns and acclimation temperature have any affect on the length of the alimentary tract.

2 To examine the general histology of the alimentary tract of turbot and determine if any adaptive changes are made in response to different temperatures or feeding regimes.

3 To examine the fine structure of the absorptive epithelium of the alimentary tract of turbot, a carnivorous fish, and compare with other fish species and also observe any differences that occur between starved and fed turbot.

4 To determine the feeding/ingestion rates of fish held under various feeding regimes and at different acclimation temperatures.

5 To study the rate of passage of food through the alimentary tract and examine the effect of temperature and feeding regime on the time taken.

6 To determine the pH of different regions of the gut and observe the condition of the gall bladder at different phases of the digestive cycle.

7 To investigate the influence of different dietary proteins, carbohydrate, temperature and different feeding regimes on the assimilation efficiency of turbot.

3. 2. MATERIALS AND METHODS

3. 2. 1 Feeding experiments

3.2.1.1 Introduction

A large group of feeding experiments were designed to examine the effects of acclimation temperature, feeding level and frequency of feeding on the feeding rate, absorption efficiency, respiration rate, growth rate, conversion efficiency, condition and body composition of juvenile turbot. The morphology and histology of the alimentary tract was also examined to discover if the different experimental factors resulted in changes at this level. The overall design of the experiments is described here but some specific details are explained in other sections of this work. The basic experimental design is depicted in Figure 3.2 and explained here.

3.2.1.2 Experimental design

Eighty-six juvenile turbot, aged six months, were obtained from Ardtoe and acclimated to laboratory conditions at 10°C under a 12 h light - 12 h dark regime, following the procedures described in Chapter 2. The fish were fed to satiation twice daily with WFA7 pellets for a period of three weeks. Feeding occurred at 1000 h and 1500h or as close to those times as possible. After this period of acclimation, the fish were separated into a control group of ten fish (which were used to determine the initial properties of the fish) plus four experimental groups of twelve fish held under the conditions described for a period of ten weeks (Table 3.1). Each group of fish was held in a 54 litre polystyrene tank. The tanks were fitted with charcoal filtered Eheim pumps and regular maintenance of the tanks was carried out as described in Chapter 2.

3.2.1.3 Feeding procedure

A preliminary experiment was carried out over a one week period to define the maximum feeding rate and hence the level of feeding that approximated to satiation for fish held

Table 3.1 Summary table of experimental conditions during feeding experiment.

Group	Acclimation Temperature	Period of Acclimation	Feeding regime
One	10°C	Ten weeks	Fish fed twice daily to satiation
Two	10°C	Ten weeks	Fish fed to satiation every four to five days
Three	10°C	Ten weeks	Fish fed twice per day on reduced ration
Four	20°C	Ten weeks	Fish fed twice daily to satiation
Five	10°C	Three weeks	Fish fed twice daily to satiation (control - representing initial condition of fish)

under laboratory conditions. Four groups of six fish held in 54 litre tanks at 10°C were fed to satiation either once, twice, three or four times per day at 0900, 1200, 1500, and 1800 h. According to de Groot, (1971), turbot mainly feed during the day with stomach filling showing the greatest peak in the morning plus a secondary peak in the afternoon. He also stated that feeding ceases at night and for this reason only a daytime feeding schedule was used in this study. It was found that satiation occurred at two meals per day for juvenile turbot held under the laboratory conditions specified and so it was decided to offer these meals at 1000 h and 1500 h for the duration of the main feeding experiments.

The following day's food ration was removed from -20°C storage and thawed overnight in a refrigerator. The food for each tank was weighed before and after feeding to determine the amount consumed. Groups fed to satiation were offered food until rejection, whereupon any remaining food was quickly removed from the tank and added to the leftover food for weighing. A reduced ration of 1.5 % body weight per day was given to group 3. This was determined from the weight of fish in the tank and was re-estimated every fortnight after morphometric measurements had been re-assessed. From these data the feeding rates and amount of food consumed per group of fish per fortnight could be determined and related to the growth rate (see Chapter 4) of the fish under the different experimental conditions.

3.2.1.4 Morphometric measurements

Morphometric measurements were assessed as described in Chapter 2.2 for each of the five groups of fish. Measurements, including the weight and length of the fish, were taken at the start and end of the experiment and at approximately two-weekly intervals throughout this period. At the end of the experiment, the fish were sacrificed and further measurements were

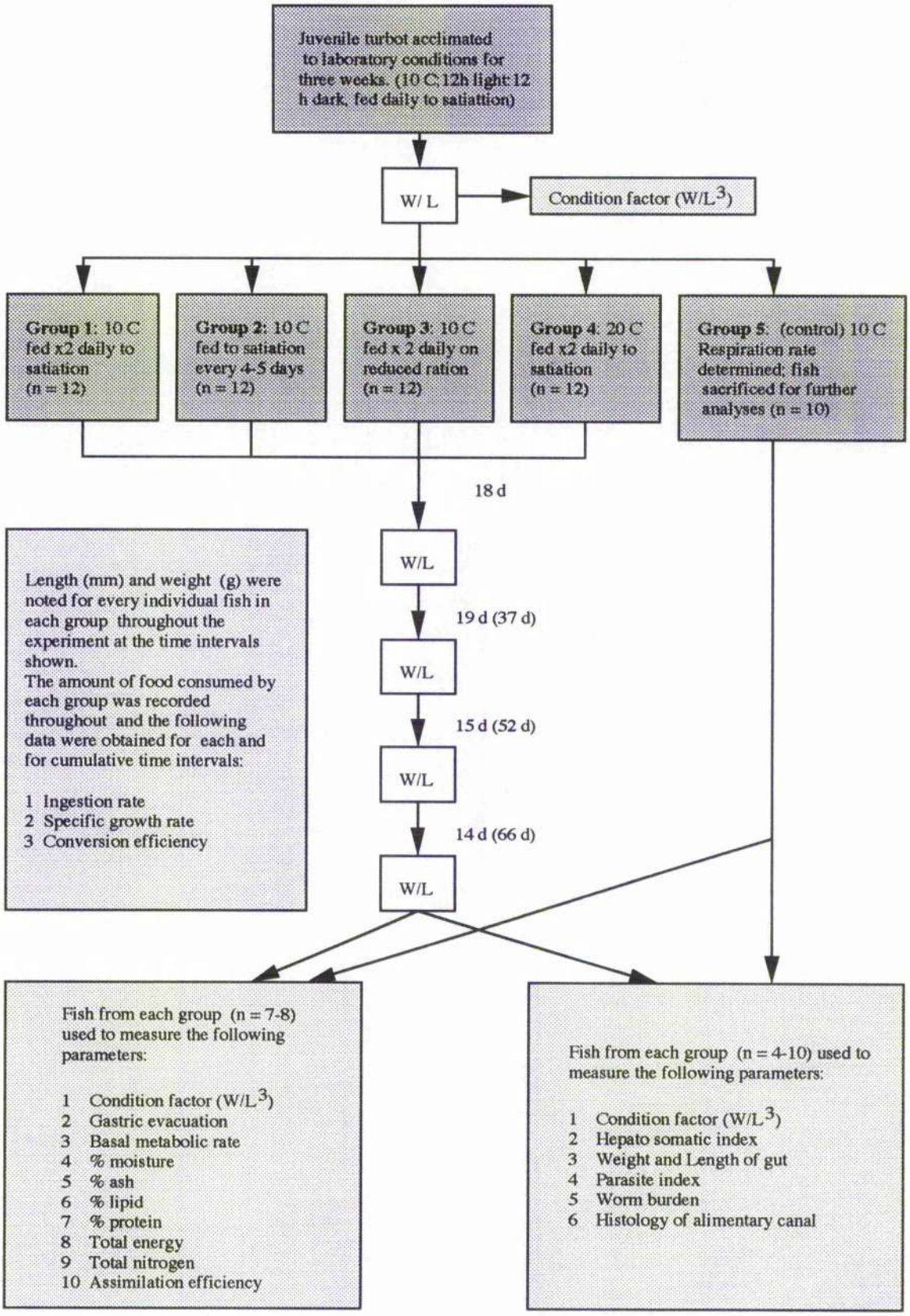


Figure 3.2 Flow chart depicting experimental design of main feeding experiments.

taken including the weight of the liver, the number, length and weight of tapeworms present to give information on the parasite status (see Chapter 6) and length and weight of the alimentary tract.

3.2.1.5 Chemical analyses

Fish carcasses and liver tissue from each group of fish were dried to a constant weight and prepared for chemical analysis as described in Chapter 2 sections 2.6-2.8. The following components were determined for each group: percentage moisture, percentage ash, percentage lipid, total protein, total nitrogen and total calories.

3.2.1.6 Determination of the effect of temperature and feeding regime on the length of the alimentary tract.

The relative length of each part of the alimentary tract and the total length of the tract from the oesophagus to the anus were assessed after three weeks for the control fish and at the end of ten weeks for the experimental fish.

Braber and de Groot (1973b) expressed the length of the alimentary tract of turbot (extending from the buccal-pharyngeal cavity to the anus, inclusive) as a percentage of the total body length. They found the length of the alimentary tract to be approximately equal to the body length in fish ranging from 5-70 cm. In their work, the buccal-pharyngeal cavity represents approximately 23-25% (≅ 24%) of the total body length in fish of ≤ 250 mm in length and 25-32% (≅ 27.7%) of the body length of fish of 250-650 mm in length. In the present work the length of the alimentary tract includes the segment from the oesophagus to the anus. However, comparisons can be made with Braber and de Groot if an estimation of the total length of the alimentary tract including the buccal-pharyngeal cavity is made based on their figures.

3.2.1.7 Parasite status

The condition of each fish was examined and the presence or absence of tapeworms in the alimentary canal was noted. The number of worms per host was recorded and the morphometric measurements of each worm were assessed as described in Chapter 2.3. These data are discussed in Chapter 6.

3.2.1.8 Gross anatomy and histology of alimentary tract

The alimentary tracts of three fish fed twice daily to satiation and maintained under laboratory conditions at 10°C for three weeks were removed for examination of gross morphological structure. The guts were dissected out, opened longitudinally and the anatomical

features observed. Samples from each group of fish were preserved and serial sections of the alimentary tract were prepared for histological examination as described in Chapter 2.10.

3.2.1.9 Fine structure of the epithelium of the alimentary tract

Samples were obtained from fed juvenile turbot after an initial three week acclimation period to laboratory conditions. Another group of fish had food withheld for two weeks and then samples were taken. These specimens were prepared for electron microscopical examination as described in Chapter 2.10.

3. 2. 2 Rate of passage of food through the alimentary tract

Three different methods were employed to examine the rate of passage of food through the alimentary tract. Firstly, independent of the main feeding experiments, a group of thirty-three fish were acclimated to laboratory conditions at 10°C and fed to satiation on WFA7 pellets for a period of three weeks. Food was withheld from them for four days and then the fish were fed voluntarily to satiation before killing groups of fish at various time intervals after feeding. The fish were killed by spinal severing, weighed, the gut quickly removed, opened and the location of the food in the alimentary tract observed.

For the second approach, fish from each group of the main feeding experiment were maintained on their specific feeding regime for the duration of the experiment and then force-fed the relevant % body weight of an artificially prepared diet containing chromic oxide, an inert dye which is not absorbed and which is readily detectable in the faeces (see Chapter 2 for details). As this procedure followed the experimental feeding pattern, it entailed Group Two fish having a longer period (4 days) without food than the other four Groups which were fed daily. The fish were observed every hour until first faeces appeared after feeding and then at 2-4 h intervals until the last faeces appeared.

Thirdly, fish from each Group were maintained under specific feeding regimes for the duration of the test after which food was withheld for 48 hours, the fish were then force-fed the chromic oxide diet and the time taken for the first and last faeces to appear was observed.

3. 2. 3 Determination of fluctuations in lumen pH in the alimentary tract during different phases of the digestive cycle

It is often the case that the pH of the substrate incubation media used in histochemical studies of enzymes in tissue sections may bear little resemblance to that existing *in vivo* (Western, 1971). The fluctuations of lumen pH of each of the separate regions of the alimentary canal during the different phases of the digestive cycle were determined so that the roles of the

enzymes possibly involved in the digestive sequence might be assessed more realistically and to complement histochemical studies and so give an insight into the digestive sequence in turbot.

Fish of weight range 33-45 g were obtained from Ardtoe and were maintained at 10°C for three weeks. The fish were fed daily to satiation with WFA pellets. The fish were then deprived of food for four days and then allowed to feed to satiation on WFA pellets before they were sacrificed at various time intervals after feeding. Group size was three and only fish observed to be healthy feeders were used for this investigation. Fish were killed by spinal severing, weighed and the gut was quickly removed, opened and analyzed. The approximate lumen pH was assessed using universal indicator test papers, followed by confirmatory tests with BDH 'narrow range' indicator papers.

3.2.4 Determination of feeding rates

The ingestion rates of fish held under the various conditions of the main feeding experiment were assessed over the ten week period. The amount of food consumed by each group was carefully measured and expressed as a percentage of the body weight per day. All fish were measured for weight and length at approximately two weekly intervals throughout the experiment.

3. 2. 5 Determination of assimilation efficiency

The assimilation efficiency for the five groups of fish held under various feeding conditions was measured using the indirect chromic oxide method (detailed in Chapter 2.7) to determine if there was any variation in the digestive efficiencies due to differences in acclimation temperature, size of fish, frequency of feeding or quantity of food given.

At the end of the 10 week acclimation period, food was withheld for 48 hours after which the fish were weighed and then force-fed 2 % of their body weight of the test chromic oxide diet. This procedure was repeated three times giving three samples from each group.

3.2.5.2 Digestibility trials

The indirect chromic oxide method was also employed to examine the assimilation efficiency of fish fed diets containing a supplement of different protein sources (see Chapter 2 for details of chemical analyses). Digestibility trials were conducted to compare the assimilation efficiency of turbot fed on diets containing Pruteen (ICI, Macclesfield) as the major protein source with those fed on diets containing other proteins and also to test the sparing effect of a carbohydrate (kaolin) on the digestive efficiency of Pruteen. Pruteen is obtained from the fermentation of the micro-organism *Methylophilus methylotrophus* (see Chapter 2.4. for composition of Pruteen and other diets used).

Juvenile turbot in the weight range 4-12 g were obtained from the fish farming unit at Hunterston where they had been weaned on to a WFA pelleted diet and maintained at ambient temperatures of approximately 10°C. The fish were transported to the laboratory where they were acclimated to laboratory conditions and fed twice daily to satiation on WFA pelleted diet and maintained at 10°C for three weeks before the feeding trials commenced. The fish were divided into three groups of between 12 - 16 fish. Each group of fish was deprived of food for 48 h then individual fish were force fed 2 % body weight of the test diet, using a 5 ml plastipak syringe. The tanks were observed regularly and faecal matter promptly removed and dried and then chemical analyses carried out to determine the chromic oxide, protein and energy content of the faeces.

The basic test diet consisted of 68 % sprats; 10 % vitamin binder; 2 % chromic oxide and 20 % of one of the following protein sources: Pruteen; fish meal; casein or albumin. To test the sparing effect of carbohydrate on the digestive efficiency of the protein source, the 20 % Pruteen or 20% fish meal component was progressively substituted with 5, 10, 15 or 20 % kaolin (starch). Samples from each diet were dried and analyses carried out to determine their chromic oxide, protein and energy content. The assimilation efficiency was then calculated using the following equation:

$$\text{Assimilation efficiency } \% = 100 \times \left(1 - \frac{Cr_2O_3 : \text{Nutrient ratio in food}}{Cr_2O_3 : \text{Nutrient ratio in faeces}}\right)$$

Details of the chemical analyses employed and the concept behind the indirect chromic oxide technique are detailed in Chapter 2.8.

The energy value of each of the food samples was also determined using the Gallenkamp bomb calorimeter. Analyses of the water, ash, and lipid content were also made (for details of methods see Chapter 2).

The condition factor, conversion efficiency and specific growth rate of the fish are dealt with in Chapter 4; the prevalence and intensity of infection with the tapeworm, *Bothriocephalus scorpii*, and the parasite index are detailed in Chapter 6.

3.2.6 Data analyses

All statistical analyses were performed using Minitab version 10.0 for Windows. Parametric tests included T-tests and non-parametric tests used included Mann-Whitney U-tests. Statistical significance has been accepted at a probability level of less than 0.05. Means are presented ± their standard deviation unless otherwise stated.

3.3 RESULTS

3.3.1 Structure of the alimentary tract of turbot

3.3.1.1 General morphology

The overall organization of the alimentary tract of juvenile turbot from the oesophagus to the anus is shown in Figure 3.3. The relative lengths of the different regions of gut are noted in the legend. The alimentary tract is relatively short which is fairly typical for carnivorous fish and is differentiated into a large oesophageal region and large stomach, pyloric valve, pyloric caeca, relatively short intestine, rectal valve and rectum. The buccal-pharyngeal cavity is not included in the photograph but is relatively large (representing approximately 24 % of the length of the alimentary tract in turbot ranging from 40-700 mm, Braber and de Groot, 1973b). Turbot possess a muscular tongue and a series of large gill rakers with four double rows of small teeth just above the entrance to the muscular oesophagus. According to Braber and de Groot (1973b), the buccal and pharyngeal cavity combined with the oesophageal and stomach region of the turbot represents roughly 50 % of the alimentary tract.

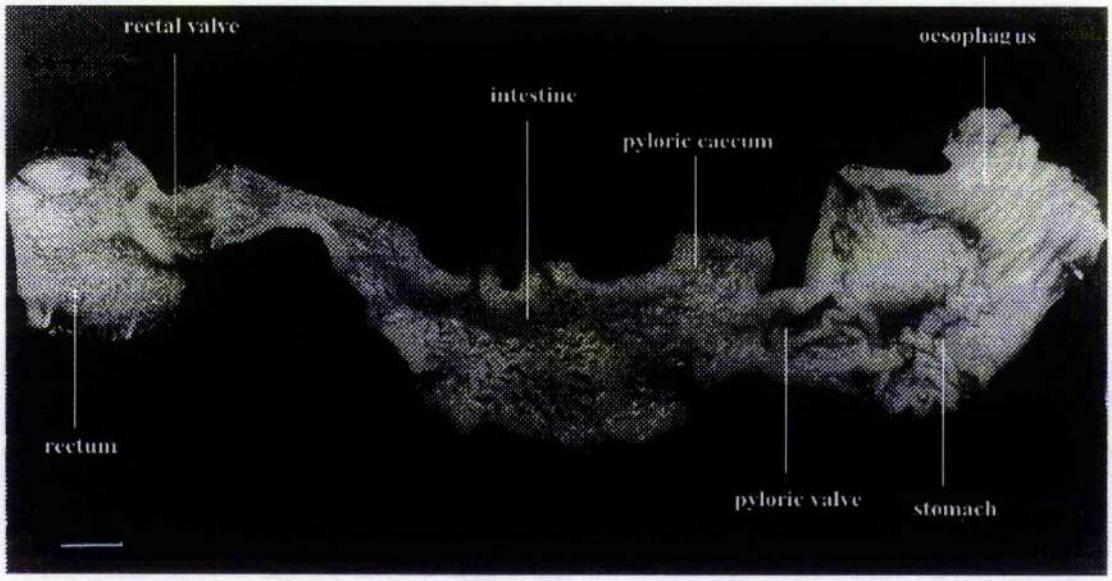


Figure 3.3 View of alimentary tract of juvenile turbot; dissected open from oesophagus to rectum. The buccal-pharyngeal cavity is not included in the figure but measured 30 mm in length. Scale bar: 10.0 mm. (Oesophagus 10 mm; stomach 17 mm; intestine 53 mm; rectum 13 mm).

No demarcation between the oesophagus and stomach is visible until the alimentary tract is opened longitudinally whereupon the large muscular oesophagus is seen to be thrown into deep muscular folds which probably help to propel captured food into the stomach. The stomach is

also large but slightly less muscular than the oesophagus and consists of a moderate number of convoluted infoldings. A pyloric valve leads into the intestine where two outgrowths are found at the anterior end. These are often referred to as pyloric caeca (only one is visible in Figure 3.3 as the opposite pyloric caecum is lying in shadow). The pyloric caeca are histologically similar to the intestine and for this reason would be more aptly named intestinal caeca. The intestine is relatively short and simple and is thrown into a loop. A rectal valve separates the intestine from the rectum which is short, broad and moderately muscular and has a convoluted lining.

Scanning electron microscopy revealed that the lining of the intestine was thrown into many complicated convolutions which increase the surface area for digestion and absorption (Figures 3.3 and 3.4). At higher magnification the apices of individual mucosal epithelial cells were seen to possess many long microvilli (2-5.0 μm), (Figure 3.5, 3.6, 3.7).

3.3.1.2 Length of alimentary tract as percentage of body length

The length of the alimentary tract from the oesophagus to the anus when expressed as a percentage of the total body length of the fish was found to be 68.2 ± 2.6 % in fish acclimated to 10°C for three weeks and fed to satiation twice daily on WFA pellets (Figure 3.8, Group 5). Following 10 weeks acclimation to different feeding regimes and temperatures, the equivalent lengths were found to be 65.5 ± 4.32 % (fish fed to satiation twice per day and acclimated to 10°C), 59.8 ± 3.2 % (fish fed to satiation once every 4-5 days and acclimated to 10 °C), and 59.1 ± 3.1 % (fed daily on a reduced ration and held at 10°C). Fish held at 20°C and fed to satiation twice per day were found to have a relatively shorter alimentary tract (53.9 ± 1.9 %) compared with fish at the start of the experiment (68.2 ± 2.6 %) (Mann Whitney U - test significant at P = 0.0059).

An estimate of the total length of the alimentary tract including the buccal-pharyngeal cavity was made based on results by Braber and de Groot, (1973b). The alimentary tract was found to be 88.8 ± 3.2 % of the body length in control fish (Group 5). This is significantly less than the 99 ± 7.8 % found by Braber and de Groot for fish captured from the North Sea (size range 40-150 mm, n = 60) (Mann Whitney U-test, significant at P = 0.0029), suggesting that fish fed on an artificial diet developed shorter alimentary tracts although stock differences may occur.

3.3.1.3 General histology of alimentary tract

The alimentary tract of the turbot consists of four main layers which may vary in form and function in the different regions of the tract. Proceeding inwards towards the lumen these are: 1) the outer serosa; 2) the muscularis externa, consisting of a thin outer layer of longitudinal muscle and a thicker layer of circular muscle; 3) the sub-mucosa or connective

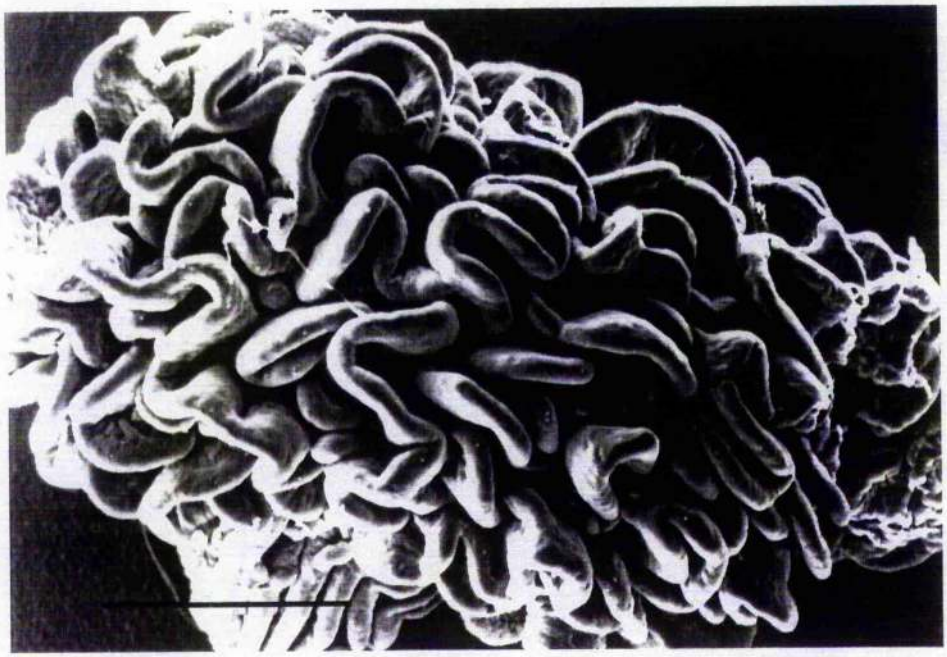


Figure 3.4 SEM of anterior intestine of juvenile turbot showing complicated folding of mucosal epithelium. Scale bar: 1.0 mm.

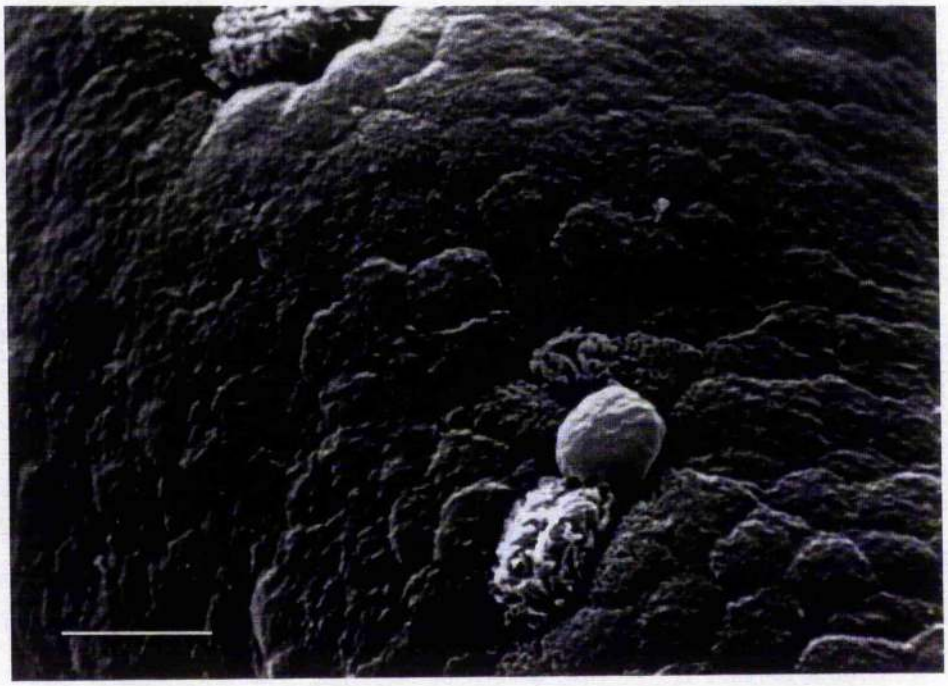


Figure 3.5 SEM of mucosal surface of anterior intestine of juvenile turbot showing apices of individual mucosal epithelial cells with many microvilli. Scale bar: 10.0 μm.

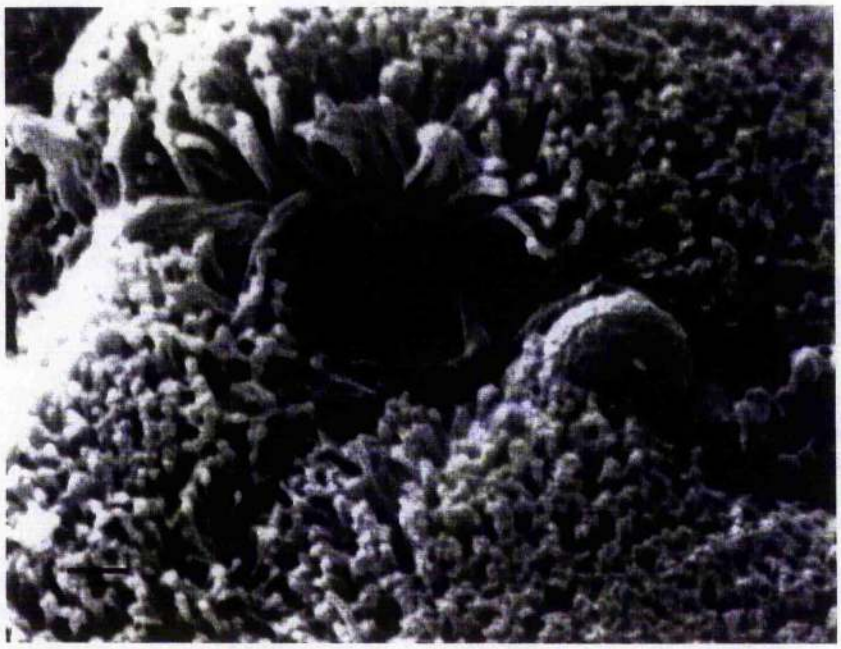


Figure 3.6 SEM showing tips of microvilli at apex of mucosal epithelial cells of anterior intestine. Gap where presumably mucous cell has been secreted; mucous droplet nearby. Scale bar: 1.0 μm.

Figure 3.7 SEM showing longitudinal view of columnar epithelial cells with long microvilli (4.0 μm). Scale bar: 10.0 μm.

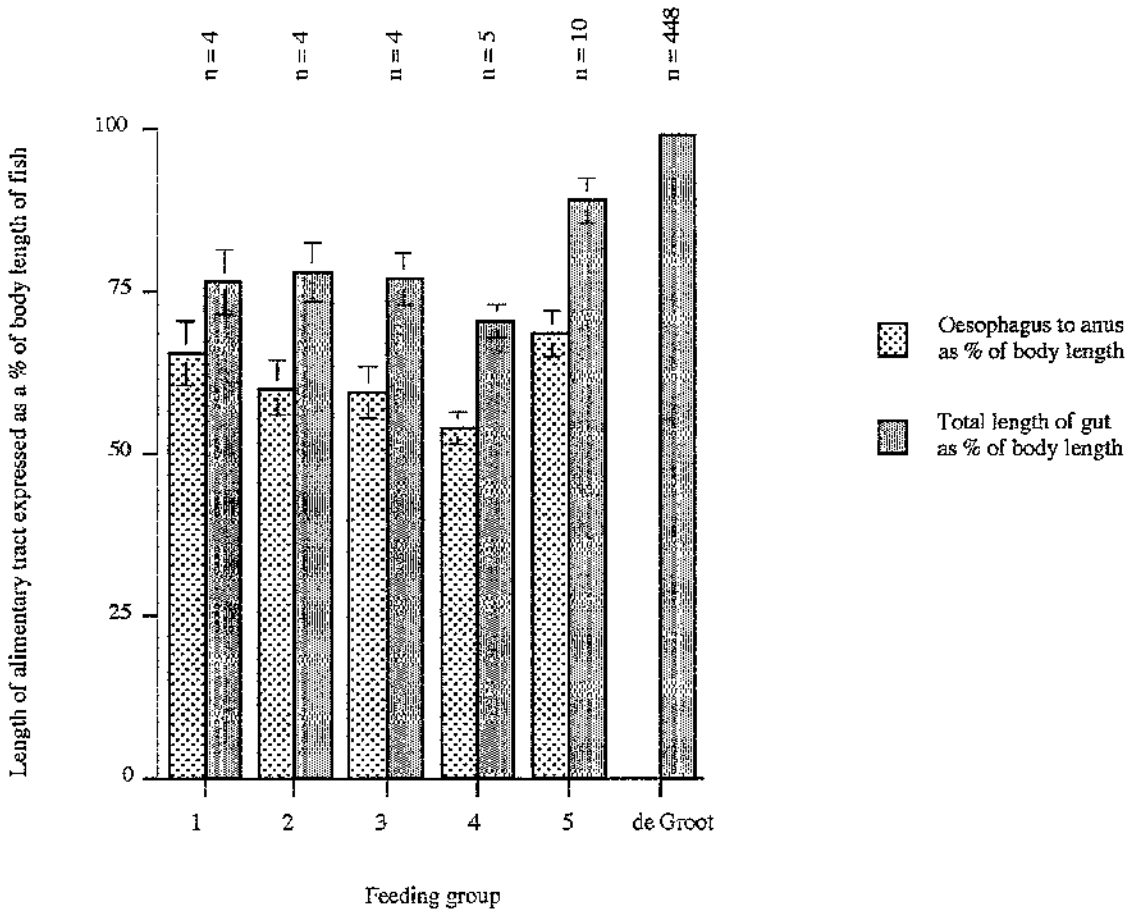


Figure 3.8 Length of the alimentary tract expressed as a percentage of the total body length of the fish. Groups 1-4 represent farmed, experimental turbot. Group 5 represents turbot on arrival from WFA, Ardtoe. Data from de Groot (1971) represents wild caught turbot.

tissue layer and 4) the mucosa, consisting of a layer of columnar epithelium. The mucosal epithelium varies in composition throughout the tract whilst the sub-mucosa and muscularis vary in thickness in the different areas of the gut, most likely in accordance with the function of each particular region.

i) Oesophagus

The mucosal layer of the oesophagus is thrown into large thick folds and consists of columnar epithelium with numerous, mucous-secreting cells. The mucous cells are so numerous in some areas that there appear to be few normal epithelial cells (Figures 3.9 and 3.10). Very few rodlet cells (see Chapter 6) are observed in the most anterior region of the oesophagus but as the oesophagus nears the stomach there appears to be little differentiation in cell composition between the two. The muscularis externa consists of a thin (30 μm) outer layer of longitudinal muscle and a thicker layer of circular muscle ranging in depth from 200-300 μm (Figure 3.10).

ii) Stomach

The stomach mucosa consists of tall columnar epithelial cells and contains many rodlet cells along with a number of typical mucous cells. The sub-mucosa has longitudinal muscle fibres scattered in places and the muscularis externa contains well developed circular muscle (150 μm) and longitudinal muscle (150-180 μm) (Figures 3.11 and 3.12).

iii) Pyloric caeca

Two pyloric caeca are situated at the anterior end of the intestine and histologically they resemble the intestine (Figure 3.13). The muscularis externa is narrower (80 - 100 μm) than in the oesophagus and stomach and the sub-mucosa is also less developed. The mucosal epithelium is thrown into many folds and consists of tall columnar epithelial cells with a brush border consisting of relatively long microvilli ranging from 3 - 5 μm in length (Figure 3.14).

iv) Intestine

The mucosal epithelium of the intestine is thrown into many filariform folds. The muscularis externa are narrower (80 - 130 μm) than in the oesophagus and stomach, as is the sub-mucosa (Figures 3.15 and 3.16). The most striking feature of the intestine is the brush border of very long microvilli (Figure 3.17). These range in length from 2.5 - 5.5 μm depending on the location in the intestine but there appears to be a general reduction in size from the anterior intestine to the posterior intestine and rectum. A muscular rectal valve leads from the intestine into the rectum.

v) Rectum

The mucosal epithelium of the rectum is supplied with numerous mucous cells as well as some rodlet cells and is similar to that of the intestine in possessing a brush border of long microvilli (2.5 - 4.0 μm) indicating an absorptive function (Figure 3.18).

3.3.1.4 Fine structure of the epithelium of the alimentary tract

The epithelium of the anterior portion of the intestine from the alimentary tract of starved and fed juvenile turbot was examined using transmission electron microscopy.

i) Fed turbot

The epithelium of the anterior intestine consisted mostly of columnar absorptive cells (Figures 3.19, 3.20). Mucous cells and rodlet cells were also observed (Figure 3.19 and Chapter 6). The most striking feature of the mucosal epithelium was the apical brush border consisting of very long microvilli (Figures 3.19, 3.20). Each microvillus is covered by a trilaminar cell membrane. The microvilli show a dark cap (capitulum of Krementz and Chapman, 1975) and have a glycocalyx or fuzzy coat of mucopolysaccharides (Figure 3.19, 3.20, 3.21). The cytoplasm of the microvilli contained longitudinal fibres and occasionally these were observed projecting into the terminal web (Figure 3.22). Pinocytotic vesicles were frequently observed between the microvilli and in the terminal web area (Figure 3.23). Apart from the pinocytotic vesicles and occasionally the filamentous extensions of the microvilli and some smooth endoplasmic reticulum, the terminal web is generally devoid of organelles and inclusions (Figures 3.19, 3.20, 3.23).

Junctional complexes consisting of tight junctions, intermediate junctions and desmasomes were observed in the apical region between neighbouring absorptive epithelial cells (Figures 3.20, 3.23).

Mitochondria were observed throughout the cells except in the terminal web area and appeared to be more concentrated in the basal part of the cell (Figure 3.24). In the basal region of the absorptive cells the lateral plasma membranes form lamellar infoldings similar to those identified by Stroband and Debets (1978) and Noaillac-Depeyre and Gas (1973) (Figure 3.24).

The nucleus of the columnar absorptive cells was basally located and contained a prominent nucleolus. Rough and smooth endoplasmic reticulum was observed throughout the cells (Figures 3.23, 3.24, 3.25).

ii) Fasted turbot

The mucosal epithelium of turbot fasted for 14 days was dramatically different from that in fed fish in certain respects. There were clear signs of cellular degeneration with sloughed cells visible in the lumen (Figure 3.26). Large supranuclear vacuoles containing cellular debris were observed in most sections (Figures 3.27, 3.28). Mitochondria were observed in abundance in the apical region of the cells and these contained dense granules dispersed throughout the matrix (Figures 3.28, 3.29). Similarities between fasted and fed fish were observed, especially in the long microvillus processes. Pinocytotic vesicles were noted between the microvilli and in the terminal web in fasted turbot as well as in the fed fish (Figures 3.30, 3.31).

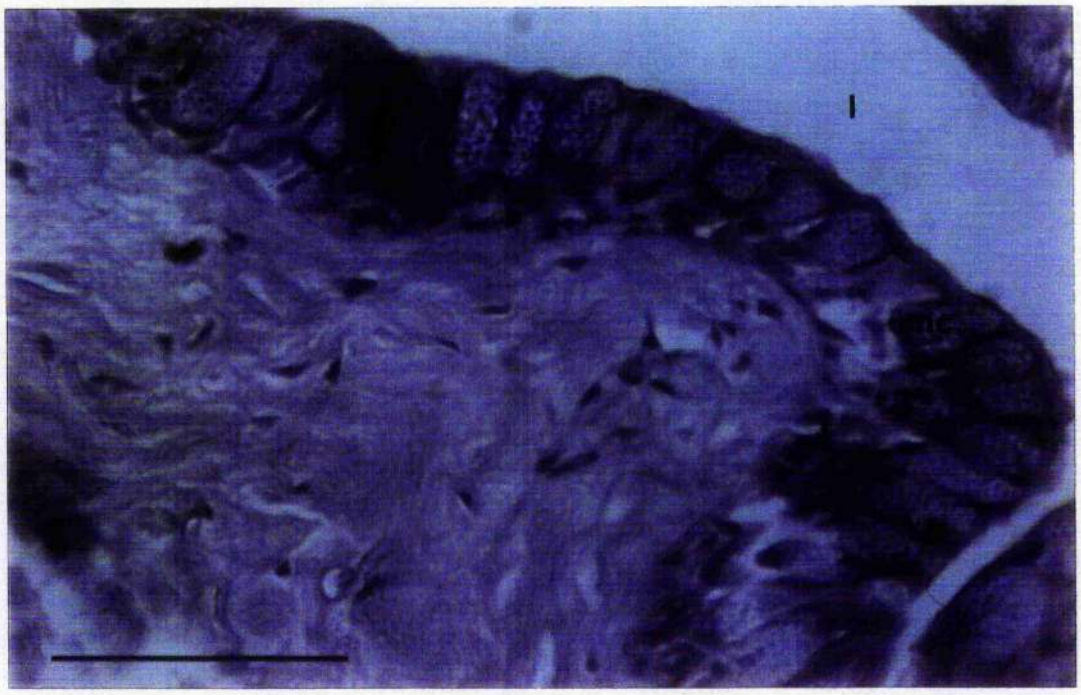


Figure 3.9 LM of transverse section through oesophageal region showing lumen (l) and numerous mucous secreting cells (mc). Scale bar: 50.0 μm.

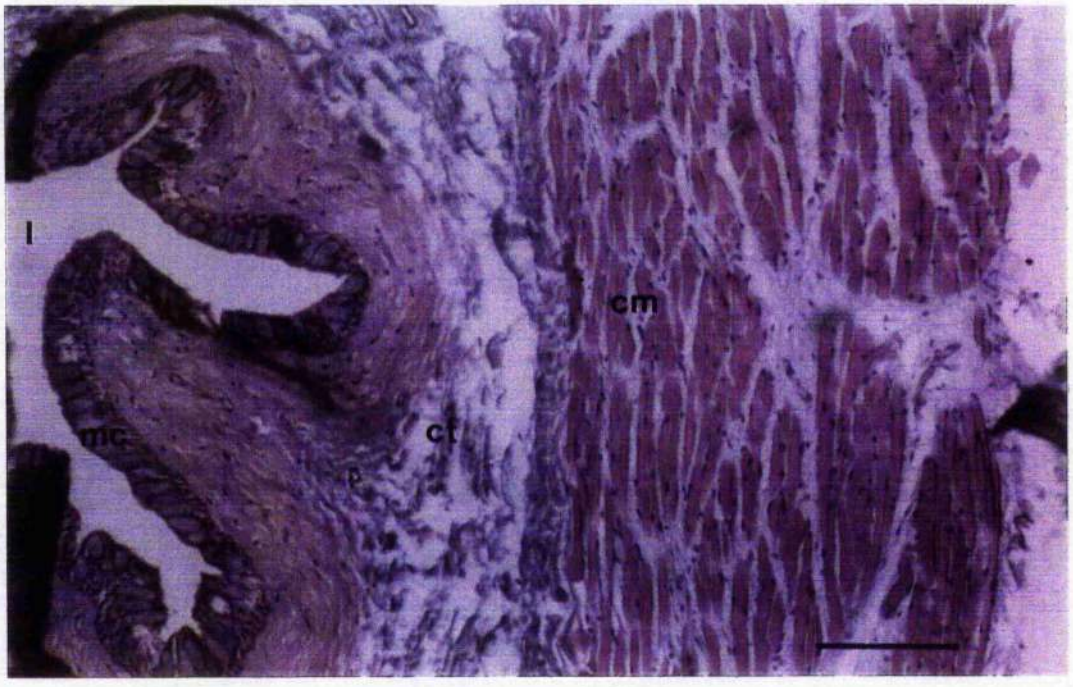


Figure 3.10 LM of transverse section through oesophagus showing lumen (l), folded mucous membrane with numerous mucous cells, connective tissue layer (ct) and thick layer of circular muscle (cm). Scale bar: 100 μm.

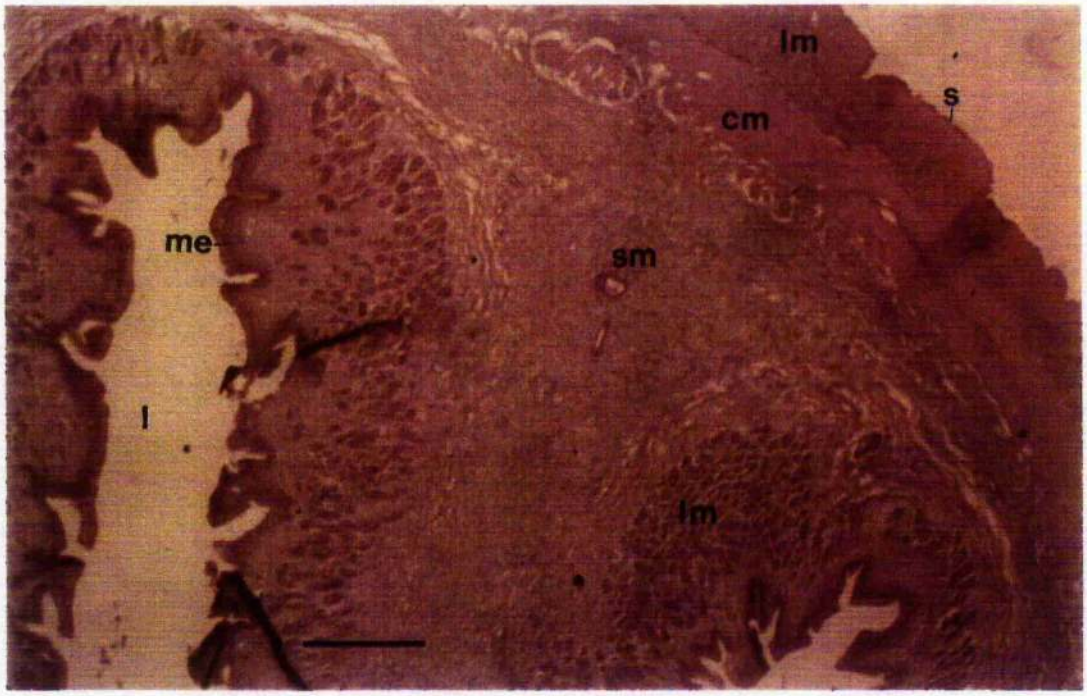


Figure 3.11 LM of transverse section through stomach region showing outer serosa (s); muscularis externa of outer longitudinal muscle (lm) and inner circular muscle (cm); sub mucosa (sm) containing scattered muscle blocks (mb); mucosal epithelium (me) and lumen (l). Scale bar: 200 μm.

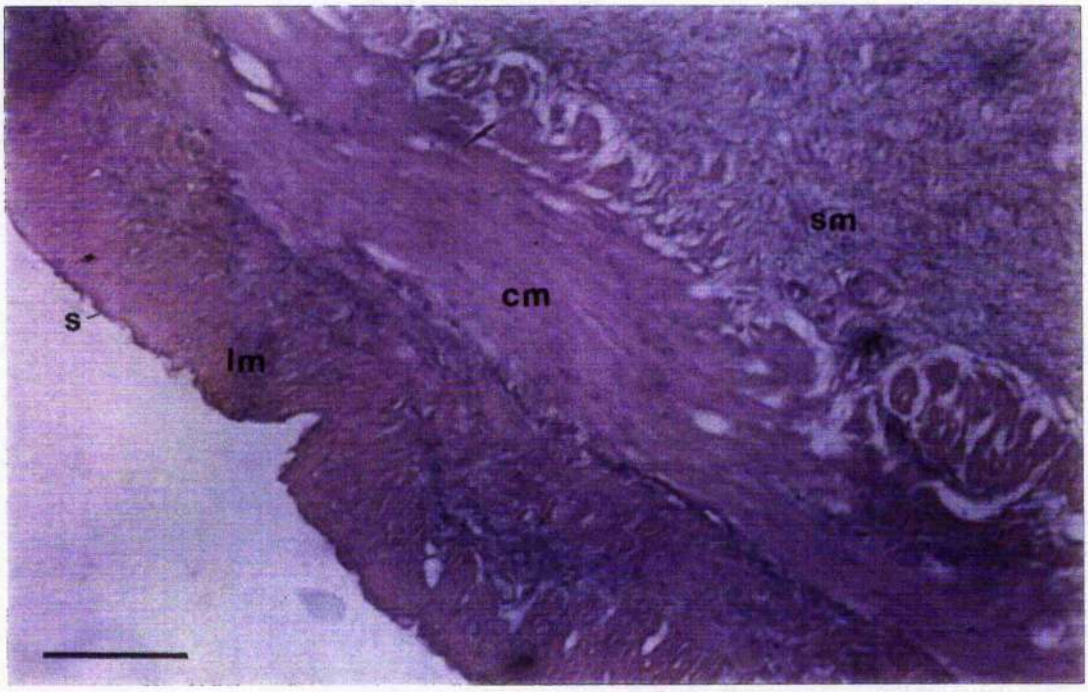


Figure 3.12 LM of transverse section through stomach showing outer serosa (s); thick muscularis externa of longitudinal muscle (lm) and inner circular muscle (cm); and submucosa (sm). Scale bar: 100 μm.

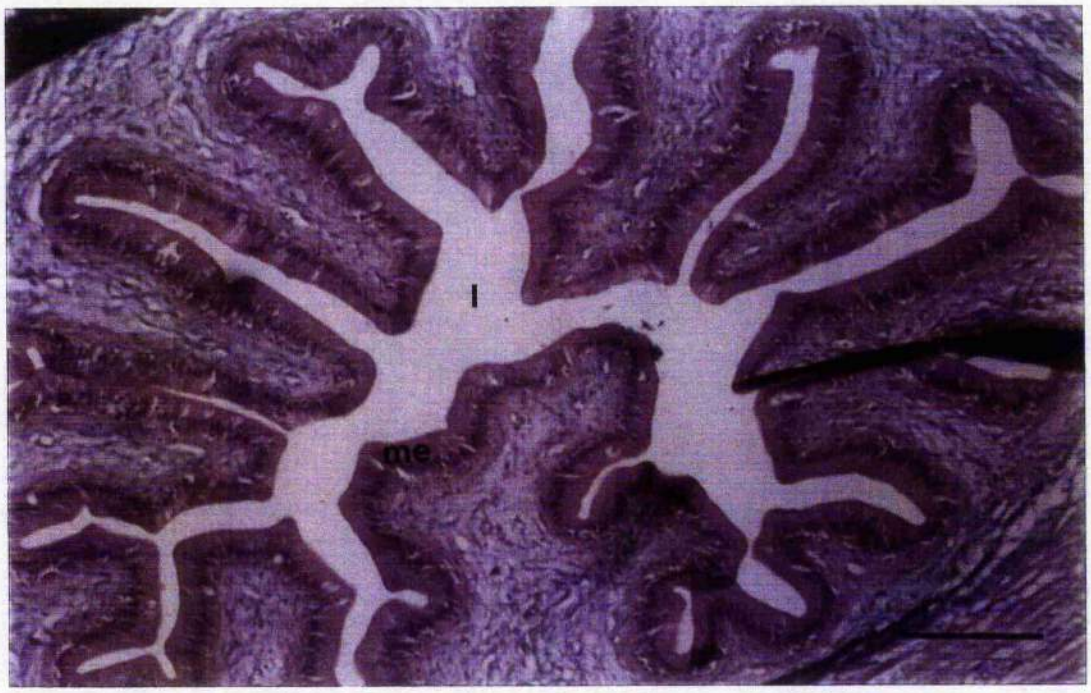


Figure 3.13 LM of transverse section through pyloric caecum showing lumen (l) and mucosal epithelium (me) thrown into folds. Scale bar: 100 μm.

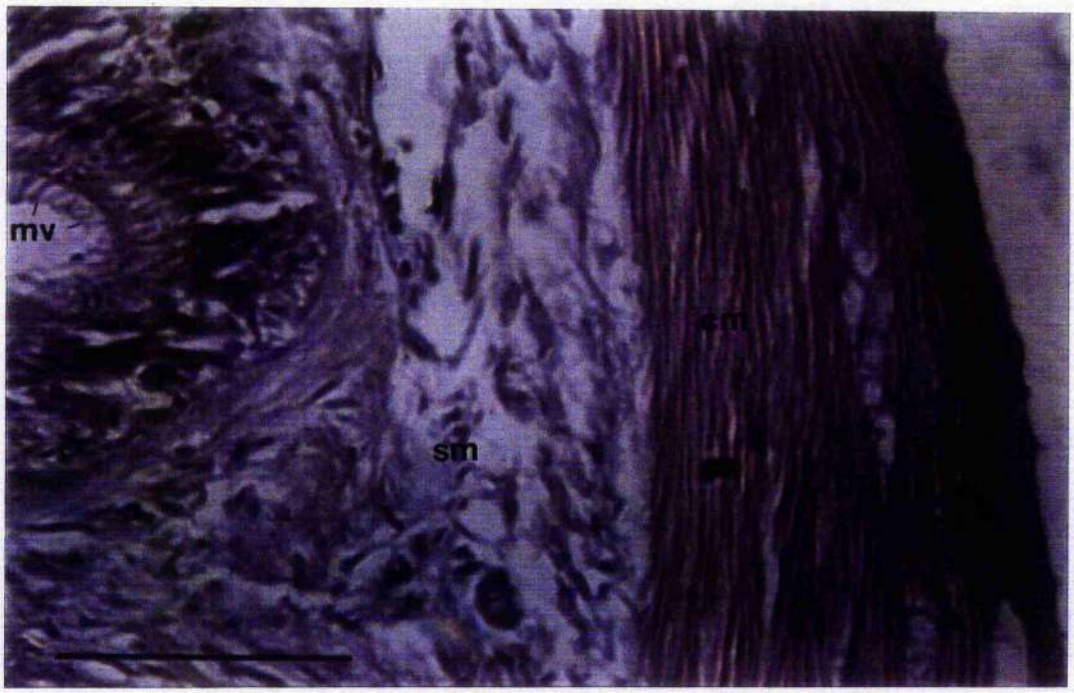


Figure 3.14 LM of transverse section through pyloric caecum showing muscularis externa of outer longitudinal muscle (lm) and inner circular muscle (cm); sub mucosa (sm) and brush border of long microvilli (mv). Scale bar: 50 μm.

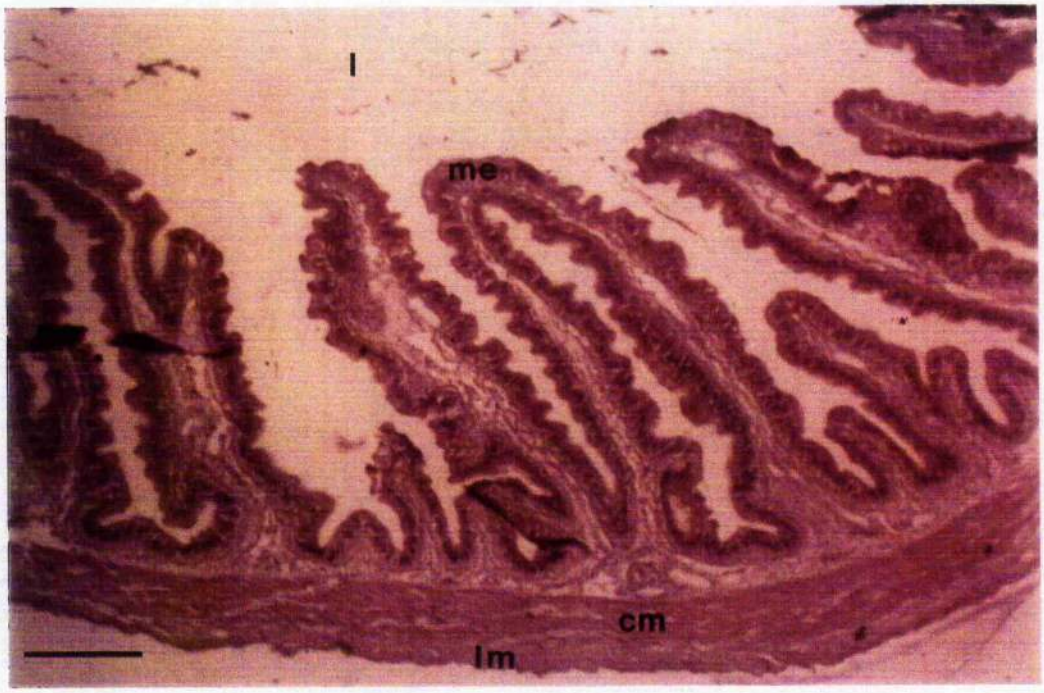


Figure 3.15 LM of transverse section through intestine showing mucosal epithelium (me) thrown into filariform folds. Scale bar: 200 μm.

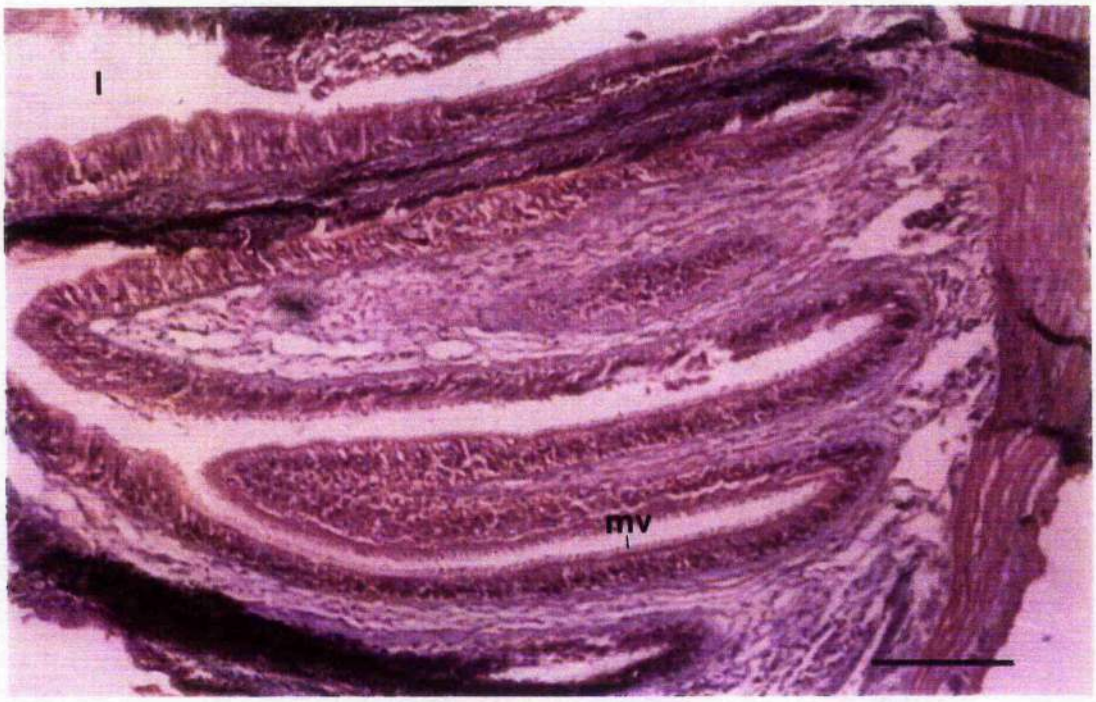


Figure 3.16 LM of transverse section of intestine showing filariform folds and brush border with long microvilli (mv). Scale bar: 100 μm.

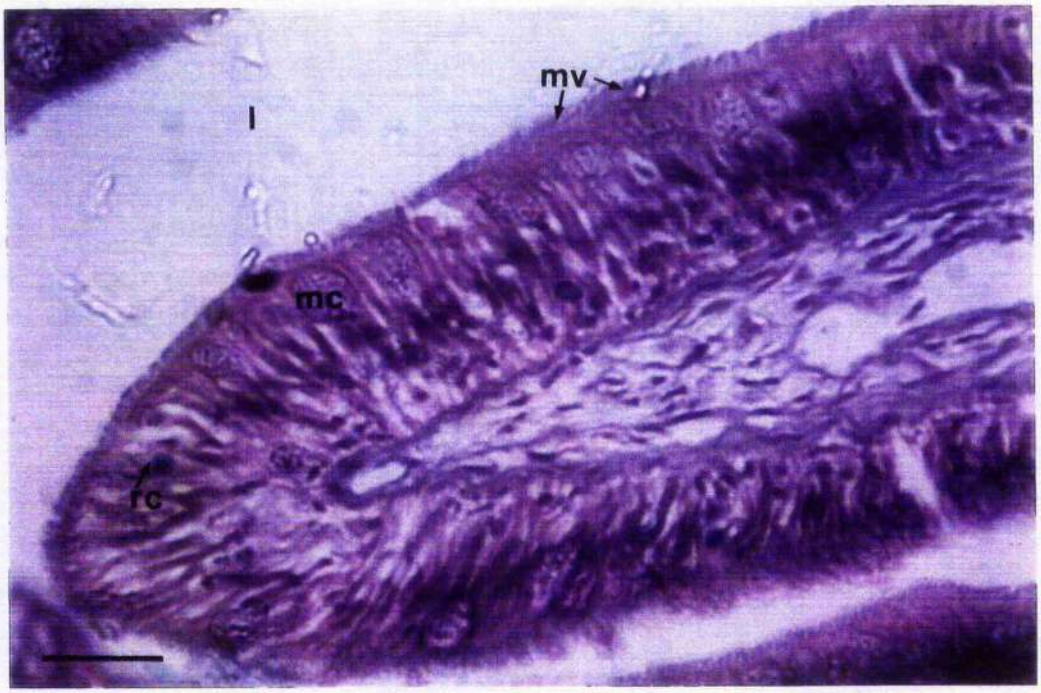


Figure 3.17 LM of transverse section of intestine showing brush border with long microvilli (2.5-4.5 μm). Many mucous secreting cells (mc) and some rodlet cells (rc) are visible in the mucosal epithelial layer. Scale bar: 20.0 μm.

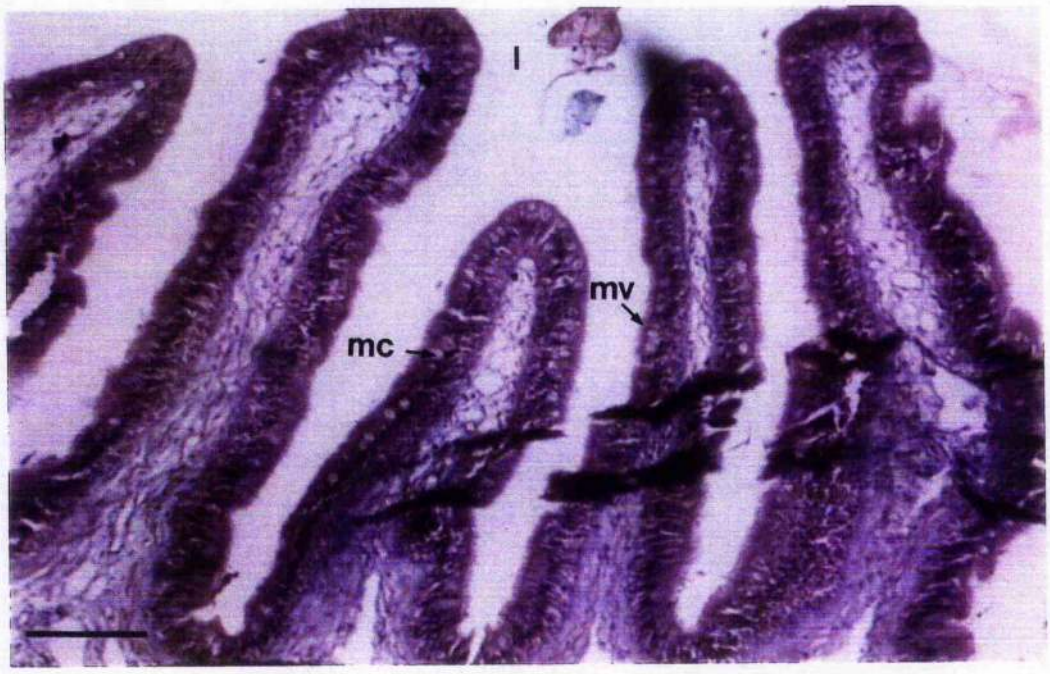


Figure 3.18 LM of transverse section of rectum showing mucosal epithelium thrown into filariform folds. Numerous mucous secreting cells (mc); and a brush border with long microvilli (mv) are visible. Scale bar: 200 μm.

3. 3. 2 Influence of feeding regime and temperature on the daily food consumption and feeding rates of turbot

Although food intake is well regulated in fish, many factors are known to influence the feeding rate of fish. An experiment was carried out to examine the effects of varying the frequency of feeding, the amount of food offered and temperature on the daily food consumption of juvenile turbot over a ten week period. The experimental design is explained in a flow chart (Figure 3.2).

3.3.2.1 Determination of satiation levels in laboratory-maintained turbot

Preliminary studies showed approximate satiation was reached with two meals per day for juvenile turbot in the weight range 10-35 g . Generally, the fish fed well at the 0900 h feed but tended to reject food offered three hours later at noon. Appetites returned by 1500 h but in fish fed a fourth meal, three hours later at 1800 h, food was again rejected. The maximum ingestion rate was quite low at 4.1 % (n = 20) body weight per day during the feeding trial.

3.3.2.2 Influence of frequency of feeding on ingestion rate of turbot

Fish fed to satiation twice daily and acclimated to 10°C for ten weeks had an overall ingestion rate (amount of food consumed per day expressed as a percentage of the body weight of the fish) of 1.69 % for the 10 week experimental period compared with an ingestion rate of 1.04 % for fish fed to satiation once every 4-5 days (Table 3.2). The overall ingestion rates of fish fed on a reduced ration twice per day and those fed to satiation every 4-5 days were quite similar for the 10 week period at 1.14 % and 1.04 %, respectively (Table 3.2).

3.3.2.3 Influence of temperature on ingestion rate of turbot

The overall ingestion rate of turbot fed to satiation twice daily and acclimated to 20°C was 1.93 % compared with 1.69 % for fish fed to satiation twice daily but acclimated to 10 °C (Table 3.2). For the first 18 days of the experiment fish held at 20°C had a lower ingestion rate of 0.94 % compared with 2.6 % for those held at 10°C. However this situation was reversed for the remainder of the experiment with the 20°C acclimated fish showing a much higher ingestion rate than the 10°C acclimated fish (Table 3.2). The initial low ingestion rate for the 20 °C acclimated fish was probably due to the sudden rise in temperature of 10°C.

3.3.2.4 Influence of ration level on ingestion rate of turbot

Fish fed daily to satiation and maintained at 10°C had a higher ingestion rate, as

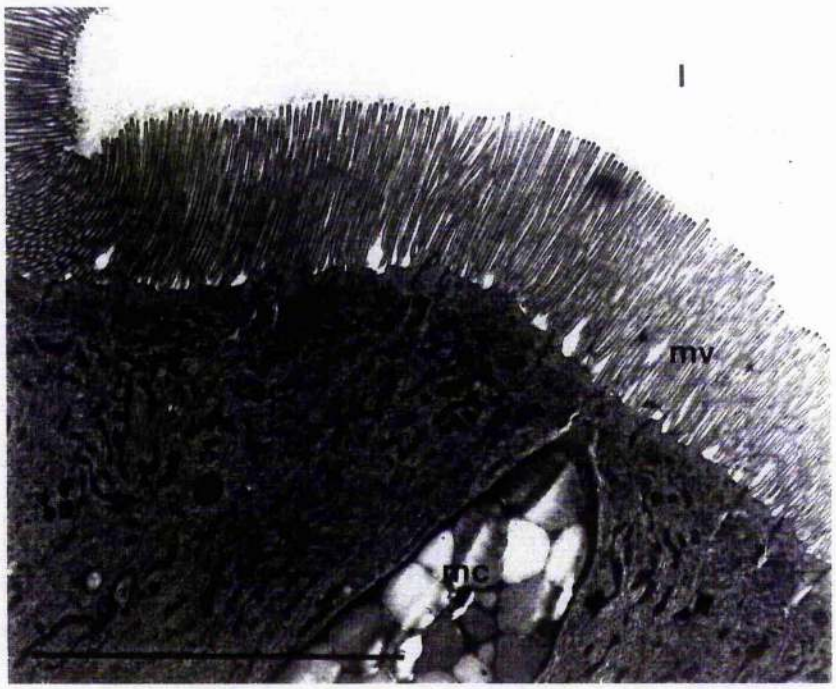


Figure 3.19 TEM of transverse section of mucosal epithelium of anterior intestine of fed turbot showing lumen (l); apical brush border with long microvilli (4.5 μm), absorptive columnar epithelial cells (ec) and mucous cell (mc). Scale bar: 10.0 μm.

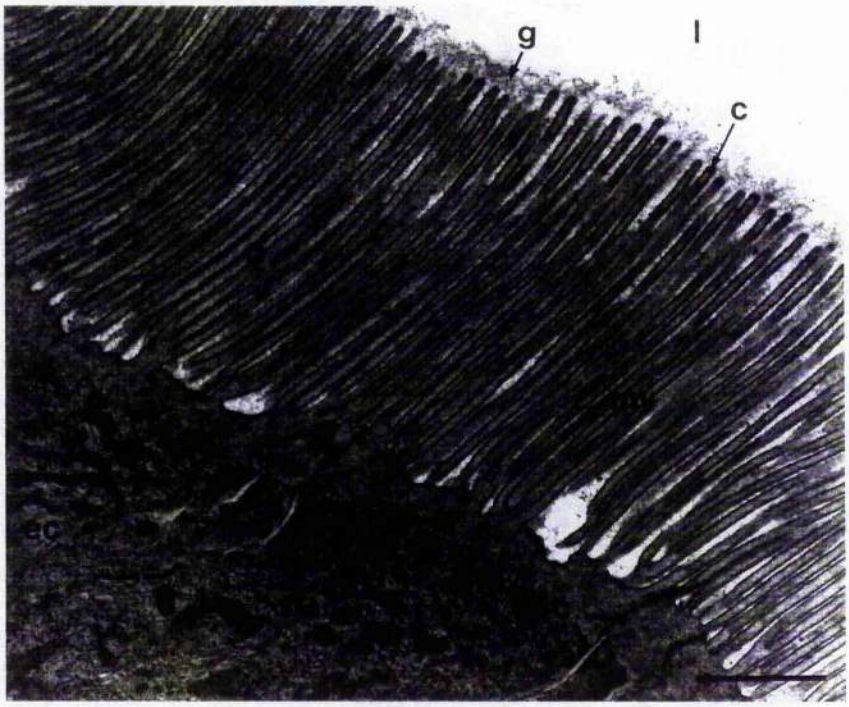


Figure 3.20 TEM of transverse section through anterior region of intestine of fed turbot showing absorptive epithelial cells. Apical microvilli are relatively long (4.7 μm) and display a dark cap (capitulum) and a fuzzy border, glycocalyx (g). Junctional complex (jc) of tight junctions and desmasomes are observed between neighbouring cells. Scale bar: 1.0 μm.

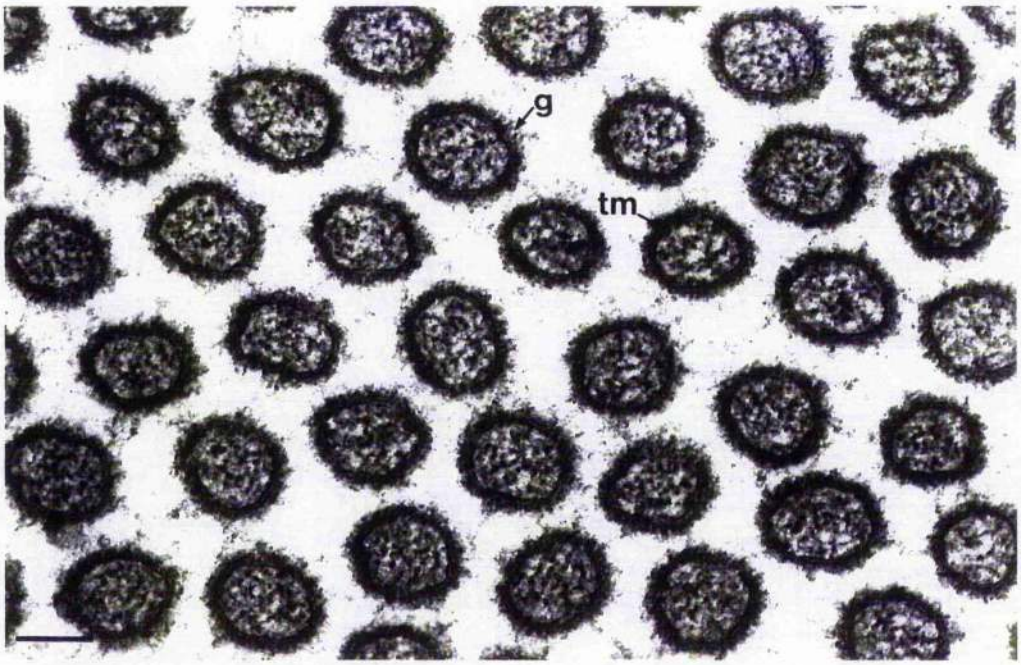


Figure 3.21 TEM of transverse section through microvilli of epthelial cells of anterior intestine showing core filaments, trilaminar cell membrane (tm) and glycoprotein surface layer, glycocalyx (g). Scale bar: 0.1 μm.

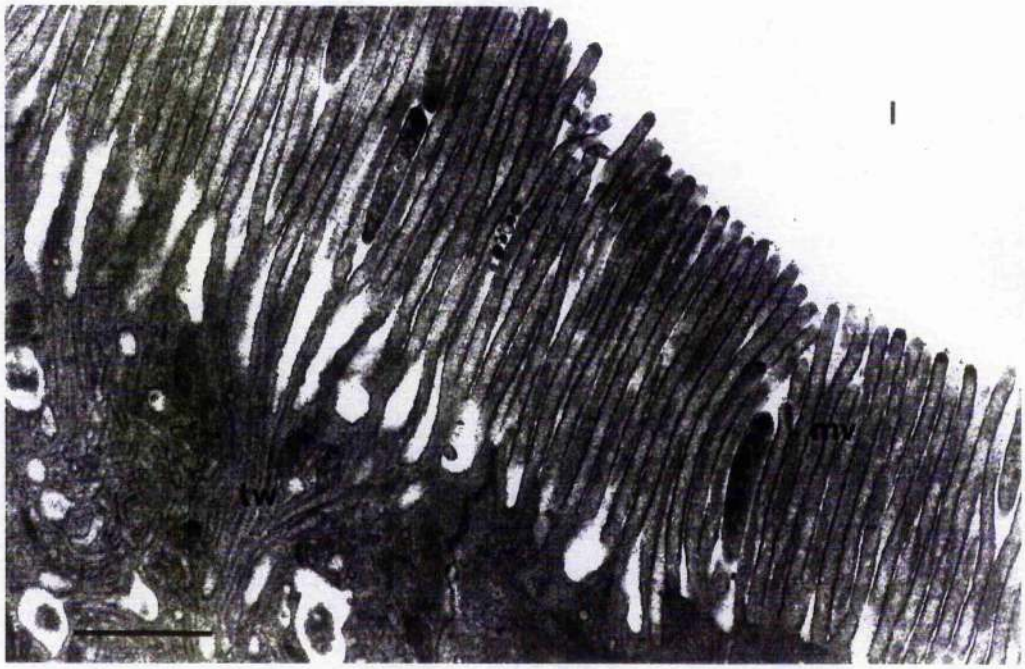


Figure 3.22 TEM of transverse section through anterior intestine showing microvilli (mv) with longitudinal fibrillar structures projecting into terminal web (tw) of epithelial cell. Scale bar: 1.0 μm.

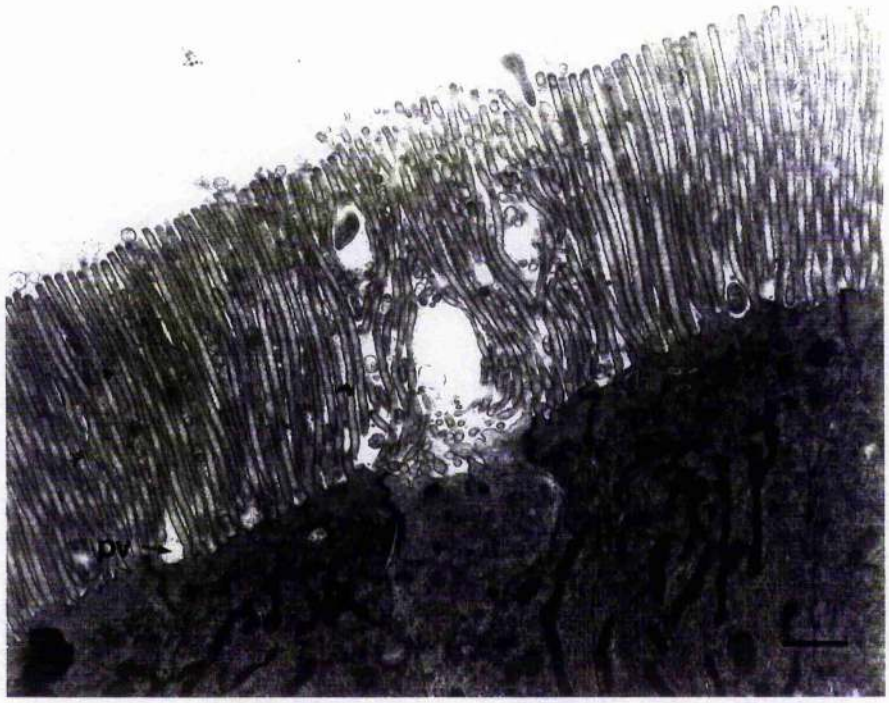


Figure 3.23 TEM of section through anterior intestine showing pinocytotic vesicles (pv) between long microvilli (5.0 μm) and in terminal web (tw). Scale bar: 1.0 μm.

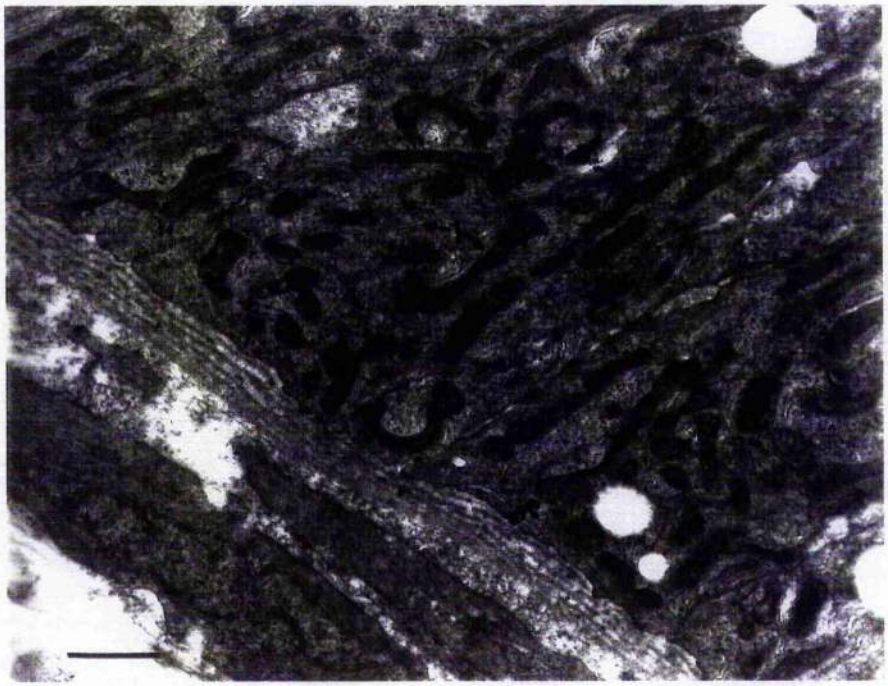


Figure 3.24 TEM of section through basal region of epithelial cell showing laminar infoldings of lateral plasma membrane. Mitochondria (m) are closely connected with lamellar structure (l). Scale bar: 1.0 μm.

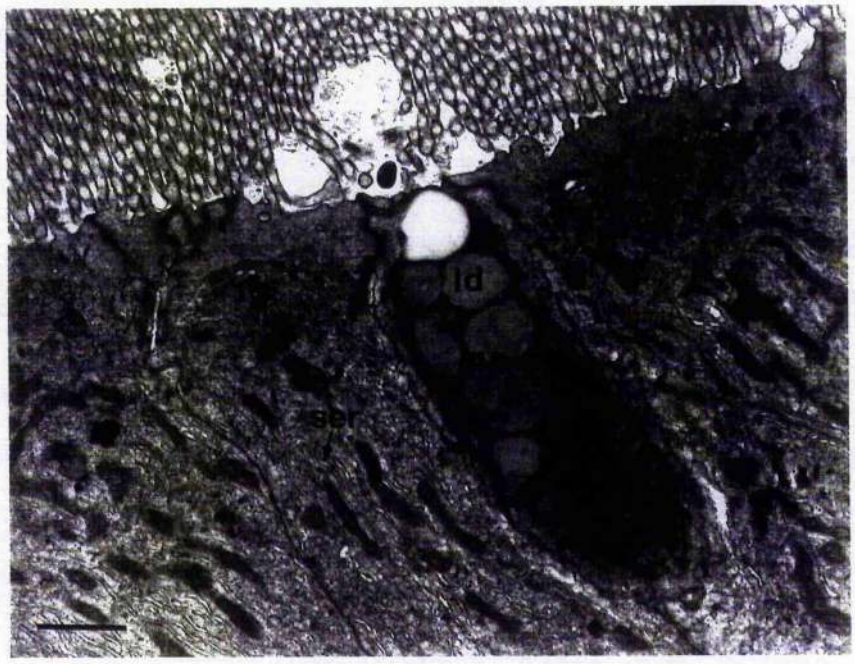


Figure 3.25 TEM of columnar absorptive epithelial cells of anterior intestine. Smooth endoplasmic reticulum (ser) scattered throughout cells. Mucous cell (mc) in process of secreting lipid droplets (ld) by merocrine secretion. Scale bar: 1.0 μm.

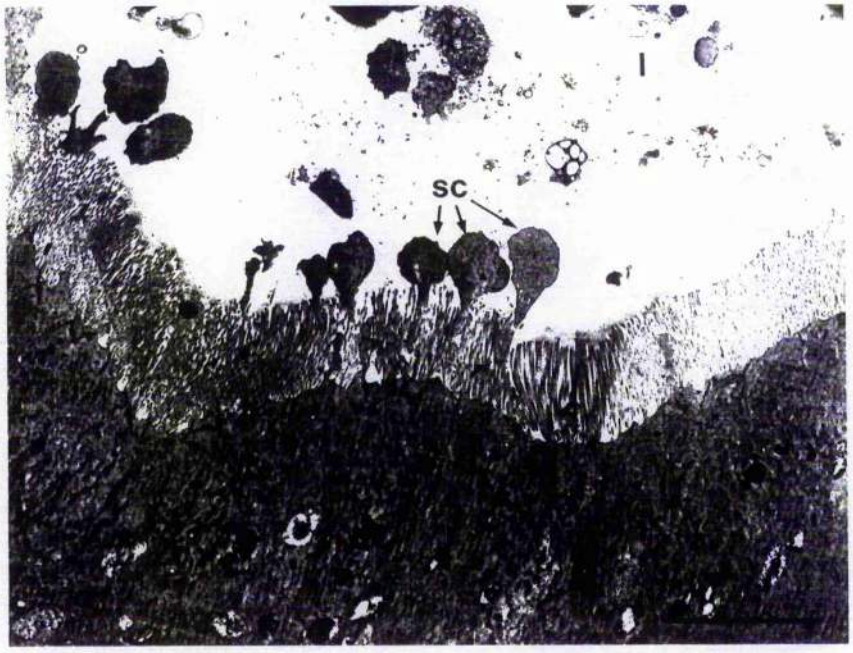


Figure 3.26 TEM of mucosal epithelium of anterior intestine of turbot fasted for 14 d showing degenerative epithelium. Scale bar: 10.0 μm.

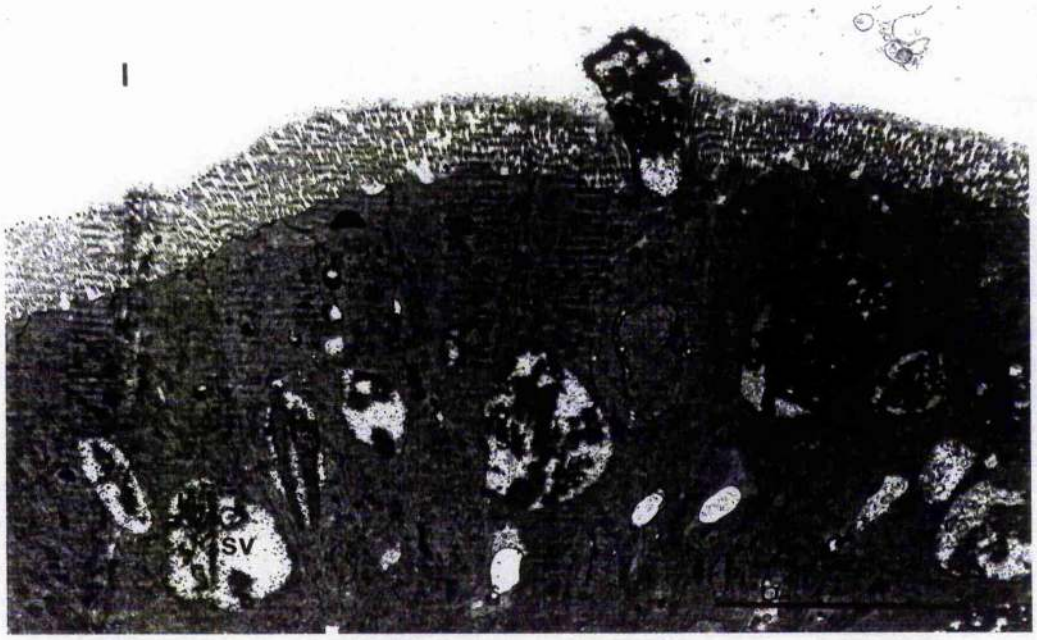


Figure 3.27 TEM of anterior intestine of turbot fasted for 14 d showing large supranuclear vacuoles (sv) containing cellular debris. Scale bar: 10. 0 μm.

Figure 3.28 TEM of anterior intestine of turbot fasted for 14 d showing large supranuclear vacuole (sv) with cellular debris. Many mitochondria (m) observed in apical region of cell. Scale bar: 10.0 μm.

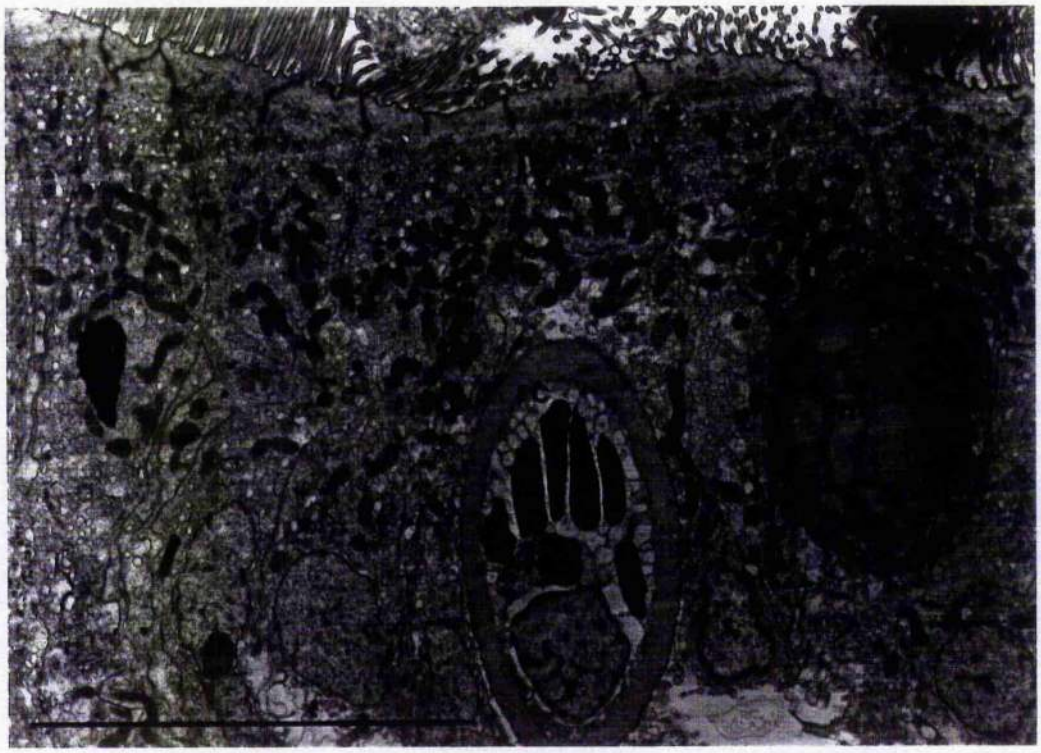


Figure 3.29 TEM of anterior intestine of turbot fasted for 14 d showing many mitochondria (m) observed in apical region of cell. Also shows part of rodlet cell (rc) and mucous cell (mc). Scale bar: 10.0 μm.

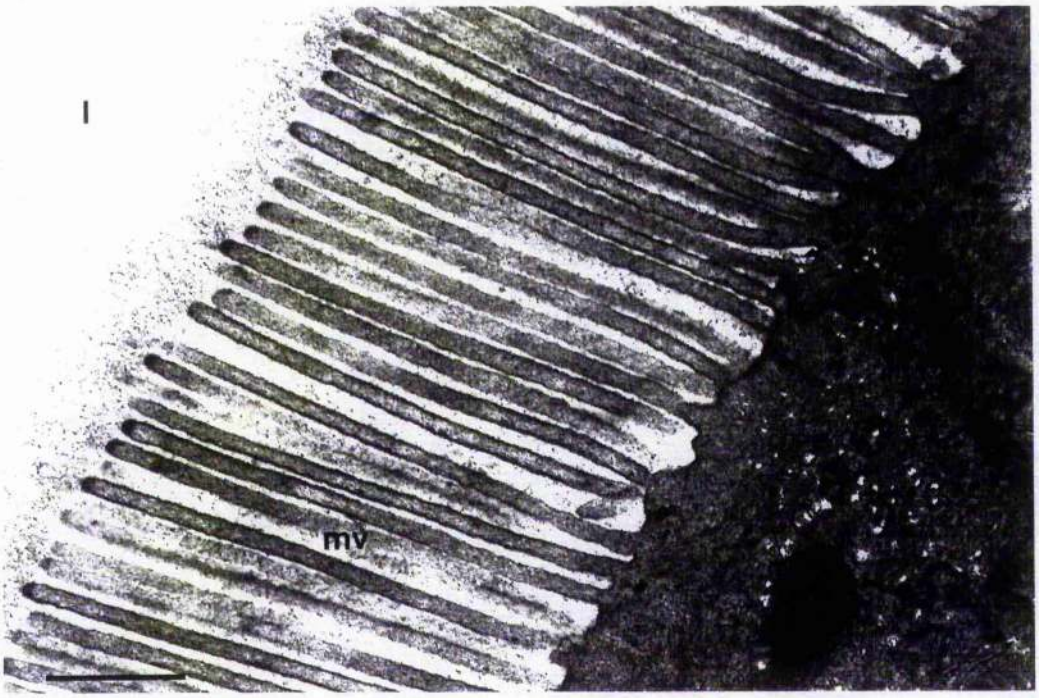


Figure 3.30 TEM of anterior intestine of turbot fasted for 14 d showing pinocytotic vesicles observed between microvilli (mv) and in terminal web. Scale bar: 1.0 μm.

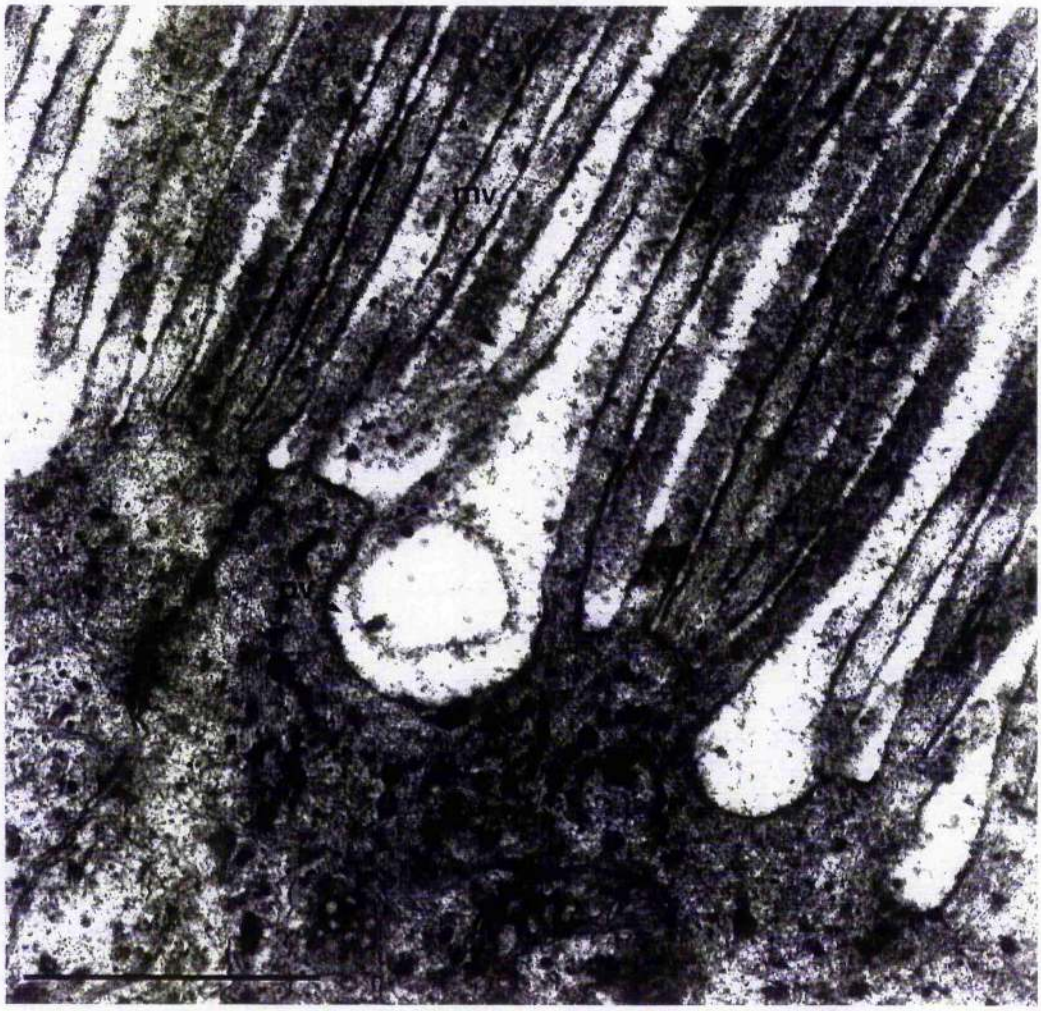


Figure 3.31 TEM of anterior intestine of turbot fasted for 14 d showing pinocytotic vesicles observed between microvilli (mv). Scale bar: 1.0 μm.

expected, than those fed on a reduced ration and kept at 10°C (Table 3.2). Figures 3.32 and 3.33 show ingestion rate against time and cumulative ingestion rate against time for each of the four experimental Groups of fish.

3.3.3 Digestion in juvenile turbot

3.3.3.1 Variations in lumen pH after feeding

The variation in pH of the different regions of the alimentary tract at various time intervals after feeding are given in Table 3.3 and Figure 3.34. At 2 h and 4 h after ingestion of food, the stomach had a pH of 3.7 ± 0.2. This fell to 3.5 at 6 h after ingestion and appeared to level off at 3.5 until 98 h after feeding when there was a slight rise to 3.7. This was followed by a decrease to 2.5 at 170 h (one week) after feeding. However, there was more variation between 98 h and 170 h than at other times. The pH in the pyloric caeca ranged from 7.75 at 2 h after feeding to 9.0 at 30 h post ingestion and fell again to 7.5 at 170 h after feeding. The anterior intestine had an initial pH of 8.2 at 2 h after feeding, rose slowly to 9.0 at 8 h after ingestion and then declined slowly to 8.5 at 98 h with a sharper decline to 7.6 one week after feeding. The contents in the posterior intestine followed a similar slow rise and decline in pH but had slightly lower values than the anterior intestine until one week after ingestion when a pH of 7.6 was recorded. The pH in the rectum fluctuated between values of 8.5 and 9.0 from 2 h to 30 h after feeding and steadied at 8.5 from 48 h to 98 h after feeding but dropped to 7.5 at one week after ingestion of food. Differences in the initial pH values (96 h after feeding) with those 98 h after feeding may possibly be accounted for by the inaccuracies of the technique used.

3.3.3.2 Rate of passage of food through the alimentary tract

Investigations were carried out using three different methods to examine the rate of passage of food through the alimentary tract. Firstly, a number of fish were killed at various time intervals after feeding and the contents of their alimentary tracts were observed (methods 3.2.3). Secondly, fish from the major feeding experiment were fed on their respective feeding regimes for ten weeks at the end of which a meal containing chromic oxide was substituted for the WFA diet. The time taken for the first and last faeces to appear was noted. Thirdly, food was withheld for 48 h from each group of fish at the end of the feeding experiment and the fish were then fed the chromic oxide diet again and time taken for the first and last faeces to appear was noted.

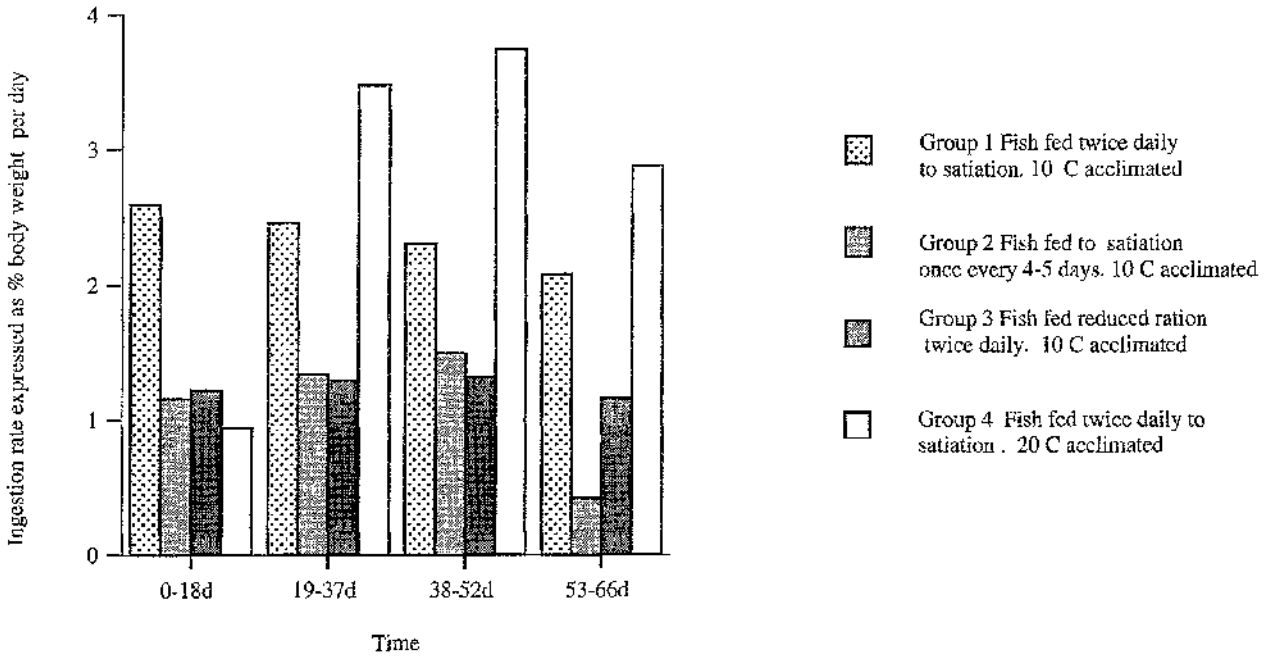


Figure 3. 32 Ingestion rate against time of fish on different feeding regimes

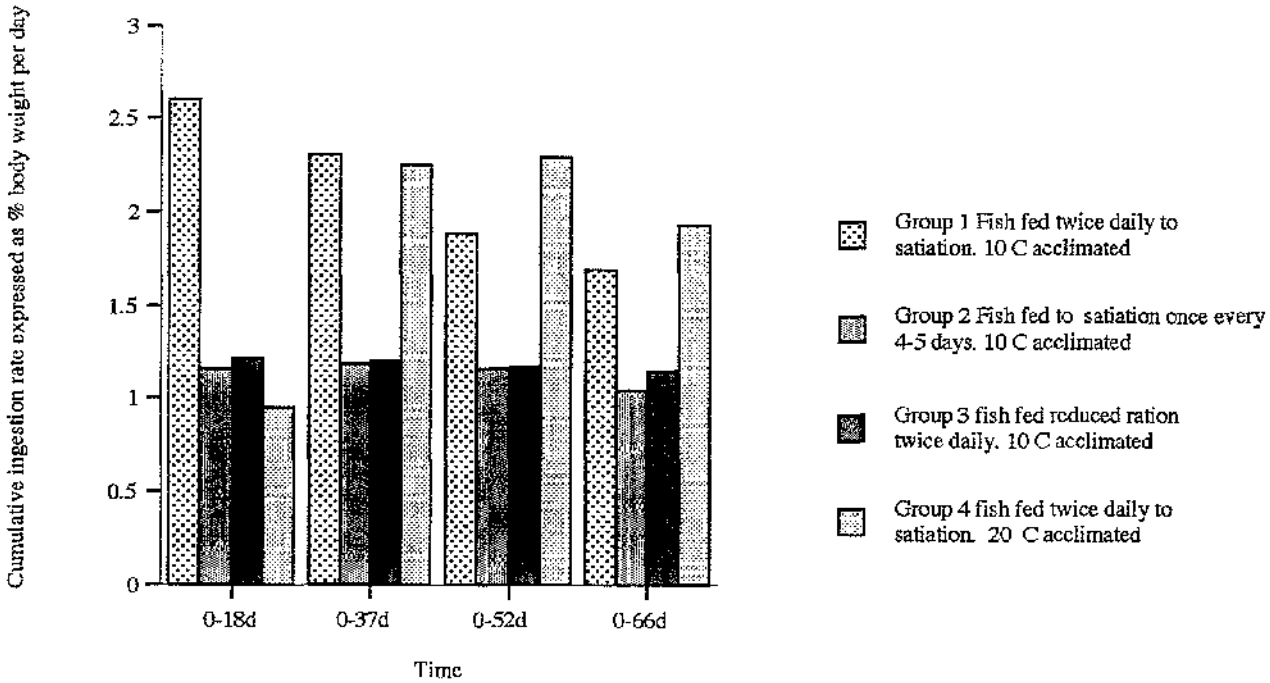


Figure 3.33 Cumulative ingestion rate against time for fish on different feeding regimes

Table 3.2 Ingestion rates of turbot held under various feeding regimes and at different temperatures. The amount of food consumed is expressed as a percentage of the body weight per day during the time period noted. Cumulative ingestion rates are given in brackets for the time periods noted.

	Period of experiment (66 d)			
Feeding/holding conditions	**0-18 d**	**19-37 d (0-37 d)**	**38-52 d (0-52 d)**	**53-66 d (0-66 d)**
1 Satiation rations twice daily acclimated to 10°C	2.60 %	2.46 % (2.3 %)	2.3 % (1.89 %)	2.08 % (1.69 %)
2 Satiation rations once every 4-5 d acclimated to 10°C	1.15 %	1.34 % (1.19 %)	1.49 % (1.16 %)	0.41 % (1.04 %)
3 Reduced ration twice daily acclimated to 10°C	1.21 %	1.30 % (1.20 %)	1.32 % (1.17 %)	1.15 % (1.14 %)
4 Satiation rations twice daily acclimated to 20°C	0.94 %	3.47 % (2.25 %)	3.73 % (2.29 %)	2.87 % (1.93 %)

Table 3.3 Hydrogen ion concentration of the contents of alimentary tract of turbot at different stages of the digestive cycle. Results are based on means ± sd of data for three fish at each time interval.

Nutritional history of fish	Stomach pH	Gall bladder pH	Pyloric caeca pH	Anterior intestine pH	Posterior intestine pH	Rectum pH
Food deprived 96 h	2.7±0.2	6.73±0.25	5.25±0.25	6.5±0	8.66±0.18	9.0±0
Fed WFA7 pellets						
2 h after feed	3.7±0.2	7.0±0	7.7±0.2	8.2±0.2	8.0±0	8.6±0.5
4 h after feed	3.7±0.2	6.2±0.2	8.2±0.2	8.5±0.3	8.5±0	9.0±0
6 h after feed	3.5±0	6.5±0.5	8.0±0	8.5±0	8.2±0.2	8.6±0.1
8 h after feed	3.6±0.1	6.0±0	8.5±0	9.0±0	8.7±0.2	9.0±0
24 h after feed	3.5±0.5	5.5±0.5	8.5±0	8.5±0	8.8±0.5	8.5±0
30 h after feed	3.5±0	6.0±0	9.0±0	8.7±0.2	8.7±0.2	8.7±0.2
48 h after feed	3.5±0	5.7±0.5	8.5±0	8.5±0	8.5±0	8.5±0
72 h after feed	3.5±0	6.0±0	8.5±0	8.5±0	8.5±0	8.5±0
98 h after feed	3.7±0.2	6.2±0.6	8.0±0	8.5±0	8.0±0	8.5±0
170 h after feed	2.5±1.0	6.5±0	7.5±0	7.6±0.3	7.6±0.3	7.5±0.5

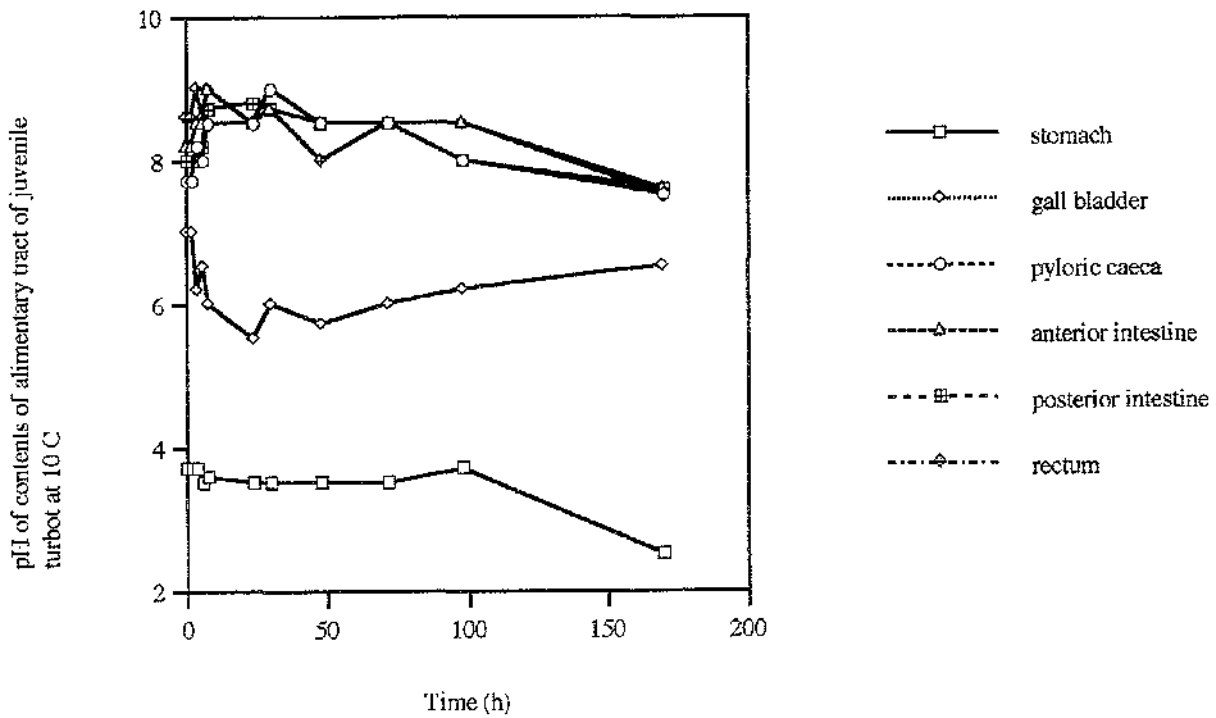


Figure 3.34 Hydrogen ion concentration of contents of various regions of alimentary tract of turbot at different phases of the digestive cycle. (n=3 for each plot).

i) Movement and digestion of food through the alimentary tract at different time intervals after feeding

The movement of food through the gut was observed and results are documented in Table 3.4. Prior to feeding the gall bladder was swollen and full of dark green fluid, the stomach was empty and the rest of the intestine contained a watery fluid. Two hours after feeding, the gall bladder was still full of dark green fluid but the stomach was very distended with a bolus of food whilst the anterior and posterior intestine and rectum still had a watery fluid. After 4 h the gall bladder and stomach were still quite full and a small amount of semi-fluid food was present in the anterior region of the intestine. Twenty-four hours later the fluid in the gall bladder was yellow in colour and some semi-fluid food was distributed throughout the intestine and rectum. A small amount of food still remained in the stomach after 72 h and a very little yellow fluid was present in the gall bladder whilst a mixture of food and watery fluid was found in the intestine and rectum. After four days, a very small amount of food and a watery fluid was present in the stomach. The gall bladder was still almost empty with just a small amount of yellowy fluid and a mixture of a tiny amount of semi-fluid food and watery fluid was present throughout the intestine and stomach. One week after feeding, the stomach was completely empty, the gall bladder was full of green fluid and a watery fluid was found in the intestine and rectum.

ii) Time taken for first and last faeces to appear using the indirect chromic oxide method

Two different approaches were used to find out if the length of time since the last meal influenced the time taken for first and last faeces to appear. In the first experiment, the time period since the last meal varied depending on the particular feeding regime of each group. The results are shown in Table 3.5 In the second approach, food was withheld from each group for 48 h before feeding to satiation with a chromic oxide meal. The results are given in Table 3.6

The results in Table 3.5 show that for fish fed to satiation twice every day at both 10°C and 20°C the initial time taken for faeces to appear was relatively short (4-6 h) and it took between 20-24 h for the last coloured faeces to appear. Comparison of the results for Groups 1 and 3 suggest that for fish held at the same acclimation temperature, the gastric evacuation rate is faster for those fed to satiation than for those fed on a reduced ration. The gastric evacuation rate for fish fed to satiation only once every 4-5 d (Group 2) was also lower than for those fed to satiation. The gastric evacuation rate of fish at the start of the experiment (Group 5) appeared to be slow compared with fish held under similar conditions for the 10 weeks of the experiment (Group 1) suggesting that the latter fish had adapted to long term regular feeding to satiation.

The results in Table 3.6 give the gastric evacuation rates of each group of fish when the test diet was administered after 48 h of food deprivation. Again Groups 1 and 4 had a relatively

Table 3.4 Movement and digestion of food within the alimentary tract of juvenile turbot at different stages of the digestive cycle. Results based on observations of three different animals at each time interval.

Nutritional history of fish	Stomach	Gall bladder	Anterior intestine	Posterior intestine	Rectum
Deprived of food for 96 h	empty	very full of dark green fluid	watery fluid	watery fluid	watery fluid
Fish fed WFA7 pellets					
2 h after feed	distended with food	very full of dark green fluid	watery fluid	watery fluid	watery fluid
4 h after feed	distended with food	very full of dark green fluid	small amount of food present	watery fluid	watery fluid
6 h after feed	distended with food	full of green fluid	some food	some food	watery fluid
8 h after feed	distended with food	full of green fluid	some food	some food	watery fluid
24 h after feed	full	yellowy - green fluid	some food	some food	some food
30 h after feed	some food	yellow little fluid	some food	some food	some food
48 h after feed	some food	yellow very little fluid	food/watery fluid	food/watery fluid	food/watery fluid
72 h after feed	some food	yellow very little fluid	food/watery fluid	food/watery fluid	food/watery fluid
98 h after feed	little food/watery fluid	yellow very little fluid	very little food/watery fluid	very little food/watery fluid	very little food/watery fluid
170 h after feed	empty	full of yellowy-green fluid	watery fluid	watery fluid	watery fluid

Table 3. 5 Time taken for first and last faeces to appear in juvenile turbot maintained on various feeding regimes for 10 weeks. A meal containing chromic oxide was delivered under the same conditions as the feeding regime. Weight of fish given as mean ± sd.

Group	Feeding regime	Acclimation temperature	Time since last feed (h)	Mean weight fish (g)	Food ingested (% body weight)	Time for first faeces to appear (h)	Time for last faeces to appear (h)
1	satiation twice daily	10°C	19	46.7±15	2.0	6.0	20-24
2	satiation once every 4-5 days	10°C	120	37.2±9.4	2.0	10.0	44-48
3	reduced ration daily	10°C	19	36.6±10.4	2.0	8.0	34-36
4	satiation twice daily	20°C	19	47.1±17.6	2.0	4.0	20- 24
5	satiation twice daily	10°C	19	26.0±8.4	2.0	10.0	44-48

Table 3. 6 Time taken for first and last faeces to appear in juvenile turbot maintained on different feeding regimes. Food witheld for forty-eight hours then fish fed to satiation with meal containing chromic oxide. Weight of fish given as mean ± sd.

Group	Feeding regime	Acclimation temperature	Time since last feed (h)	Mean weight fish (g)	Food ingested (% body weight)	Time for first faeces to appear (h)	Time for last faeces to appear (h)
1	satiation twice daily	10°C	48	46.7±15	2.56	5.5	20-24
2	satiation once every 4-5 days	10°C	48	37.2±9.4	2.62	5.0	44-48
3	reduced ration daily	10°C	48	36.6±10.4	1.36	9.0	34- 36
4	satiation twice daily	20°C	48	47.1±17.6	2.63	4.5	24-28
5	satiation twice daily	10°C	48	26.0±8.4	3.07	9.5	44- 48

fast gastric evacuation period and there appeared to be little difference in gastric evacuation rates due to temperature except that the time taken for the first faeces to appear was again faster in fish acclimated to 20°C. The time taken for the first faeces to appear in Group 2 was also fast but the last coloured faeces does not appear until 48 h. Group 3 (on a reduced ration) had a slower gastric evacuation rate as did Group 5, the fish at the start of the experiment.

The results suggest that fish fed to satiation on a regular basis have a faster gastric evacuation rate than those fed on a reduced ration or those fed to satiation only once every four to five days.

3.3.4 Assimilation efficiency in juvenile turbot

The assimilation efficiency is the proportion of food (expressed as protein or energy content) which is absorbed by the fish. A proportion of the ingested food will pass through the alimentary tract unabsorbed and will be lost in the faeces. Along with the undigested component, the faeces also contains cells sloughed from the tract, mucous, bacteria and possibly shed proglottids from tapeworms if the fish are infected. However, it is generally assumed that this component, known as metabolic faecal nitrogen (MFN), contributes a minor amount to the faecal material (100-200 mg N per 100 g dry diet consumed (Jobling, 1994) and neither MFN nor excretory losses through urine were taken into account in this study.

The experimental procedure used in this study necessitated the pooling of samples from each group of 16 or of 12 fish. Three separate samples were taken from each group and chemical analysis of each sample was replicated 5 times. The results are presented in Tables 3.7, 3.8 and 3.9.

3.3.4.1 The effect of body size on assimilation efficiency

There is no significant difference in assimilation efficiency (when expressed in terms of protein) with variation in size of juvenile turbot. The assimilation efficiency was generally very high: 95.9 % ± 0. 6 se for small fish (3 months old) in the weight range 4-13 g (x = 6.33 g ±1.4 se); 93.1 % ± 1.4 se in fish (6 months old) ranging from 14-38 g (x = 23.7 g ±8.4 se) and 94.1 % ± 0.8 se in fish (9 months old) ranging from 24-75 g (x = 46.7 g ± 15.0 se). When the assimilation efficiency is expressed in terms of energy content, the smallest fish (3 months) show a significantly higher level of digestive efficiency at 95.8 % than the 6 months old fish at 81.7 % (T = 9.86 P = 0.0022 DF = 3) and also higher than the 9 months old fish at 82.2 % (T = 7.95 P = 0.0042 DF = 3). When the smallest size group is compared to the next two size ranges collectively, there is no significant difference in their assimilation efficiencies (protein)

but again the smaller fish show a higher assimilation efficiency in terms of energy than the other two groups (T = 8.1; P = 0.0002; DF = 6). (Tables 3.7 , 3.9).

Table 3.7 Assimilation efficiency in fish fed composite diet containing various protein supplements

Protein supplement	Number of fish in group	Mean Assimilation efficiency + se (%) (Protein)	Mean Assimilation efficiency ± se (%) (Energy)
Fish meal	16	95.9±0.6	95.8±0.9
Pruteen	16	96.0±1.2	97.3±0.9
Casein	16	95.8±0.2	95.1±0.9
Albumin	16	92.1±0.80	94.5±0.7
Kaolin	16	90.5±3.5	94.2±0.8

3-month old turbot (6.3 g ±1.4) acclimated to 10°C

Table 3. 8 Effect of varying carbohydrate source on the assimilation efficiency

Carbohydrate source	Number of fish in group	Mean Assimilation efficiency ± se (%) (Protein)	Mean Assimilation efficiency ± se (%) (Energy)
Starch 20%	16	93.0±0.7	93.7±0.7
Starch 15%, Pruteen 5%	16	94.9±0.9	94.0±0.9
Starch 10%, Pruteen 10%	16	94.1±0.8	93.2±1.6
Starch 5%, Pruteen 15%	16	96.3±0.4	94.3±0.7
Starch 15%, Fish meal 5%	16	94.5±1.0	94.8±0.3
Starch 10%, Fish meal 10%	16	95.9±0.6	95.7±0.5
Starch 5%, Fish meal 15%	16	96.7±0.4	96.2±0.4

3-month old turbot (6.3 g±1.4) acclimated to 10°C.

3.3.4.2 The effect of temperature on assimilation efficiency

The assimilation efficiency of fish held at 10°C was not significantly different from those held at 20°C when expressed in terms of protein content (T = 1.79 P = 0.22 DF = 2). However, when expressed in terms of energy content there is a significant difference between the two temperatures with fish held at the lower temperature (10°C) having a higher assimilation efficiency than those held at the higher temperature (T = 3.27 P = 0.047 DF = 3). (Table 3.9).

3.3.4.3 The effect of feeding frequency on assimilation efficiency

The assimilation efficiency (expressed as protein) of fish fed to satiation twice per day was significantly higher than those fish fed only once every 4-5 days to satiation (T = 3.27 P = 0.047 DF = 3) (Table 3.9). However, there appeared to be no significant difference between these two feeding regimes when assimilation efficiency was measured in calories (T = 3.12 P = 0.052 DF = 3). There was no significant difference in assimilation efficiency for protein and energy content between fish fed every day on a reduced ration and those fed to satiation once every 4-5 days.(Table 3.9).

3.3.4.4 The effect of ingestion rate on assimilation efficiency

There was no significant difference in the assimilation efficiency of fish fed to satiation twice daily and those fed on a reduced ration (Table 3.9).

3.3.4.5 The influence of different dietary protein supplements on assimilation efficiency

Alteration of the dietary protein supplement in the food of turbot (weight range 5.1 g ± 1.37) had very little effect on the assimilation efficiency (energy and protein) of these very small fish. Generally, the digestibility coefficient remained very high at 90.53 - 97.3 % for all the dietary supplements, including kaolin, showing the assimilation efficiency of juvenile turbot is very high (Table 3.7)

3.3.4.6 Investigation into the sparing effect of carbohydrate on assimilation efficiency

No significant difference in assimilation efficiency was detected when the protein supplement was replaced in part with a starch component although the assimilation efficiency gradually decreased with increasing starch content (Table 3.8).

Table 3. 9 Effect of feeding regime and temperature on the assimilation efficiency of juvenile turbot

Feeding regime	Temperature	Number of fish in each group	Age of fish (months)	Mean weight of fish(g) ± se	Mean Assimilation efficiency ± se (%) (N_2)	Mean Assimilation efficiency ± se (%) (Energy)
(1) twice daily to satiation (10 weeks)	10°C	12	9	46.7±15.0	94.1+0.9	82.2±1.5
(2) satiation once every 4-5 days (10 weeks)	10°C	12	9	37.2±9.4	90.0±0.9	72.82±1.6
(3) reduced ration twice daily (10 weeks)	10°C	12	9	36.6±10.4	93.9±0.9	82.3±0.6
(4) twice daily to satiation (10 weeks)	20°C	12	9	47.1±17.6	88.7±2.9	74.7±1.7
(5) twice daily to satiation (3 weeks)	10°C	10	6	23.7±8.4	93.1±1.4	81.7±1.1

3.4. DISCUSSION

A knowledge of the factors which influence the feeding rate, digestion and absorption of nutrients in fish is important in order to establish the conditions for optimum health and production in farmed fish. Although fish to an extent regulate their food intake, various factors may influence this. Once ingested the food must be digested and this will depend on the structure and functional properties of the alimentary tract (*e.g.* the length and degree of folding of the gut, the number and length of microvilli, and the nature of the digestive enzymes present). These properties may also be influenced by a number of factors. The rate of passage of the food through the gut is also of consequence in digestion and assimilation and is known to vary considerably under different conditions. The proportion of food energy and of particular nutrients in the food assimilated under different intrinsic and environmental conditions are also very important as the assimilated energy and nutrients are used for maintenance and growth in the fish.

Each of the features of fish nutrition can be influenced by a number of interrelated environmental or intrinsic factors. It is necessary to determine the part played by individual factors for any fish species as their importance varies widely with different species. It was therefore considered necessary to study these features in juvenile turbot.

Study of the morphology of the alimentary tract showed that its length is a variable entity which reacts to changes in feeding condition (Kapoor *et al.*, 1975). The degree of such adaptability is probably limited by the degree of specialization of the fish species, with omnivorous fish displaying the greatest plasticity. Gut length tends to be correlated with feeding habits, especially with the quantity of indigestible matter. Carnivorous species generally have the shortest gut lengths whilst species that feed on detritus and algae have the longest (Kapoor *et al.*, 1975). The long length of the alimentary tract in some species may compensate for the lack of development of folds in the lining and may allow a relatively unimpeded passage of roughage through the intestine (Kapoor *et al.*, 1975). Species with short intestines often have complicated branched folds in the intestine and this was found to be the case with juvenile turbot in this study (Figures 3.3, 3.4). Very long microvilli were also observed in the intestine and rectum of turbot thus further increasing the area for digestion and absorption.

In some fish, especially omnivorous species, the gut length varies with the components of the diet. Vickers (1962) demonstrated that the length of the intestine in *Carassius auratus* varied between individuals of the species and concluded that this was probably associated with the specific character of the diet during development. Turbot also display a variation in the relative lengths of different regions of the gut during development, although the total length of the alimentary tract remains the same (approximately equal to the body length in fish fed a natural

diet, Braber and de Groot, 1973b). Juvenile turbot of 60-110 mm in length feed on mysids, those of 110-300 mm rely mainly on fish as food, whilst turbot of 310-650 mm are exclusively fish feeders with the size of prey captured increasing with the size of the fish (Braber and de Groot 1973a). Fish feeders which swallow large prey at once and swallow it whole tend to possess a large buccal-pharyngeal cavity. The relative length of the buccal-pharyngeal cavity in turbot changes in relative proportion to the rest of the gut probably due to this modification in diet (Braber and de Groot, 1973b). The oesophagus and stomach are also large and this may be correlated with the requirement of a long period for digestion in the stomach and with the short length of the intestine. The present study also indicated that their may be a shortening of overall gut length in juvenile turbot maintained on an artificial diet and acclimated to 10°C in comparison with fish fed on a natural diet. There appears to be an even more pronounced shortening of the tract in turbot acclimated to 20°C. Fish maintained under intensive fish farming conditions also tend to be fed a highly nutritious diet rich in protein which is very easily digested in comparison to the natural diet which contains more indigestible material, and so it seems likely that they would be similar to the turbot in this study and have relatively short guts.

The histological organization of the turbot alimentary tract was examined and found to bear many similarities to that of other teleost fish. Four main layers were observed, the serosa, a double layered muscularis, a sub-mucosa and mucosa. The size and composition of these layers was found to vary in relation to the function of the particular region. The muscularis of the oesophagus was very thick and the sub-mucosa also contained blocks of longitudinal muscle fibres which is probably related to its role as a transit tube transporting food into the stomach. The muscularis of the stomach was also well developed as was the sub-mucosa which may act as a strengthening layer and may help to regulate the distension of the wall. This has been described by some researchers as an adaptive feature of carnivorous fish (Al-Hussaini, 1949a,b; Bucke, 1971; Murray *et al.*, 1994b).

The mucosal epithelium of the alimentary tract is composed of columnar epithelial cells and plays an important role in the digestion and absorption of food substances. Each region of the tract has mucosal specializations which maximize the efficiency of the secretion, digestion and absorptive functions (Buddington and Diamond, 1986; Stinson and Colhoun, 1993; Murray *et al.*, 1996). The mucosal layer of the turbot oesophagus contains many mucous secreting cells which indicates a large output of mucin. This probably replaces the secretion of salivary glands which are absent in fish and may aid in the emulsification of food for rapid transmission into the stomach. Eight to ten folds were seen in the mucosal layer of the oesophagus and a moderate amount of folding was also observed in this layer in the stomach. This is common in fish which

capture live prey allowing expansion on ingestion of the prey and also allows a large quantity of food to be stored.

The mucosal layer of the stomach contains many rodlet cells, a moderate number of mucous cells and also has some short, stubby microvilli. The mucous cells of the stomach probably play a digestive role, helping to break down the bolus of food into a chyme which is released into the intestine in regulated amounts. The muscular pyloric sphincter allows only chyme to pass through to the intestine and prevents solid food particles from entering the intestine (Bromley, 1987).

Although the length of the intestine in turbot is short, the area for digestion and absorption is increased by infoldings of the mucosal epithelium (Figure 3.3). This is greatest in the intestine and rectum where many convoluted folds are found and the surface area is further increased by numerous, unusually long microvilli. Microvilli are known to increase the digestive and transportive surface of the epithelial cells and form the basis for membrane digestion (Ugolev, 1972, 1985). The muscularis and sub-mucosa of the anterior and posterior intestine are poorly developed in comparison with the oesophagus and stomach. The anterior and posterior intestine also contain mucous secreting cells the number of which is seen to increase dramatically in the rectum, probably as an aid in the lubrication of faecal substances.

The ultrastucture of the anterior intestinal epithelium of the alimentary tract of both fed and fasted turbot was examined to gain further insight into the process of digestion and absorption in turbot. Generally, the mucosal epithelium was composed of typical columnar, absorptive epithelial cells (enterocytes) with mucous secreting cells and some rodlet cells (see Chapter 6) dispersed throughout. The most striking feature was the apical brush border of very long microvilli which greatly increases the surface area of the intestine for digestion and absorption. Pinocytotic vesicles were observed between the microvilli and in the terminal web area indicating protein. Mitochondria were observed throughout the cells except in the terminal web area and appeared to be more concentrated in the basal part of the cell in the fed fish.

Turbot fasted for two weeks differed in some aspects from fish fed 24 h previously. In particular, there were clear signs of cellular degeneration and sloughed cells were observed in the lumen. Large, supranuclear vacuoles containing cellular debris were observed in most sections. Kuperman and Kuz'mina (1994) noted degenerative changes in the enterocytes of bream, *Abramis brama* L., during winter starvation and McFadzen *et al.* (1994) demonstrated cellular degeneration and loss of microvilli in larval turbot fasted for 48 h. Fasting for two weeks did not appear to reduce the number or height of the microvilli in the anterior intestine of juvenile turbot. However, this feature has been demonstrated previously in carp, *Cyprinius carpio* L., fasted for six months, where the microvilli were reduced in height (0.5-0.6 μm) compared with the

microvilli of fed carp (1.2 μm) (Noaillac-Depeyre and Gas, 1973). The age of the fish and the extent of the period of fasting is probably crucial to the amount of damage incurred by the mucosal epithelium. It is possible that autophagy (use of endogenous energy sources by larval turbot, Quantz, 1985) occurs in fish as a survival procedure against starvation as many fish species are subjected to fluctuating food supplies or periods of winter fasting. Large supranuclear vacuoles were observed in the intestine of fasted turbot, possibly indicating autophagy.

Mitochondria were noted in abundance in the apical part of the cells of fasted turbot and they contained dense granules dispersed throughout the matrix. Stroband and Debets (1978) also noted the apical localization of mitochondria with dense granules dispersed throughout the matrix in fasted juvenile grass carp, *Ctenopharyngodon idella,* and thought this may be related to the accumulation of calcium ions and a decrease in oxidative phosphorylation as described by Hackenbrock and Caplan (1969).

Recently, Kuperman and Kuz'mina (1994) examined the ultrastructure of the intestinal epithelium of three species of fish with different feeding habits. They discovered the most substantial variations occurred in the height of the microvilli with the pike *Esox lucius* and the burbot *Lota lota* having the longest microvilli in the posterior part of the intestine whilst the bream *Abramis brama* had the longest microvilli in the anterior region. Previous studies have shown the length of the microvilli to decrease from the anterior to the posterior region of the intestine (Anderson, 1986; Noaillac-Depeyre and Gas, 1979 and Stroband, 1977). In the present study, the anterior intestine of fed turbot possessed microvilli measuring up to 5.6 μm in length, although the range was from 3.0-5.6 μm. The microvilli in the posterior intestine and rectum appeared to be shorter at 2.5-4.0 μm in length. Short, stubby microvilli were found in the stomach of both fed and unfed turbot. The length of the microvilli in turbot is much greater than the length of the microvilli in many other fish species which generally measure around 0.6 - 2.0 μ m in length (Iwai, 1968a,b; Krementz and Chapman, 1975; Stroband and Debets, 1978; Kuperman and Kuz'mina, 1994). Since microvilli are known to increase the digestive and transportive area of the intestine, it would be reasonable to assume that the total area for these purposes is greatly increased in turbot and that this possibly contributes to a highly efficient digestive system.

Digestive enzymes are produced in the mucosal epithelium of the alimentary tract and in the pancreas and liver (stored in gall bladder) and are secreted into the lumen of the tract. Together they degrade the proteins, polysaccharides, lipids and nucleic acids of the ingested food into smaller molecules which can then be absorbed and assimilated. Protein digestion probably begins in the stomach of some fish rendering protein soluble and more readily digested by

pancreatic and intestinal proteases. In fish possessing a stomach, the main gastric proteolytic enzyme is probably a pepsin with maximum activity at pH 2.0 with a second peak between pH 3.0 and 4.0 (Barrington, 1957; Kapoor *et al.*, 1975). The low optimal activity of the enzyme is due to the presence of HCl produced by gastric mucosal glands (Western and Jennings, 1970).

Preliminary investigations were carried out on the pH conditions of the mucosae of the alimentary tract of turbot to give some indication of the enzyme activity present. In turbot which had been deprived of food for one week the pH of the stomach mucosa was approximately pH 2.5, whereas in fish two hours after feeding and up to 72 hours after feeding the pH reading was 3.5 - 3.75. This would appear to indicate a pepsin-like enzyme activity. Glass *et al.*, (1989) demonstrated that Atlantic halibut *Hippoglossus hippoglossus* which have a clearly defined stomach have a pepsin-like enzyme optimally active at pH 1.0-2.0 plus another peak of activity at pH 5.5, whilst Munilla-Moran and Stark (1990) showed turbot to have a maximum gastric activity at pH 2.5. In the present work, the pyloric caeca, intestine and rectum all demonstrated a pH of between 7.75 and 9.0 from two hours after feeding until 98 hours after feeding. Tryptic activity is maximal between pH 7.0 and 9.0 and trypsin and chymotrypsin from the intestine and pancreas are probably the major digestive enzymes in the intestine. This is consistent for carnivorous fish on a high protein diet. More recent work on digestion in larval and juvenile turbot has shown a slow but significant increase in protease activity occurs after ingestion of rotifers suggesting a nutritional control of the rate of digestion (Munilla-Moran and Stark, 1989). Later work by the same authors demonstrated a constant high activity of pH 8.0-10.0 throughout the intestine (Munilla-Moran and Stark, 1990).

In fish the pancreatic juice also contains amylase, maltase and lipase which break down carbohydrates and fats. Lipase has been demonstrated in the stomach, pyloric caeca, intestinal and pancreatic fluid and in the bile. The role of these and other enzymes varies in different species of fish and tends to reflect the diet of the fish. For example, high chitinase levels exist in fish that feed on crustaceans or insects (Fänge and Grove, 1979) whilst in plant feeding fish such as *Tilapia* and carp, the amylase activity is much higher than in the perch, *Perca fluviatilis*, or in the pike, *Esox lucius* (Vonk, 1941; Fish, 1960). In the present study, the reduction in gall bladder volume was correlated with the passage of food from the stomach into the intestine commencing approximately 4-6 h after feeding and emptying over a 98 h period. The pH of the bile fluids fell from pH 6.5-7.0 before feeding and 2 h after feeding to pH 5.5-6.0 between 8-98 h after feeding. This agrees with more recent work by Bromley (1987) who suggested this is consistent with the role of the gall bladder as a source of bile assisting in the emulsification and absorption of lipids. These pH conditions are also consistent with the presence of amylase, esterase and lipase

activities, all of which have been demonstrated in turbot in more recent research (Cousin *et al.*, 1987; Munilla-Moran and Stark, 1990; Koven *et al.*, 1994a,b).

The amount of food and frequency of food consumed by a fish (feeding rate) and the rate of passage of food through the gut (gastric evacuation / digestion rate) are closely linked. The food intake of fish is controlled by metabolic debt and by foregut fullness (Colgan, 1973; Elliot, 1975a,b; Grove *et al.*, 1978; Grove and Crawford, 1980; Godin, 1981). Metabolic debt arises from food deprivation but the rate progressively decreases as the fish adapts both behaviourally and physiologically to conserve its energy (Jobling, 1981a,b; Talbot, 1985). The rate of gastric emptying in fish depends on the quantity of food consumed, frequency of feeding, fish size, temperature, gastric secretion, gastric motility and the capacity of the intestine. A wide range of gastric evacuation patterns have been reported from different fish in reponse to varying biotic and abiotic factors demonstrating a surprising plasticity in digestive properties (Edwards, 1971; Elliott, 1972; Grove *et al.* 1985; Jobling *et al.* 1977; Tyler, 1970; Windell *et al.*, 1976).

Various methods have been used by researchers to measure the rates of gastric evacuation in fish. For example, sacrificing fish at different time intervals after feeding to satiation (Western, 1971), removal of stomach contents by flushing or by emetics (Meehan and Millar 1978; Strange and Kennedy, 1981), radiographic methods (Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Grove *et al.*, 1985) and the production of coloured faeces (Rozin and Mayer, 1964; Hadjichristophorou and Grove, 1983). These methods were reviewed by Talbot (1985). Many results are based on fish that have been force-fed and this may have a profound effect on evacuation rate with force-fed fish having an evacuation rate twice that of voluntary-fed fish in some cases (Swenson and Smith, 1973). Force-feeding has also been shown to physically damage the fish whilst radiographic techniques can prove quite stressful to the fish (Windell, 1978; Swenson and Smith, 1973).

Generally, digestive rates increase with increased food intake (Windell *et al.*, 1969; Tyler, 1970, Elliot, 1972; Steigenberger and Larkin, 1974; Windell, 1978). In the present study on turbot, increased food intake appeared to have the greatest effect on the rate of gastric evacuation. This is consistent with studies on other fish where increased food intake and rate of feeding gives faster rates of evacuation (Pandian and Vivekanandan, 1985).

The food particle size may influence evacuation rates whilst food with a lipid concentration greater than 15% of the dry weight has been shown to have an inhibitory effect in evacuation rates (Kapoor *et al.*, 1975). Turbot of equal size range fed on meals of varying energy contents were able to increase their food intake to partly offset the decreased energy supply (Flowerdew and Grove 1979; Grove *et al.* 1985) and this was correlated with an increase in gastric evacuation rate with increasing dilution of the energy content of food. The proportion

of digestible to indigestible material in the food is also important as the indigestible proportion has been shown to remain in the gut for longer in turbot (Bromley, 1987). Grove *et al.* (1985) showed the digestion rate of turbot fed on fish fillets was 40 % of that in turbot fed on pellets. They pointed out that data obtained from fish fed on pelleted diets cannot be applied to natural populations.

Small fish tend to exhibit greater rates of gastric evacuation than larger fish (Pandian, 1967; Brett, 1971; Moriarty and Moriarty, 1973; Flowerdew and Grove, 1979). Bromley (1987) demonstrated that the relative rates of evacuation of wet material and protein were greater in smaller turbot. A decrease in relative evacuation rate with increase in body size in turbot was noted by Flowerdew and Grove (1979). However, Bromley (1987) demonstrated that the relative rate of evacuation in energy terms was greater in turbot of 2.8 kg than in smaller turbot of 0.42 kg. In the present study no difference in evacuation rate was noted between turbot of 20 g and turbot of 45 g probably because the size range was too similar.

Temperature tends to have a large influence on digestive rates with a temperature rise of 10°C resulting in a threefold increase in digestion rate, Windell *et al.* (1976), however, found temperature changes in the range between 0 and 5°C had a much greater influence on gastric evacuation rates than changes in the higher temperature ranges of 15-20°C. Flowerdew and Grove (1979) found the gastric evacuation rate of turbot increased with increasing temperature. In the present study a rise in temperature of 10°C (10-20°C) only produced a slight increase in gastric evacuation rate which is more in agreement with Windell *et al.* (1976) than that of other workers and may partly reflect the only slightly greater ingestion rate of the 20°C acclimated fish. The secretion and activity of digestive enzymes, gastric motility and intestinal absorption are all influenced by temperature (Molnár and Tölg, 1962; Molnár *et al.*, 1967; Smit, 1967; Edwards, 1971). However, it is possible there may be a compensatory effect with long term acclimation to higher temperature.

The time elapsed since the last meal also influences the subsequent consumption of food and evacuation rate with fish fasted for long periods generally having slower rates of evacuation than fish fed more recently (Windell, 1966; Sarokon, 1975). In the present study, turbot fasted for periods of 5 d had a slower evacuation rate than those fed every 24 h which is consistent with the observation that increased feeding frequency accelerates the passage of food through the gut (Talbot, 1985).

Grove *et al.* (1985) showed that turbot fed through demand feeders regulate their food intake so that feeding activity starts when the stomach is 10-20 % full and that feeding ceases when the fish are 80-90 % full. When the turbot were fed regularly via automatic feeders they fed steadily, maintaining the stomach at approximately 85% fullness. Bromley (1987) suggested

turbot may have a high degree of control over the process of digestion and evacuation and this may regulate the flow of chyme into the small intestine, possibly at an optimum rate for completion of digestion and absorption of nutrients. Although the mechanisms of control of gastric evacuation in fish are not completely understood, Bromley's work indicates an inhibitory reflex is in operation in turbot. Work on mammals has shown that control of gastric evacuation may be achieved by suppression of gastric secretions which are regulated by a neuro-hormonal feedback which is triggered by mechanical and chemical stimulation of the foregut.

The ingested food is only useful to a fish if it can be digested and absorbed by the intestinal epithelium. The amount of energy available for growth and maintenance for the fish depends on the amount of food consumed, its energy value and its digestibility (assimilation efficiency). If the assimilation efficiency is low then much of the energy and nutrients are lost in the faeces. This occurs in plant-eating fish, where a large proportion of the food is indigestible. The digestive efficiency is found to be in the range of 40-80 % for herbivorous fish, whilst the assimilation efficiency tends to in the range of 70-95 % for carnivorous fish where a large amount of protein is present in the food (Jobling, 1994).

In the present study the assimilation efficiency of juvenile turbot at 10°C was generally found to be very high and the results are very similar to the more recent results obtained by Grove *et al.* (1985) for turbot held at 16°C. For turbot of a similar weight range, the protein assimilation and energy assimilation efficiencies in this study were 96 % and 95 % respectively. Grove *et al.* (1985) reported similar high assimilation efficiencies (the equivalent figures being 97.5 % and 91 %). When expressed in terms of protein assimilation, there was no significant difference between 3, 6 or 9 month old fish in the present work. When expressed in terms of energy value, however, the smallest fish showed a significantly higher assimilation efficiency than the 6 and 9 month old fish, indicating an ontogenic shift in the energy assimilation efficiency of turbot. A temperature increase of 10°C significantly decreased the assimilation efficiency of turbot when expressed as energy value, but no difference was found in terms of protein assimilation. Increased feeding frequency significantly increased the protein assimilation efficiency but not the energy assimilation efficiency in 6 and 9 month old turbot in the present work. In contrast, Grove *et al.* (1985) found no difference in the protein or energy assimilation efficiencies of fish fed single meals and those fed multiple meals, although the exact feeding frequency was not described by the authors and the fish used in their experiment were much smaller. The present study shows however, that very young turbot generally have a very high assimilation efficiency and for these fish no significant difference was found either for protein or energy assimilation efficiency between fish fed daily on different ration levels. This is in agreement with data noted in Grove *et al.* (1985).

Generally, the assimilation efficiency of very young turbot is very high but there appears to be a very slight decrease in the energy assimilation efficiency as the fish increase in size whilst the protein assimilation remains high. The energy assimilation efficiency also appeared to be decreased by increasing temperature whilst the protein assimilation remained unaltered. This may be a reflection of the very high protein content of the diet as some studies have shown the assimilation efficiency to be reduced at lower temperatures (Jobling, 1994). Lower feeding frequency on the other hand appeared to decrease the protein assimilation efficiency but not the energy assimilation.

Finally, incremental replacement of the protein content of the diet by starch had no significant difference in the protein or energy assimilation in 3 month old turbot. This reflects the high efficiency with which very young turbot assimilate energy and nutrients from food. Presumably, this efficiency will depend on the relative proportions of nutrients in the food and its energy value and it could be that the efficiency is reduced if the values fall below a critical level. This could be tested by adding progressively more kaolin to the diet.

In conclusion, different species of fish display a wide range of feeding patterns. The type of food consumed is reflected in the structure of the alimentary tract and is dependent on the evolutionary history of the fish. However, within certain limits, it has been shown that the gross and fine structure of the alimentary tract may be altered by a number of factors including the type or amount of food consumed and the temperature of acclimation. It has been shown that the ingestion rate, the process of digestion, including the rate of gastric evacuation, gastric enzyme secretion and assimilation in fish are dependent on a number of intrinsic and extrinsic variables, are all closely related, and are in fact very much interdependent. The amount of time the fish is subjected to the various conditions is also important as the fish may show some ability to acclimate to some long term conditions. For instance, fish may reduce their activity level in response to a decrease in food supply in order to reserve their resources. Ultimately the fish may adapt metabolically by reducing the basal metabolic rate (See Chapter 4). The degree these processes are altered by the amount of available food, proportion of nutrients and energy value of the food, food size and consistency, frequency of feeding, temperature, light regime, size of fish, condition of fish including parasitic status, quality of water and holding conditions will vary for different species of fish. However, an insight into the factors that affect feeding, digestion and absorption in fish and how these are related is very important if optimum conditions for economic growth under intensive fish farming conditions are to be achieved. Some of the factors that affect the structure of the alimentary tract, feeding, digestion and absorption in juvenile turbot have been discussed in this Chapter in relation to other work. Generally, the alimentary tract of turbot is typical of carnivorous fish and would appear to have a certain degree of plasticity in its

gross morphology. The length of the intestinal and rectal microvilli are exceptionally long and are perhaps, in part, responsible for the very high assimilation efficiencies recorded for turbot. A more detailed study of the effects of diet and temperature on the number and height of the microvilli, using digital analyses, would be interesting. It is important, however, to remember that the results found here and by other workers have been obtained under laboratory conditions and should be interpreted with this in mind. They give an insight into the very complicated relationship between feeding and growth in fish and they should be applied to fish farming conditions cautiously.

3.5 SUMMARY

1 The alimentary tract of turbot is typical of carnivorous fish that capture live prey. The buccal-pharyngeal cavity, oesophagus and stomach are relatively large to accommodate large prey. The intestine and rectum are relatively short and simple with their lining thrown into many complicated convolutions which increase the surface area for digestion. The ratio of gut length to body length of farmed turbot maintained in the laboratory, acclimated to 10°C and fed on WFA pelleted diet, was found to be 0.88 : 1.0. This is significantly shorter than the ratio of 1.0:1.0 found by Braber and de Groot (1973b) for wild caught fish feeding on natural prey items and may possibly reflect adaptation to the highly nutritious, easily-digested pelleted diet. Fish acclimated to 20°C had a ratio of gut length to body length of 0.7:1.0, which was significantly shorter than that for 10°C acclimated fish. This possibly reflects the greater proportion of easily-digested food eaten by the 20°C fish.

2 The histological organization of the turbot alimentary tract has many similarities to other teleost fish. There are four main layers, the outer serosa, a muscularis of longitudinal and circular muscle, a sub-mucosa and mucosa. The muscularis of the oesophagus is very thick as is the sub-mucosa which contains blocks of longitudinal muscle scattered throughout the connective tissue. The mucosal epithelium of the oesophagus contains many mucous cells indicating a large output of mucin which probably aids in the production of chyme. The stomach also has a relatively thick muscularis and sub-mucosa whilst the mucosal epithelium of this region shows large numbers of rodlet cells and a moderate number of mucous cells and also has short, stubby microvilli. In contrast the muscularis and sub-mucosa of the intestine and rectum are thin whilst the mucosal epithelium is thrown into filoform folds and is characterized by the brush border of numerous, long microvilli (2.5-5.6μm). The microvilli greatly

increase the surface area for digestion and absorption. The intestine also contains mucous-secreting cells. The number of these increases dramatically in the rectum, possibly to aid in the lubrication of faecal material. The microvilli of the anterior intestine of turbot appear to be longer (3.0- 5.6 μm) compared to those of the posterior intestine and rectum (2.5-4.5 μm).

3 Cellular sloughing as well as large supranuclear vacuoles containing cellular debris were observed in fish fasted for 14 d. This is possibly due to autophagy. Fish fed 24 h previously possessed typical absorptive columnar epithelial cells. The microvilli had a glycocalyx where possibly proteins are adsorbed to the coat and taken into the cell. Pinocytosis was observed showing active digestion.

4 Fish acclimated to 10°C and fed twice daily to satiation for 10 weeks had a higher ingestion rate (1.69 % body wt/day) than fish held under the same conditions and fed to satiation only once every 4-5 days (1.04 % body wt/day). However, they had a lower ingestion rate than fish acclimated to 20°C (1.93 %).

5 The pH of the stomach of turbot was 2.5 in fish fasted for 7 days whereas in fish fed between 2 h and 72 h previously, the pH of the stomach was found to be 3.5-4.0. This suggests the possible presence of a gastric proteolytic enzyme such as pepsin which has a maximum activity of pH 2.0 and a second peak at between pH 3.0 - 4.0. The pyloric caeca, intestine and rectum had a pH of between 7.75 - 9.0 from 2 h after feeding to 98 h after feeding which would be consistent with trypsin as the active enzyme.

6 After fasting for seven days it took approximately 98 hours for a meal of 2 % body weight to pass completely through the alimentary tract of turbot acclimated to 10°C. The stomach remained very distended with a bolus of food for 8 h and only after 4 h was the first food visible in the anterior part of the intestine. There was a reduction in the size and contents of the gall bladder during gastric evacuation indicating the involvement of bile which assists in the emulsification and absorption of lipids.

7 Increased feeding frequency produced faster evacuation rates in turbot. The greater the period of fasting, the longer the period for complete evacuation. The time taken for the first appearance of coloured faeces in fish acclimated to 20°C was less than for fish acclimated to 10°C, but the time taken for the last coloured faeces to appear was the same.

8 The assimilation efficiency for juvenile turbot at age 3, 6 or 9 months old is generally very high and there is no significant difference when expressed in terms of protein content (95.9, 93.1 and 94.1 % respectively). When expressed in terms of energy content the smallest fish show a significantly higher assimilation efficiency at 95.8 % compared with

81.7 % and 82.2 % for the 6 month and 9 month old fish respectively, possibly indicating an ontogenic shift.

9 A temperature increase of 10°C significantly decreased the assimilation efficiency of turbot when expressed in terms of energy content, but no difference was found in protein assimilation efficiency.

10 Increased feeding frequency significantly increased the protein assimilation efficiency of turbot but did not appear to influence the assimilation efficiency when expressed in terms of energy content.

11 No significant difference was found between the assimilation efficiencies (expressed in terms of protein or energy content) of fish fed to satiation daily or those fed on a reduced ration daily.

12 Ideally, larger numbers of fish should have been employed for each of the experimental treatments described in this chapter and replicates of each experimental set-up used. Constraints, however, in time and the number of juvenile turbot available for this research combined with limitations in number and size of tanks due to shortage of aquarium space, imposed severe restrictions in experimental design. The lack of a re-circulating sea-water system necessitated the use of an Eheim charcoal filter pump for each tank and involved lengthy daily maintenance procedures (see Chapter 2).

CHAPTER 4

ASPECTS OF THE ENERGY METABOLISM OF TURBOT

4.1 INTRODUCTION

4.1.1 Bioenergetics and the energy budget

Fish farming is a business and the ultimate aim of the fish farmer is to make money; this is achieved by producing the maximum amount of healthy fish flesh in a given time with a minimum amount of food input and other forms of expenditure. A highly efficient and speedy growth rate for their stock, with negligible losses due to disease, stress and predators is desirable. A comprehensive understanding of the fishes' performance capacity under various environmental conditions and at different ontogenic stages is essential. Increase in fish mass depends on the energy made available to the fish in the form of food and how that energy is utilized within the fish. Energetics is the study of the energy requirements and flow of energy, or energy partitioning, in a system (De Silva and Anderson, 1995).

In chapter one the concept of fish energetics and the energy budget was introduced and the utilization of food energy for metabolism and growth was explained on the basis of thermodynamically balanced energy equation (Ivlev, 1939; Winberg, 1956; Beamish *et al.*, 1975; Webb, 1978; Brett and Groves, 1979; Cho *et al.*, 1982; Calow, 1985; Weatherly and Gill, 1987; Jobling, 1994; Cho and Bureau, 1995; De Silva and Anderson, 1995). Basically this means that all the energy entering and leaving a system should be accounted for and can be represented in its simplest form in the following equation as:

$$E_i = E_o + E_g$$

where E_i = food consumed

E_o = energy lost in faeces (F), urea and ammonia (U), through gills (G), mucous and sloughed cells (MSC) and energy cost of metabolic processes (R): (U, G, MSC and R = catabolism).

E_g = energy used in growth (anabolism)

With this approach, growth (either somatic or reproductive) is represented as an anabolic component of an animal's total metabolism. Each aspect of metabolism can be represented in energy units (joules). The respiratory metabolism (R) can be divided into the following subcomponents: standard metabolism (R_S) = fish at rest; routine metabolism (R_r) = routinely active fish; feeding metabolism or specific dynamic action of fish = (R_{sda}) and active metabolism (R_a) = actively swimming fish. The oxidation of foodstuffs during metabolism can be estimated by measuring the oxygen consumption of the fish which in turn can be converted to energy units (1l O_2 = 19.4 kJ). Thus, each component of metabolism can be determined and

insight gained into the relationships between the food consumed and the various components of utilization of that food including growth under different conditions such as temperature, stress, disease, feeding regimes, diet, and at varying levels of activity. The energy budget equation is a means of balancing the energy intake (food consumed) against energy expenditure (loss in faeces, urine, mucous and sloughed cells, through gills, energy used in metabolic processes and the component used in the production of fish tissue - growth).

Brett and Groves (1979) carried out an extensive review of published data by workers on physiological energetics (Mann, 1965; Paloheimo and Dickie, 1966; Warren, 1971; Solomon and Brafield, 1972; Elliot, 1976a,b,c) and from these data devised formulae to serve as a general indication of energy usage in carnivorous and herbivorous fish. These are:

Carnivores: $100\ I = (44 \pm 7)\ M + (29 \pm 6)\ G - (27 \pm 3)\ E$

Herbivores: $100\ I = 37\ M + 20\ G + 43\ E$

Where: I = ingested energy; M = metabolism; G = growth and E = excretion.

These formulae are helpful for comparing the general energy utilization in different feeding types of fish and suggest marked variations in the metabolic use of ingested food between these large trophic levels. They do not, however, account in detail for the energy use of the different subcomponents of metabolism which are of particular importance to fish farmers and field biologists examining a certain species of fish.

Various schemes of energy utilization by fish have been devised by a number of authors and many are discussed by Weatherley and Gill (1987). Most agree with the basic assumption made in the energy equation used by Brett and Groves (1979) but differ in the components they attribute to excretion or to metabolism. In the present study faeces are not considered to be metabolic waste, although it is recognized they consist of both undigested food plus a metabolic component of mucous and sloughed epithelial cells. In practice it is difficult to separate these components of faecal energy and generally the metabolic faecal content is thought to be very low. The digestible energy component is therefore equal to the ingested food energy minus the energy lost in the faeces. In this study the urinary energy is considered as the energy products of metabolic processes *e.g.* urea and ammonia. The gill excretion energy is equal to the energy of the compounds excreted through the gills. A diagrammatic representation of the energy utilization of a fish is shown in Figure 4.1.

Energetic approaches and the use of predictive energy budgets have flourished over the past 15 years. They are particularly useful in aquaculture where they can be tested over relatively short time periods. For instance the effect of a new dietary formulation, change in

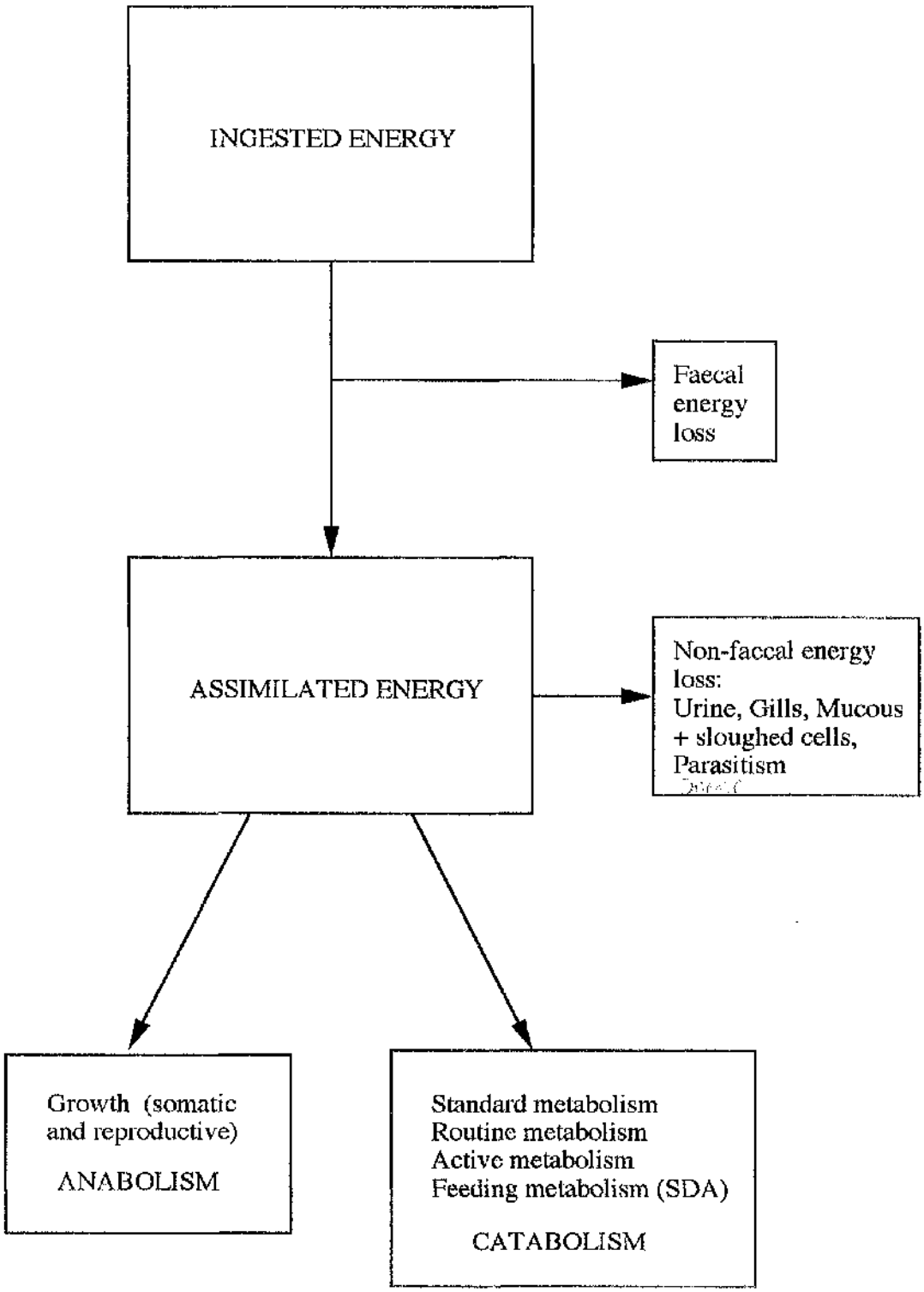


Figure 4.1 Diagrammatic representation of the utilization of ingested energy by a fish

temperature, feeding regime, stress and disease on the growth rate of fish can be predicted quite rapidly. Data can also be gathered at intervals over longer time periods to build up a picture of seasonal changes in growth but without experiencing the inherent problems involved in long-term growth experiments. This approach was adopted to study the energetics of sockeye salmon, *Oncorhynchus nerka,* (Brett, 1983). Schreck (1981) produced models for the effects of stress on smolting in salmonids and, more recently, Davis and Schreck (1997) investigated the metabolic cost of stress due to handling in juvenile coho salmon *O Kisutch.* A study on the energetics of striped bass, *Morone saxalilis,* bluefish, *Pomatomus saltatrix,* and weakfish, *Cynoscion regalis,* showed the growth of the three species appeared to be related to the water temperatures experienced during estuarine residency (Hartman and Brandt, 1995). The proportion of energy devoted to growth in six species of teleosts was shown to range from 21.3 % to 63.4 % (Cui and Liu, 1990). The Chinese catfish, *Siluris meridionalis,* was demonstrated to use around 60 % of the ingested energy on growth (Xie and Sun, 1993). Bioenergetic approaches were used to assess the conditions pertaining to the long-term successful maintenance of the bull shark, *Carcharhinus leucas,* (Schmid and Murru, 1994) and to estimate the food consumption of roach, *Rutilus rutilus* (L) in a eutrophic lake (Horppila and Peltonen, 1997)

Bioenergetics models are also very important in the understanding and modelling of marine ecosystems since the food consumed by a fish and its relationship to growth can be applied directly in the study of transfer of energy from one trophic level to another in the food chain (Steele, 1974). Such uses of bioenergetics models along with new applications have recently proliferated *e.g.* predictions of larval survival, estimates of the intensity and dynamics of predator-prey interactions, nutrient cycling within aquatic food webs of varying trophic structure, and analysis of ecosystem function (Bevelhimer and Adams, 1993; Brandt and Hartman, 1993; Hansen *et al.,* 1993; Labar, 1993; Ney, 1993; Rudstam *et al.,* 1994).

Some critics of bioenergetics models claim the models lack sufficient detail to produce reliable results in field applications whilst others have claimed they are too complicated and too species specific and so it is unlikely that the general hypotheses of fish energy budgets will be of great use to the aquaculturalist (De Silva and Anderson, 1995). However, some studies have combined more conventional growth studies to test or corroborate predictions of bioenergetics models for certain fish. For example, Fox (1991) compared food consumption and growth rates of young of the year walleye, *Stizostedion vitreum vitreum,* with predictions of a bioenergetic model for walleye by Kitchell *et al.* (1977) and found that maximum walleye growth rates were adequately predicted by the model but food consumption was much lower than predicted. Boggs (1991) estimated the proportion of food energy used for growth and maintenance was 65 % in adult anchovy, *Engraulis mordax,* tested at different swimming speeds and noted that the

bioenergetics model derived for adult anchovy was consistent with observed growth and reproduction rates, and the few measurements of ration and swimming speed in nature. Boisclair and Sirois (1993) tested the hypothesis that activity rates can represent a large and variable component of a fish's energy budget by directly comparing model predictions with direct estimations of growth, consumption, and activity rates. In general, the more comprehensive the study, the more accurate the bioenergetic model will be (Jobling, 1983b).

To achieve a more comprehensive picture of the bioenergetics of juvenile turbot, aspects of the respiratory metabolism under varying conditions were investigated and compared with direct estimations of the food consumed, digestive efficiency, growth and body composition of the fish.

4.1.2 Respiratory Metabolism

In homeotherms, the main demand for food before any somatic or reproductive growth can be achieved is to meet the maintenance demands of the animal *i.e.* the 'basal metabolism' or, in other words, the minimum rate of energy expenditure to keep the organism alive (Brett and Groves, 1979). Poikilotherms have resting metabolic rates 10-30 times lower than mammals and up to 100 times less than small birds of the same weight (Brett, 1972). Due to such low maintenance demands, fish may survive long periods without food. However, the ease of elevation of the resting metabolic rate in fish along with the accompanying problems in measuring the resting rate has resulted in a variety of methods and a number of terms being used synonymously. The terms used in the present study are explained to avoid any confusion. Generally, these are the definitions given by Brett and Groves (1979).

The term 'standard metabolism' was used in early studies to describe the minimum rates observed in fish (Krogh, 1914). 'Standard metabolism' will be retained in the present study and refers to the minimum rate of oxygen consumption of an organism at rest in the postabsorptive state and thermally acclimated, or in other words, maintenance metabolism. It is difficult to determine the post absorptive state exactly, as the time taken for the effects of the last meal to completely clear are dependent upon the size of the fish, the quantity, quality and consistency of the food, water temperature, stress levels and disease status. A period of between 48-72 h after feeding is commonly allowed before attempting to measure standard metabolism in fish. 'Routine metabolism' is defined as the metabolic rate of an organism during normal spontaneous activity. The 'feeding metabolism', or, 'specific dynamic action' (SDA) is the metabolic heat loss associated with the digestion and biochemical transformation of food following a meal (Beamish, 1974). The active oxygen consumption is defined as the maximum oxygen consumption observed during sustained cruising swimming (Priede, 1985). The conditions and experimental set-up used

to determine 'standard', 'routine' and 'feeding metabolism' (SDA) in the present study were all standardized and are detailed in section 4.2.1.1.

Metabolic rate in mammals is often recorded directly by calorimetry measuring 'heat loss', however, this is unsuitable for fish due to their relatively low heat production and the high thermal capacity of the water where the 'heat loss' would be estimated. In fish, metabolic rate is usually determined by measuring oxygen consumption. Various methods have been devised for measuring the oxygen consumption of fish and these are reviewed in section 4.21.1. Once the oxygen consumption of a fish at rest has been recorded, the amount of energy required for maintenance of bodily functions of the fish can be calculated by using an oxycalorific coefficient. A figure of 19.4 kJ per l of oxygen consumed is recommended for fish (Jobling, 1994). This is based on the assumption that the metabolism is aerobic and that the primary respiratory substrates are protein and lipid.

4.1.2.1 Factors affecting metabolic rate

i) Body size

A number of factors may affect the metabolic rate of the fish such as body size, temperature and oxygen availiblity. The relation of body weight to standard metabolic rate has received much attention (Winberg, 1956, 1961; Fry, 1957). The metabolic rate is greater per g of tissue in smaller fish than that for larger fish measured at the same temperature. This scaling effect can be described mathematically by the formula: $Y = aX^{-b}$, where, Y is the metabolic rate, a is a constant, X is body weight, b describes the effect of body size on the metabolic rate (= the slope of the regression line).

Values of between 0.7 - 0.8 have been reported for b for fish. A slope of around 0.7 has been recorded for several species of flatfish (Edwards *et al.*, 1969, 1970; Jobling, 1982; Brown *et al.*, 1984; Waller, 1992), whilst in roundfish the exponent is reported at values of around 0.8 (Winberg, 1956; Brett, 1965; Brett and Glass, 1973; Waller, 1992; Jobling, 1994; De Silva and Anderson, 1995).

ii) Temperature

Temperature may affect the metabolic rate of fish in two ways. Generally, if a fish is acclimated to a certain temperature and is then moved to a higher temperature there will be an acute change in metabolic rate which, if the fish is returned to its original temperature of acclimation, its original metabolic rate will be restored. If, however, the fish is kept at the new higher temperature for a period of a few weeks there will be a decrease in the fish's metabolic rate as it becomes acclimated. Temperature fluctuations may be considerable and may occur rapidly in an intensive fish farming situation and therefore the affect of temperature on the metabolic rate

is very important. The term Q_{10} is often used to describe the effects of temperature on the metabolic rate. This refers to the increase in the rate of physiological process accompanying a 10 °C rise in temperature *e. g.* if the rate doubles Q_{10} is 2 (Prosser, 1973).
The Q_{10} can be derived from the formula:

$$Q_{10} = (V_2/V_1)^{10/[T_2-T_1)]}$$

where V_1 and V_2 are the rates of the physiological process at times T_1 and T_2.

iii) Feeding

An increase in metabolic rate following feeding is common to all vertebrates. The rise in metabolic rate due to feeding may vary with the composition of the diet, the energy level, the size of the meal and the temperature of the water (Jobling, 1981a,b). The period of time the metabolic rate remains elevated varies in relation to these factors and increases with increasing period of food deprivation (Soofiani and Hawkins, 1982). Each of the aforementioned factors influences the rate of passage of food through the alimentary tract and it is possible therefore that a relationship exists between rate of gastric evacuation and the heat increment (Jobling, 1981a,b). The proportion of the energy intake dissipated as heat increment may be as much as 30 % (Pierce and Wissing, 1974). It is therefore important to assess the factors leading to an increase in heat increment and to observe those feeding procedures which result in the minimum energy cost and consequently leave more energy for growth.

The above factors may affect the metabolic rate in the short term over hours or days but there is some evidence to suggest that the long-term nutritional history of an animal may influence the standard metabolic rate. This would have the effect of a well fed fish having a higher standard metabolic rate than a poorly fed conspecific of equivalent age. To investigate if some form of physiological compensation to ration level occurs in turbot, the standard metabolic rate was determined for fish maintained on a reduced ration for 10 weeks and this was compared with fish fed to satiation daily for 10 weeks.

iv) Activity

There has been a great deal of debate over the years as to what proportion of the energy intake of a fish is used in active metabolism. Some authors claim that the energy expended in active metabolism accounts for a large proportion of a fish's energy intake (Brett and Groves, 1979). Others claim that levels for active metabolism are often obtained by forcing fish to swim against a current and that this does not relate to activity levels in nature (Jobling, 1994). Some authors say the energy cost of activity may be closer to that described as 'routine metabolism' as defined by Brett and Groves (1979). Recent consensus is that relatively little time and energy are

expended on swimming activity and that this is approximately equal to 10 % and probably no more than 20 % of the energy intake (Jobling, 1994). However, the energy cost of activity is likely to vary between species depending on the behaviour and body shape of the fish (De Silva and Anderson, 1995). It is worth noting that activity does not only result in a negative effect on growth. It has been shown in Arctic charr fry, (*Salvelinus alpinus*) that sustained swimming was related to increased growth rates (Christiansen *et al.*, 1989). A method for measuring the routine activity in turbot was investigated in the present study.

v) Stress

Various factors may be attributed to causing stress in fish (see Chapter 1.9 for details) all of which have the effect of increasing the metabolic rate of the fish. This means a greater proportion of the energy intake is used in catabolic metabolism rather than growth. The salinity of the water has a marked effect on the metabolic rate of fish as osmoregulation is metabolically costly at salinities above and below the iso-osmotic point between body fluid and ambient water, for a given species (Farmer and Beamish, 1969; Von Oertzen, 1984; Waller, 1992; Gaumet *et al.*, 1995).

Other forms of stress such as handling, crowding, acute changes in temperature, low oxygen tension or in some cases saturated levels of oxygen may increase the metabolic rate and this in turn may reduce the capacity for growth in fish (Wedemeyer and McLeay, 1981; Pickering 1993; Waring *et al.*; 1996; Davis and Schreck, 1997). There may also be a natural fluctuation in metabolic rate following seasonal cycles (Karas, 1990) or even following Lunar cycles (Farbridge and Leatherland, 1987).

In the present study precautions were taken to ensure any stress suffered by the fish was kept to a minimum.

4.1.3 Growth

The proportion of energy available for somatic and reproductive growth is dependent on the amount of assimilated energy gained from the food consumed minus the proportion used for maintenance, feeding and active metabolism and the energy lost in excretion. Although growth may appear to have the lowest immediate priority, it is ultimately the feature that dictates the survival of a species. Many factors affect the growth rate of fish but, in general, there is a decrease in growth rate with an increase in size and age of the fish. Digestion, absorption, assimilation, metabolic expenditure and excretion are all influenced by many factors and, in turn, these affect the growth rate of fish. A knowledge of how these factors affect the growth rate of fish species is essential to achieve optimum growth.

4.1.3.1 Measurement of growth

Growth in fish generally refers to a change in magnitude. This may be measured in terms of length or weight of the whole fish, or of its various tissues and organs. It may also be measured in terms of the relative proportion of the protein, lipid, carbohydrate, ash and water content of the body, or, in terms of the energy content of the body. In fish farming, it is valuable to measure somatic growth in terms of efficiency of food utilization (conversion efficiency), or, in terms of the fate of the assimilated food in respect to the relative growth of the body tissues and the composition of these tissues. The latter method is perhaps the most reliable as this gives a deeper insight into the efficiency with which the ingested food energy is deposited as body tissue. For example, a fish fed maximally will tend to have a higher lipid content than one fed on reduced rations and older fish tend to lay down more lipid than younger fish. However, the conversion efficiency (growth/ration x 100) is perhaps the quickest and simplest indicator of the nutritional adequacy of a particular diet and ration level, the suitability of the environment the fish is held in and the health of the fish (Brett and Groves, 1979).

Growth studies on fish usually concern the change in magnitude (length/weight/energy content) of a sample of individuals over a period of time; that is, growth is usually expressed as the growth rate. This may be expressed as a percentage body weight gain per day, or, as an instantaneous percentage growth rate per unit of time using both length and weight as indicators of changes in size *i.e.* the specific growth rate. This is also known as the relative growth rate and is generally greatest in the smallest fish (Brett and Groves, 1979) (See 4.2 for details).

Other indirect methods of assessing the energy status of fish which are commonly used by fish biologists are, the condition factor (CF), which is an index of the body condition or 'fatness' or 'well-being' of fish, that is the extent to which the total body weight of a fish is heavy for its length, the somatic condition factor (SCF) which is the extent to which the carcass weight is heavy for a given length, and the hepato-somatic index (HSI) which is a measure of the relative weight of the liver in relation to body weight (Chellappa *et al.*, 1995) (see 4.2. for details).

4.1.3.2 Growth-ration curves

It might be expected that the more food a fish consumes the faster it will grow with the maximum growth rate being equal to the maximum amount of food a fish can consume. The relationship between the growth rate and the ingestion rate in fish was examined by Paloheimo and Dickie (1966) for several species of fish and they concluded there was a tendency for gross conversion efficiency to fall with increasing ration size. That is, maximum growth rate and maximum conversion efficiency occur under different feeding conditions.

The development of a mathematical equation to describe the relationship between growth and ingestion has progressed over the years (Paloheimo and Dickie, 1966; Ursin, 1967; Warren and Davies, 1967; Brett *et al.*, 1969; Kerr, 1971a,b,c; Ware, 1975). However, Brett *et al.* (1969) in their study on the growth of sockeye salmon used a simple method in which they plotted growth rate against rations (GR curve) and from this the ration levels approximating to starvation, maintenance, optimal and maximal growth could be obtained.

The use of the gross conversion efficiency equation was further developed by Brett and Groves (1979) who estimated the effect of various environmental factors (temperature, light, salinity, oxygen tension) and biotic factors (ration, size, competition) on the growth-rations curve.

If ration is expressed in the same units as growth rate (% body weight per day) then the relation for gross conversion efficiency (GCE) is found to be a dome shaped curve and the value of rations that produce the highest value for GCE can be estimated. At maintenance rations, GCE is zero; at optimal rations, GCE is equal to the peak of the dome. Variations in the shape of the curve occur under different environmental conditions and biotic factors.

Brett *et al.* (1969) investigated the effect of temperature on the growth-rations curve of fingerling sockeye salmon. At 5°C the curve is far to the left with a low growth rate and small maintenance ration. As temperature increases the curve shifts to the right and is elevated as optimal temperatures are approached and hence maximum growth rates are increased and maintenance rations are increased. Temperatures above optimum produce a further shift to the right as maintenance costs are increased but are accompanied by a decrease in maximum growth rate despite an increase in maximum rations; that is, conversion efficiency has declined (Brett, 1979). In other words, the efficiency of digestion and absorption decrease with increasing temperature and ration size. Allen and Wootton (1982) studied the relationship between growth rate and ration size at different temperatures for the three-spined stickleback, *Gasterosteus aculeatus*. They found that at 3°C the maintenance ration was 1 % body weight per day, whilst at 15°C the maintenance ration was 2 % body weight per day. This indicates that basal metabolic rate increases with increasing temperature. This occurs in poikilothermic animals where body temperatures and hence the rate of metabolic reactions are increased with temperature. The authors also showed that maximum ration was equal to 2.5 % at 3°C, whilst maximum ration was equal to 8 % at 11°C. The increased ration was accompanied by an increased growth rate. However, at above optimal temperatures for fish, growth rate is limited by an increase in metabolism (De Silva and Anderson, 1995).

Osmoregulatory processes have an energy cost (Brett and Groves, 1979; Waller, 1992; Gaumet *et al.*, 1995) and therefore finding the salinity conducive to optimum growth rates is important in fish farming. The capacity to adapt to various salinities has been studied mostly in

euryhaline species living in freshwater, brackish or sea water, for example in the plaice, *Pleuronectes platessa* (Hutchinson and Hawkins, 1990; Nonnotte and Truchot, 1990). Recent studies on the turbot have shown they have an ability to osmoregulate between 10‰ and 35‰ (Waller, 1992; Gaumet *et al.*, 1995) and, although they are a marine fish, data suggest growth for 'o' group fish may be improved by acclimation to lower salinities.

Photoperiodic influences on growth have been observed in many species of fish and have been shown to be independent of temperature changes for some species of salmonids (Jobling, 1994). Growth rates of Atlantic salmon (*Salmo salar* L.) subjected to natural daylight were depressed during the autumn and winter, whilst no change in growth rate was observed in fish held under a continuous light regime (Forsberg, 1995). A photoperiodic effect on the routine oxygen consumption of turbot has been observed (Waller, 1992) indicating the importance of studying the scope for metabolic adjustments under changing environmental conditions in order to establish the parameters conducive to optimum growth for different size turbot.

In the present study, standardized methods for measuring oxygen consumption were developed in order to obtain the standard metabolic rate of juvenile turbot. The relationship between body size, temperature, ration level and feeding frequency and the standard metabolic rate were investigated. Continuous recordings of standard metabolic rate were carried out to observe if any daily rhythms occurred. Feeding metabolism was measured by providing turbot with different sized ration levels and length of time taken for metabolic rates to return to standard levels noted. A method was devised to measure the routine metabolic rate of juvenile turbot. From these data the amount of energy used per day for standard, routine and feeding metabolism could be estimated.

Feeding experiments (see Chapter 3 for details) carried out in the present study provided data on the amount of food consumed per day by fish held under different temperature and feeding regimes. The assimilation efficiency of each group was analysed. Data were gathered on changes in body weight and length for each group during a 10 week period, providing information on differences in growth rates and condition factor (W/L^3) of the fish. Growth-rations curves were then produced for each group of fish. Conversion efficiencies were obtained for each group and were used to investigate the relationship between certain biotic and environmental factors and growth. Finally, the body composition of each group was analysed for differences in protein, lipid and moisture content of the fish and the hepato-somatic index obtained.

Data on ingestion rates, digestive efficiencies, respiratory metabolism and parasitic status were used to construct a bioenergetics model of energy partitioning of food consumed by turbot. These data were also used to predict the proportion of the energy consumed available for growth in turbot held under different conditions. It was also decided to test this model by direct

measurement of growth rates, using weight/length measurements and by measuring changes in total energy content.

4.1.4 Research aims

1 To develop a method for measuring the standard metabolic rate of juvenile turbot.

2 To determine the relationship between body size and standard metabolic rate.

3 To ascertain the effect of the length of time since feeding on metabolic rate

4 To examine the effect of acute and long term (acclimation) temperature changes on the standard metabolic rate.

5 To assess the affect of different feeding levels on standard metabolic rate (determination of specific dynamic action).

6 To discover if long term adaptation to different feeding levels occurs in the standard metabolic rate.

7 To devise a method for measuring routine oxygen consumption in turbot.

8 To measure the ingestion rates of fish held under various conditions.

9 To determine the growth rate of fish held under different conditions.

10 To establish the conversion efficiencies of fish at different temperatures and on different feeding regimes.

11 Derive growth-rations curves from data to explain relationship between food intake and growth under different environmental conditions.

12 Construct bioenergetics model of energy partitioning in turbot, including estimate of energy consumed used by tapeworms in infected turbot.

13 Use model to predict proportion of energy consumed that is available for growth under different conditions.

14 Use direct measurements of ingestion rates, growth rates and energy content at start and finish to test precision of bioenergetics model.

15 Determine condition factor, hepato-somatic index and carry out analyses of body composition of fish held under varying conditions to assess type of growth occurring under different conditions.

4.2. MATERIALS AND METHODS

The overall experimental design, feeding procedures employed and many of the experimental procedures used in this Chapter are described in detail in Chapters 2 and 3.

4.2.1 Respiratory metabolism

The measurement of the amount of oxygen consumed by fish is a convenient, indirect method of monitoring the energy costs associated with maintenance metabolism, feeding metabolism and active metabolism. The energy equivalent is estimated by using an oxycalorific coefficient value of 19.4 kJ l^{-1} of oxygen, which assumes lipid and protein are the main substrates and that the fish is respiring aerobically (Jobling, 1994).

Various methods of measuring the oxygen consumption of aquatic animals have been developed including the measurement of oxygen consumption in a closed chamber, the oxygen depletion during flow through a chamber, and also the measurement of oxygen consumption using manometric methods such as the Gilson system. Problems arise in closed systems due to decreasing O_2 concentrations and the build up of metabolic waste products such as CO_2, NH_3 and H^+. These problems are exacerbated during long term experiments and therefore limit the use of closed systems for such purposes. Despite the limitations of closed systems in aquatic respirometry, von Oertzen (1984) stated that, until 1984, approximately 90 % of all measurements on aquatic animals were based on these methods.

Open-flow respirometers allow continuous measurement of oxygen consumption whilst maintaining relatively stable chemical and dynamic conditions. Constant flow rates can be maintained in open-flow systems either by a high precision pump or by a constant head of water. These features make it possible to carry out long term measurements of oxygen consumption. However, a number of practical problems are encountered in flow-through respirometers which may influence the results of experiments. For example, the animals may react to the geometry of the respirometry chamber and to the flow regime. According to Hughes *et al.* (1983), these factors give widely varying results in rates of oxygen consumption. Although the development of stable oxygen sensors and pumps allows recording of oxygen over long time periods (Soofiani and Hawkins, 1982), problems of starvation and bacterial contamination should be recognized and dealt with. The design of some aquatic respirometers and the type of oxygen electrode sensors used may involve large time delays or lags between the animal chamber and the electrode sensor and this should be taken into account (Fry, 1971). The delays in the recorded signals may be due to the oxygen sensor's inertia, to the type, length and diameter of the tubing used and to the flow rate and mixing efficiency within the animal chamber. Hughes et al. (1983); Niimi (1978) and Propp *et al.* (1982) all described mathematical correction calculations to deal with lag in specific situations and Kaufmann *et al.* (1989) described a correction formula for a more general situation. Steffanssen (1989) reviewed the problems and sources of errors involved in closed and flow-through respirometry and how to correct them. However, each individual study should design the most suitable apparatus for their experimental animals and experimental design. The

optimum design should be a small respirometer chamber and high flow-through rate with efficient mixing. The apparatus should be cleaned thoroughly and frequently and tubing should be replaced regularly to prevent bacterial build up. The conditions should be standardized to decrease variability as far as possible.

4.2.1.1 Description of constant-flow respirometer

The apparatus used in these experiments was the same as that described by Jobling (1978), except for a number of refinements and modifications made to suit the experimental animals. A diagrammatic representation of the constant-flow respirometer used for this research is shown in Figure 4.2.

The respirometer consisted of an animal test chamber and a polarographic chamber containing a 'Clark-type' oxygen probe connected to a chart recorder. A supply reservoir of sea water located above the animal chamber contained an overflow tube connected to a catchment reservoir below. Water was recycled to the supply reservoir via an Eheim pump, thus maintaining a constant water level and hydrostatic pressure. The water from the supply reservoir flowed by gravity through the animal chamber, then through the polarograph chamber and back to the catchment reservoir. Both reservoirs were supplied with air stones to ensure air saturated water was used.

The oxygen probe was fitted and sealed into a Perspex chamber containing a magnetic stirrer which allowed a sufficiently fast flow rate to ensure the membrane was insensitive to flow rate variations but slow enough to detect the oxygen consumption of the experimental fish.

The supply reservoir, animal and polarograph chamber and catchment reservoir were connected together using vinyl tubing (Portex NT12), whilst rubber laboratory tubing was used to connect the Eheim pump to the supply reservoir.

Two animal chambers of 650 ml and 850 ml respectively, were used to test the oxygen consumption of juvenile turbot (Figure 4.2). The size of chamber used was dependent on the size of the fish. Each contained an inlet at the bottom of one side of the chamber and an outlet at the top of the opposite side and to ensure adequate mixing of water occurred, the chambers also housed a perforated perspex tube containing a magnetic stirrer. The tubing on either side of the animal chamber was fitted with three-way stop cocks so that the water could be shunted directly to the oxygen probe. This enabled probe readings to be taken prior to and during each test. Also, the sodium dithionite reservoir allowed probe readings to be zeroed both during and after tests with the stopcock preventing leakage of dithionite into the animal chamber. Since the probe was calibrated using sodium dithionite and fully saturated sea water it was possible to adjust the flow rate through the chamber such that the experimental fish consumed not more than 30 % of the

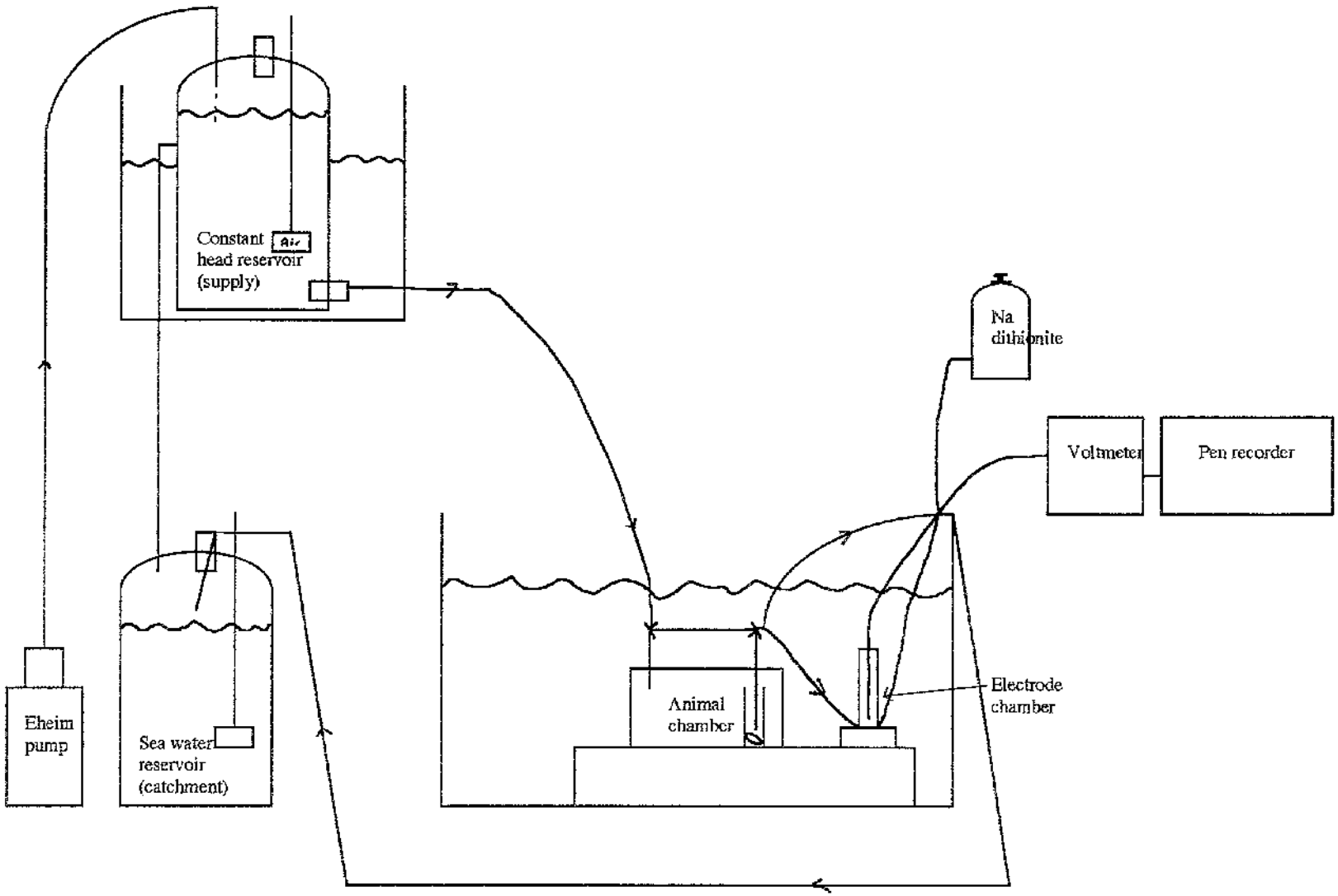


Figure 4.2 Diagrammatic representation of constant-flow respirometer.

oxygen. Flow rate was measured by shunting the water supply to the animal chamber into a measuring cylinder for a period of one minute.

The supply reservoir, the animal and polarographic chambers were surrounded by a thermal jacket and the temperature of the system was controlled using an aquacooler in combination with a temperature control unit. The experimental chamber was always removed from the system whilst still sealed and opened and closed under sea water so that no air bubbles were introduced into the system.

After the introduction of the animal to the system a period of time was required for the system to equilibrate and for the animal to settle. Standardization experiments were conducted to determine a suitable length of time for equilibration to take place. Equilibration time was dependent on a number of variables but an attempt was made to control for as many of these as possible and to reduce the delay between introduction of the animal to the system and the first reading. These steps included:

1 Handling of fish reduced to minimum prior to experiment;

2 Animals fasted for 48 h prior to experiment;

3 A two hour period for the animals to become acclimatized to the chamber;

4 Prevention of wide fluctuations in flow rate;

5 Temperature kept at constant level;

6 Laboratory air conditioning unit remained on and kept at constant temperature

The design of the apparatus allowed continuous recordings of the oxygen consumption of experimental animals to be taken over long time periods. Standardization experiments demonstrated that approximately two hours was an adequate interval for the animal to settle into the experimental chamber and for the apparatus to equilibrate. The oxygen consumption of the resting animal then remained fairly constant for a period of approximately 12 hours although fluctuations were observed over a 24 h period. The results of these preliminary experiments also showed that diurnal variations in oxygen consumption were not strongly evident in juvenile turbot, although a slight increase in respiration between 1200 h and 1800 h was observed. Disturbances caused by human activity in the laboratory did have an effect on oxygen consumption and these were controlled for as far as possible. The experimental design is described in detail in Chapters 2 and 3 and depicted in a flow chart (Figure 3.2).

4.2.2 Growth

Methods for measuring the weight and length of the fish are described in Chapter 2.

4.2.2.1 Specific growth rate

Growth rate in turbot was expressed as an instantaneous percentage growth rate per unit time (Ricker, 1979) using weight and length as indicators of change in size:

$$\text{Specific growth rate} = \frac{(\ln W_2 - \ln W_1)}{t_2 - t_1} \times 100$$

where: W_1 = the mean weight of sample 1(g)

W_2 = the mean weight of sample 2(g)

t_1 = the date of sample 1 (d)

t_2 = the date of sample 2 (d)

To determine the specific growth rate in terms of length the same formula was used but mean lengths (mm) were used instead of mean weights. Both give measures of growth relating to the sampling time scale.

4.2.2.2 Conversion efficiency

The gross conversion efficiency measures the growth rate of fish in relation to the feeding rate. It is commonly used as an indicator of the physiological energetics of a fish held under experimental conditions instead of determining a complete energy budget or in order to test the energy budget of a fish (De Silva and Anderson, 1995). The gross conversion efficiency was determined in the present study using the following equation:

$$\text{Gross conversion efficiency (K)} = \frac{\text{Growth increment (G)}}{\text{Ration (R)}} \times 100$$

where growth increment and ration were both measured in terms of energy content (joules).

4.2.2.3 Growth-ration curves

Growth-ration curves depict the overall relation of growth-rate to the rate of food uptake and give important information about optimum and maintenance rations. Both growth and ration are expressed in terms of % body weight per day.

4.2.2.4 Condition factor

The condition factor (CF) is an index of the body condition or 'well-being' of fish and is based on the hypothesis that the heavier the fish is for a given length the better the

condition than the lighter fish. The relationship between the weight and the length of fish may be expressed by the following equation:

$$(1) \qquad W = aL^b$$

where W is weight, L is the length and *a* and *b* are constants. The linear form of the equation is:

$$(2) \qquad \text{Log } W = \log a + b \log L$$

where b is the slope of the line and log a is the intercept on the y axis.

If the fish is growing isometrically then the length exponent is thought to take the value of 3.0 where weight increases as the cube of the length. If this value is significantly higher or lower than 3.0 then the growth is allometric (Ricker, 1979). A value for the slope of greater than 3.0 indicates the fish are becoming heavier for their length whilst a value of less than 3.0 indicates they are becoming lighter. If the slope of the line does not deviate much from 3.0 it can be substituted in equation (1):

$$(1) \qquad W = a L^3$$

this may then be rearranged to give:

$$a = \frac{W}{L^3}$$

The constant a is therefore a measure of the condition of the fish. Many authors have found it convenient to use the following simple equation for the determination of condition factor:

$$\text{condition factor (CF)} = \frac{W}{L} \times 100$$

where CF is the condition factor, W refers to the weight in g and L is equivalent to the fork length of the fish measured in cm. This formula tends to fit the body plan of salmonid fish better than other fish *e.g.* the condition factor for eels using the above calculation is significantly less than unity, whereas less elongated species may be greater than unity (Weatherly and Gill, 1987).

4.2.2.5 Hepato-somato-index

The hepato-somatic-index (HSI) is a measure of the relative weight of the liver expressed as a percentage of the total fish weight. The liver is an important store of energy reserves, especially in non-fatty fish, and the HSI is often used (where dissection is possible) as an indirect measurement of the energy status of the fish (Wooten *et al.*, 1978; Campbell and Love, 1978; Chellappa *et al.*, 1995). The HSI is calculated using the following formula:

$$\text{Hepato - somatic index (HSI)} \quad \frac{\text{weight of liver}}{\text{weight of fish}} \times 100$$

4.2.2.6 Analyses of body composition

The methods used for determining the protein, lipid, water, ash and energy content of turbot under various conditions are described in Chapter 2.

4.2.3 Data analyses

All statistical analyses were performed using Minitab version 10.0 for Windows. Parametric tests include Regression analysis and Analysis of covariance. Mann-Whitney non-parametric tests were used. Statistical significance has been accepted at a probability level of less than 0.05. Means are presented ± their standard deviation unless otherwise stated.

4.3 RESULTS

4.3.1 Respiratory metabolism

In order to test the effect of varying intrinsic and external factors on the standard metabolic rate of turbot, a standardized method was first devised. The respiration rates of fish fed 48 h previously were measured continuously for periods of time ranging from 20-48 h. It was hoped that any possible daily fluctuations in standard metabolic rate would become apparent using this method.

4.3.1.1 Standardization and continuous measurement of standard metabolic rate (daily rhythms)

Line diagrams of the respiration rate versus time over a period of 24 h, for six turbot, are depicted in Figure 4.3. All the fish were fed 48 h previously and allowed two hours to

settle before measurements were started at mid-day. Some fluctuation in the respiration rate of the fish did occur, but no particular pattern or daily rhythm was obvious.

The same procedure was followed for another five fish (Figure 4.4) only this time the respiration rate was followed from 1000 h and the readings were started immediately after the fish entered the respiration chamber and continued for 24-48 h. The results showed a drop in respiration rate for the first two hours for four of the five fish with some fluctuations around a standard rate during the following 24 h.

Results for three fish where the readings were started at 1400 h and the fish were allowed a 2 h period to settle into the animal chamber and for three fish where the readings began at 1800 h and no settling-in period was given are shown in Figure 4.5. Again no particular daily rhythm could be followed apart from a possible fall in standard metabolic rate in the early hours of the morning between 0200 h and 0400 h and a possible rise from about 0400-0700 h.

Generally, it was considered that a two hour acclimation period was sufficient before taking a reading to represent the standard metabolic rate of the fish and that these readings should be taken, where possible, between 0900 h and 1600 h. However, it was also noted that this was perhaps not the ideal method as there was a certain amount of fluctuation during any day. Possibly the most accurate method of assessing the standard metabolic rate of the fish would be to take continuous readings over a 24 h period and work out the total oxygen consumption per 24 h.

4.3.1.2 Effect of body size on standard metabolic rate

The results obtained from experiments on the relation between standard metabolic rate and body weight show a positive relationship. However, the increase in oxygen consumption does not increase with equal magnitude for each additional unit of tissue and so smaller fish were found to have a higher rate of oxygen consumption per gram of tissue than larger fish. This scaling effect was found to be represented by the following regression equations for fish acclimated to 10°C and 20°C (Figures 4.6 and 4.7):

$$10°C: \quad LnY - 0.77LnX + 2.027 \quad (r^2 = 0.89, sd = 0.032, n = 75, p = <0.001)$$

$$20°C: \quad LnY = 0.81LnX + 2.152 \quad (r^2 = 0.86, sd = 0.72, n = 22, p = 0.000)$$

From these data the amount of energy used per day for standard metabolism of a specific size of fish at 10°C and at 20°C was estimated (Table 4. 1).

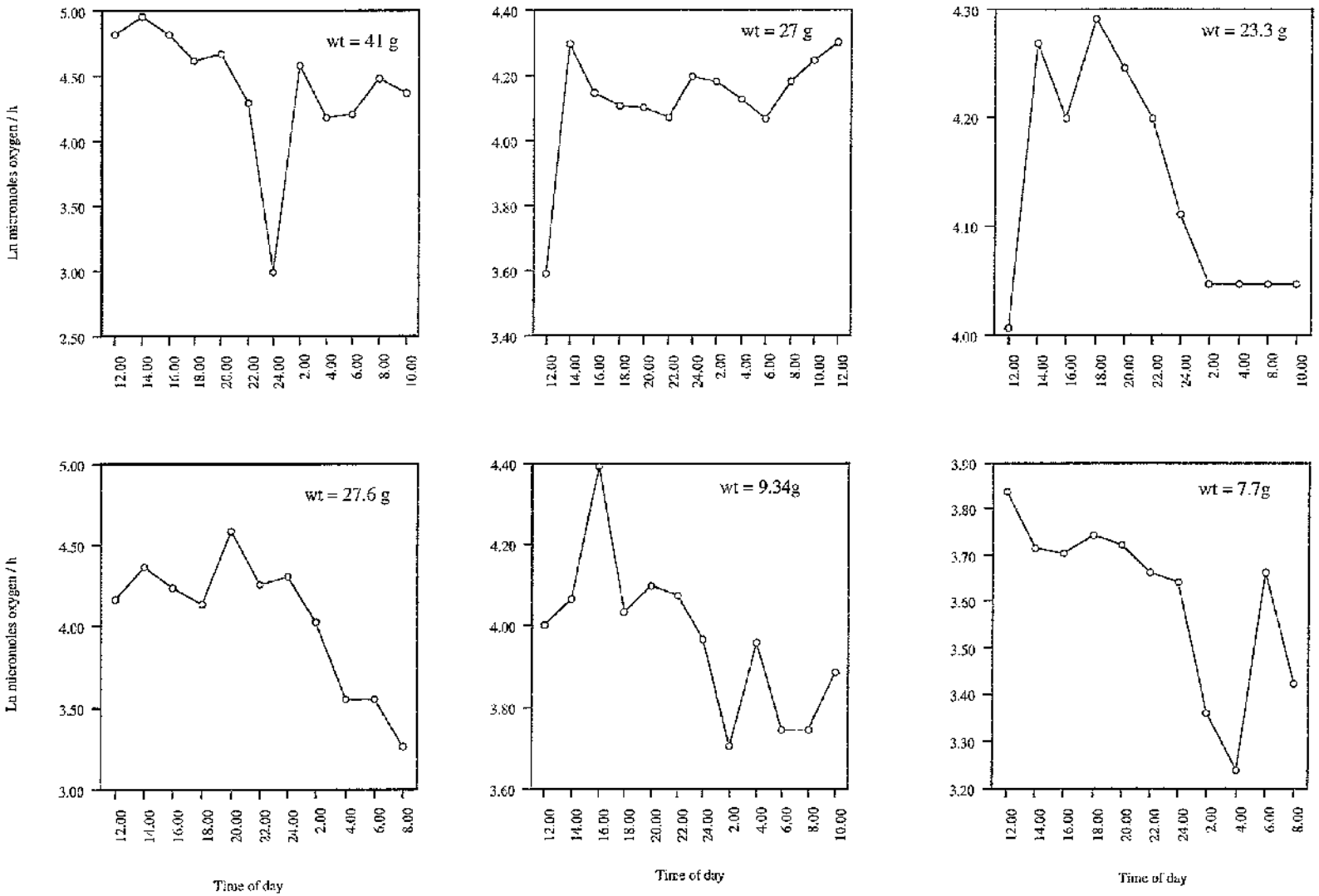


Figure 4.3 Continous measurement of respiration rate of turbot over 24 h period. Fish fasted for 48 h followed by a 2 h period to settle into animal chamber.

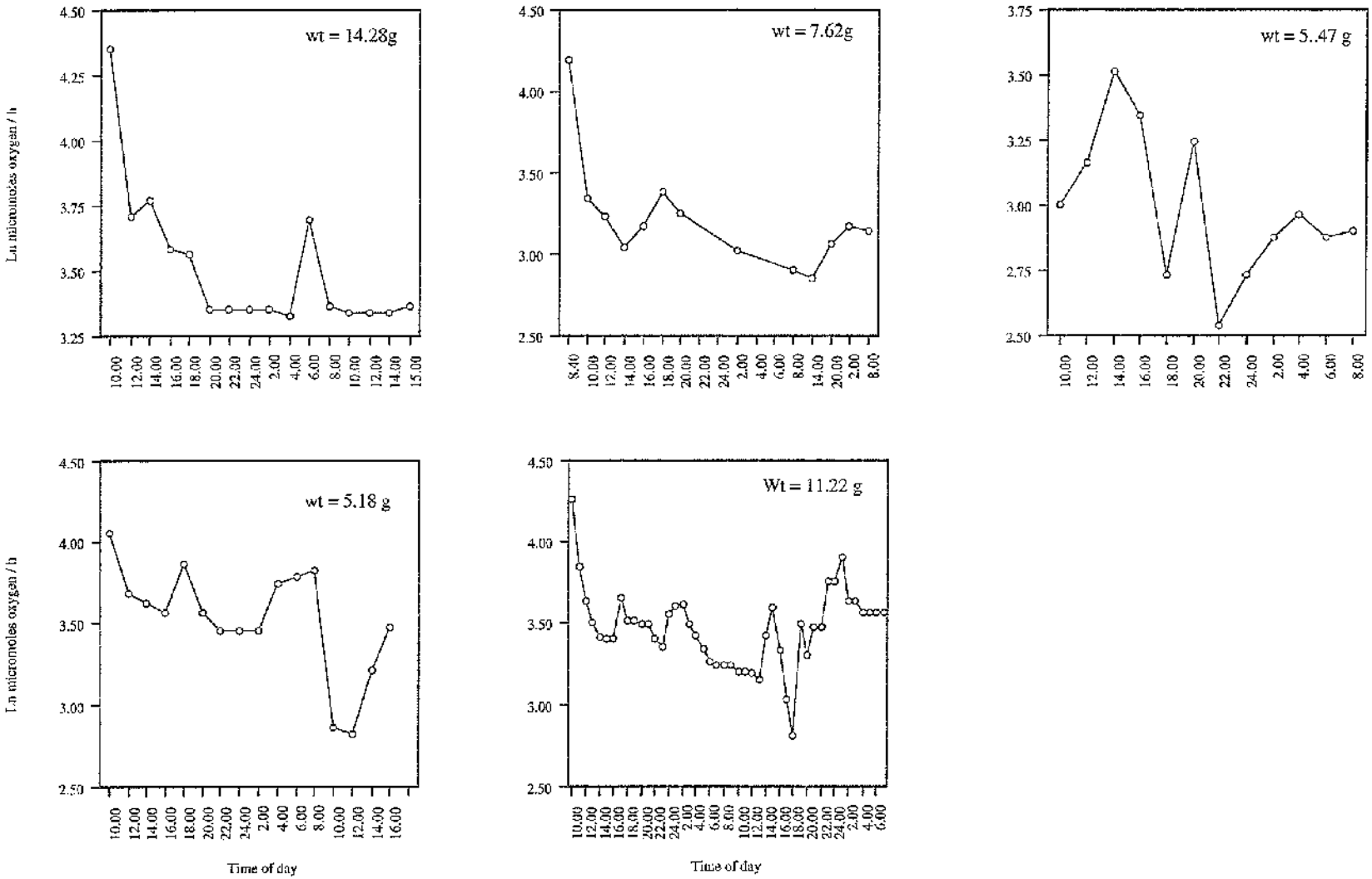


Figure 4.4 Continous measurement of respiration rate of turbot . Fish fasted for 48 h, readings started immediately after entry into chamber.

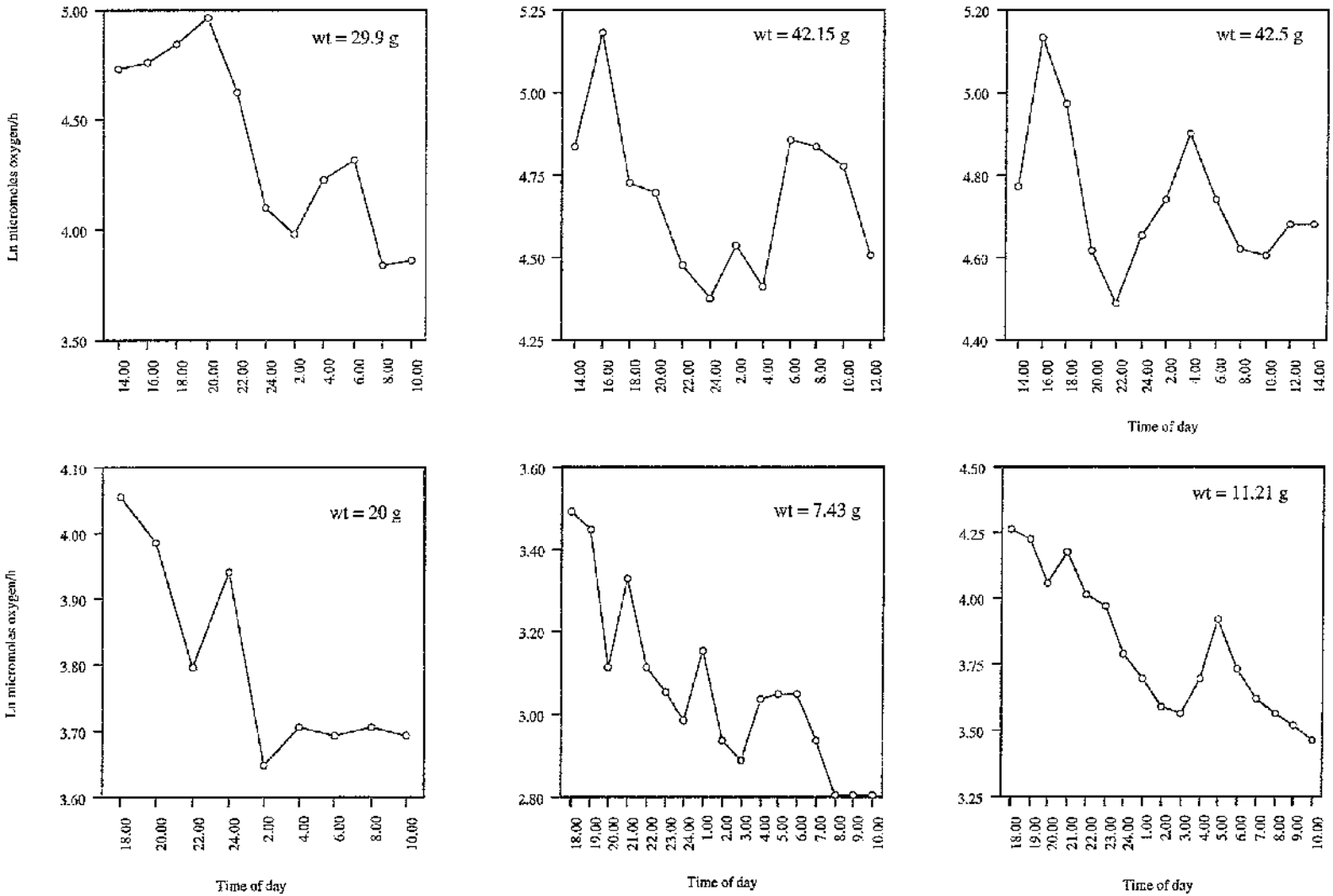


Figure 4.5 Continuous measurement of respiration rate of turbot. Fish fasted for 48 h, the fish in the top three graphs were allowed a 2 h settling in period whereas readings were taken immediately in the bottom three graphs.

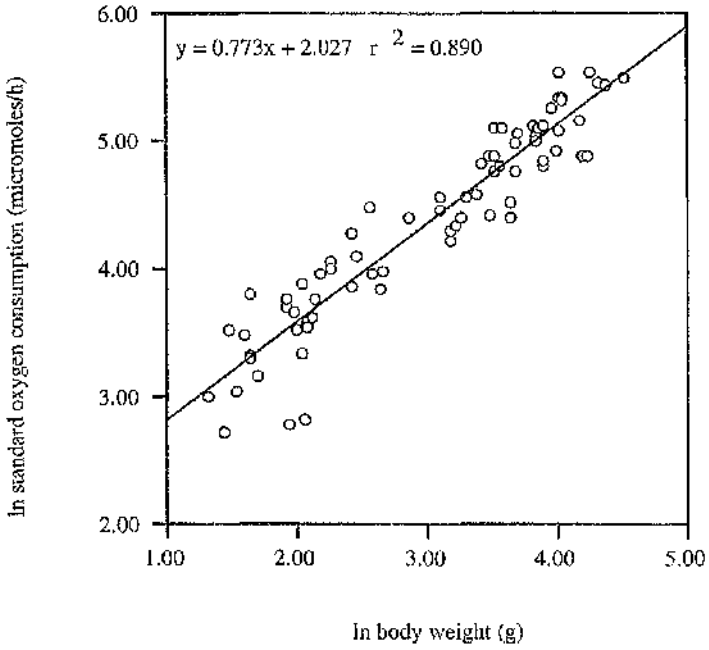


Figure 4.6: Relationship between standard metabolic rate and body weight in turbot. Regression equation is shown in graph. Natural logs (n=75).

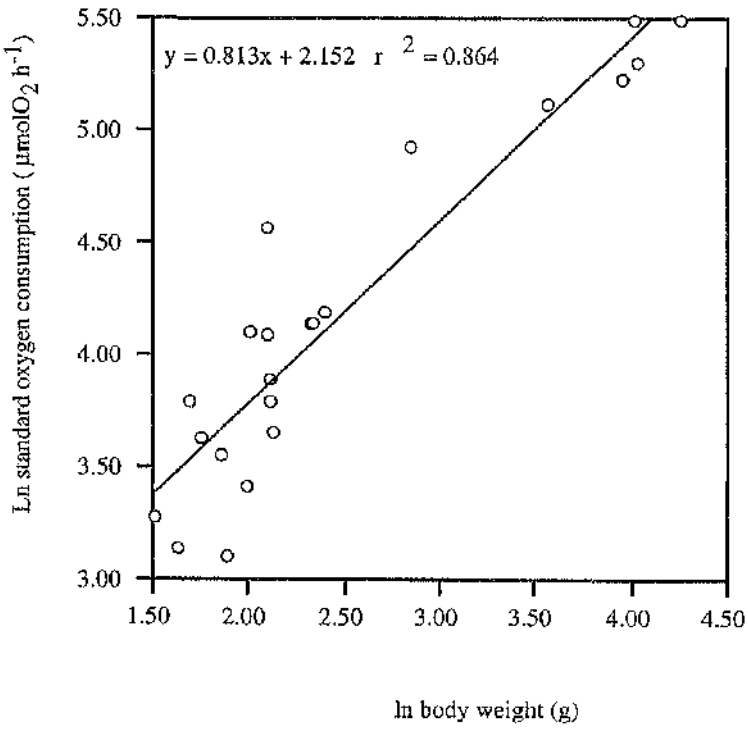


Figure 4.7 Relationship between standard metabolic rate and body weight in turbot acclimated to 20 C. Reggression equation is shown in graph. (n=22).

Table 4.1 The effect of body size on standard and routine metabolic rate of turbot plus energy equivalents used per day. Values are means ± se.

Wt (g)	T (°C)	n	µl O_2 $g^{-1}h^{-1}$	µmol O_2 $g^{-1}h^{-1}$	mg O_2 $g^{-1}h^{-1}$	kJ d^{-1}	Reference source
3-10 (x = 6.7±0.3)*	10	24	114.4 ± 6.7	5.18	0.0856	0.34	Present study
11-20 (x = 14.4±0.8)*	10	10	118 ± 11.2	5.27	0.0829	0.79	Present study
21-30 (x= 25±0.9)*	10	8	75.3 ± 3.9	3.36	0.0543	0.88	Present study
31-40 (x =35.4±0.8)*	10	13	77.4 ± 5.0	3.45	0.0538	1.27	Present study
41-50 (x = 46.3±1.0)*	10	13	72.2 ± 3.7	3.25	0.0553	1.57	Present study
51-60 (x = 55.6±0.5)*	10	7	78.1 ± 5.5	3.48	0.0522	2.02	Present study
61-70 (x = 66.5±0.8)*	10	2	50.9 ± 7.6	2.27	0.0364	1.60	Present study
71-80 (x=73.8±2.2)*	10	4	64.1 ± 8.3	2.86	0.0460	2.20	Present study
81-90*	10	-	-	-	-	-	Present study
91-100*	10	1	58.9	2.63	0.0421	2.51	Present study
45†	9-10	2	128.2	5.72	0.0916	2.69	Brown *et al.* (1984)

* Standard metabolism of turbot fed 2.0 % bwt d^{-1} on WFA pellets with energy value of 12.385 kJ g^{-1} wet weight
† Routine metabolism of turbot fed moist pelleted diet to satiation with energy value of 20.2 kJ g^{-1} wet weight
~ Routine metabolism of turbot fed moist pelleted diet at 3.0 % bwt d^{-1}; energy value unknown and exact temperature and salinity not given

Table 4.1 The effect of body size on standard and routine metabolic rate of turbot plus energy equivalents used per day. Values are means ± se.

Wt (g)	T (°C)	n	µl O_2 $g^{-1}d^{-1}$	µmol O_2 $g^{-1}d^{-1}$	mg O_2 $g^{-1}d^{-1}$	kJ d^{-1}	Reference source
48.1±7.6*	20	6	101.65	4.54	0.0726	2.26	Present study
98.6~	17.0~	5	98.0	4.37	0.071	4.56	Waller (1992)

* Standard metabolism of turbot fed 2.0 % bwt d^{-1} on WFA pellets with energy value of 12.385 kJ g^{-1} wet weight
ŧ Routine metabolism of turbot fed moist pelleted diet to satiation with energy value of 20.2 kJ g^{-1} wet weight;
~ Routine metabolism fed moist pelleted diet at 3.0 % bwt d^{-1}; energy value unknown and exact temperature and salinity not given.

4.3.1.3 Effect of time since last meal on metabolic rate

The relationship between oxygen consumption and body weight was examined for turbot at different time intervals after feeding to find out if nutritional history affected the standard metabolic rate (Figure 4.8). Regression equations representing the relationship between oxygen consumption and body weight are shown in the graphs. The relationship between oxygen consumption and body weight was significant showing a decrease in oxygen consumption for a given weight in fish deprived of food for over 192 h compared with those deprived of food between 13-60 h. A significant difference in the slopes of the two regression lines (ANCOVA $F_{1, 56,} = 14.11$, $P = 0.000$) and a significant difference in their elevations (ANCOVA $F_{1, 57,} = 12.09$, $P = 0.001$) was observed.

4.3.1.4 Effect of temperature on standard metabolic rate

Groups of turbot were acclimated to temperatures of 10°C and 20°C for periods of three and ten weeks respectively. The fish were then respired at their respective holding temperatures and at either a lower or higher temperature to examine their response to acute and long term temperature changes.

The response in rate of oxygen consumption of fish acclimated to 10°C and 20°C for a period of three weeks then measured at their respective holding temperatures and also at 15°C is shown in Figure 4. 9. The respiration rate of the 20°C fish measured at 20°C is higher than that for 10°C adjusted fish measured at 10°C. When measured at 15°C, the 10°C acclimated fish had a higher rate of respiration than the fish held at 20°C, suggesting partial acclimation. Also, when measured at 10°C, the 20°C adjusted fish have a higher rate of respiration than the 10°C acclimated fish again suggesting partial acclimation.

The pattern of acclimation of fish adjusted to 10°C and 20°C for 10 weeks then respired at their respective holding temperature followed by measurements at a higher or lower temperature is represented in Figure 4.10. The pattern of acclimation was different from the three week acclimated fish. There was translation of the curve whereby the 10°C acclimated fish had a higher rate of respiration than the 20°C adjusted fish at all temperatures and there was no (little) change in the slope of the line.

There is therefore some compensation in respiration rate to accommodate an increase in temperature of 10°C over ten weeks which results in the respiration rate of 20°C acclimated fish being only slightly higher than that of 10°C acclimated fish. There is therefore only a small increase in the amount of energy used in standard metabolism at 20°C over that required at 10°C.

This result was further investigated by comparing the standard metabolic rate versus body weight of Group 1 (fed daily to satiation and held at 10°C for 10 weeks) and Group 4 (same

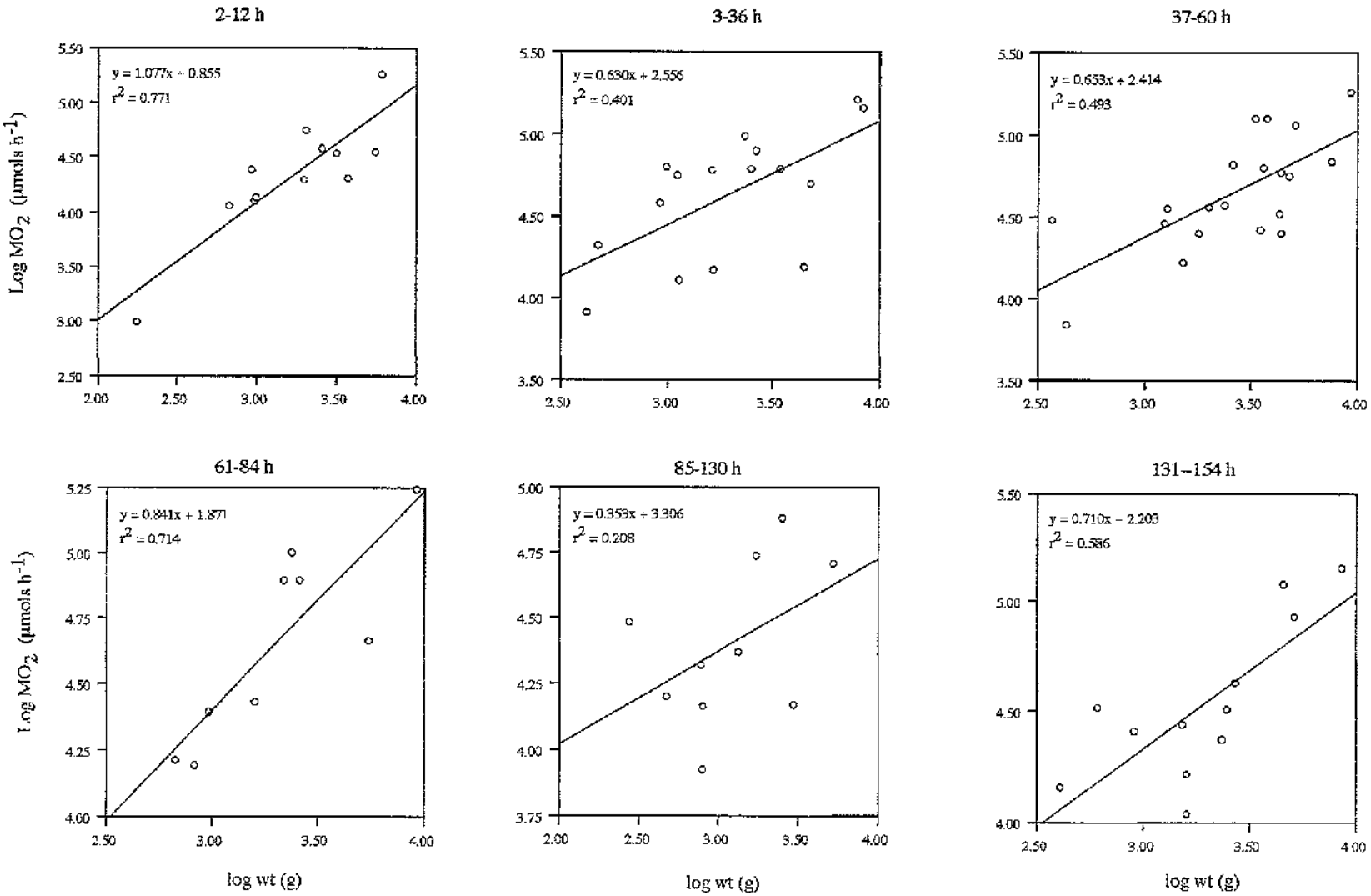
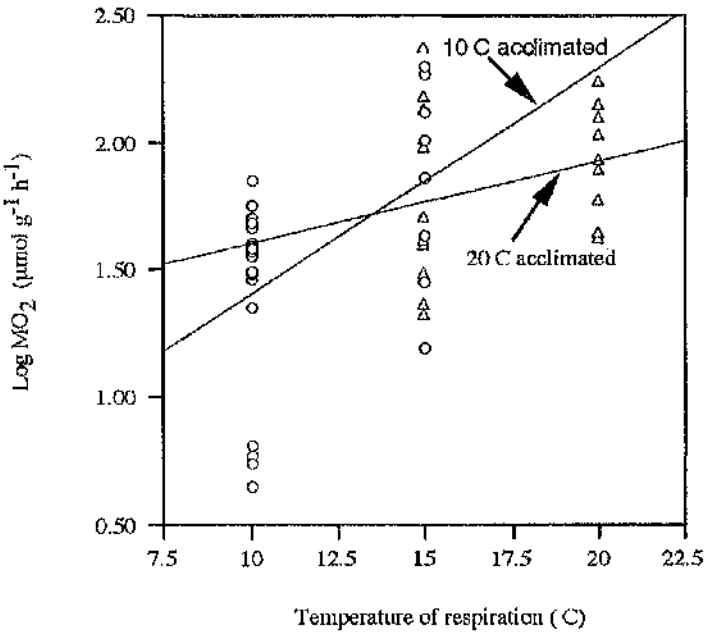


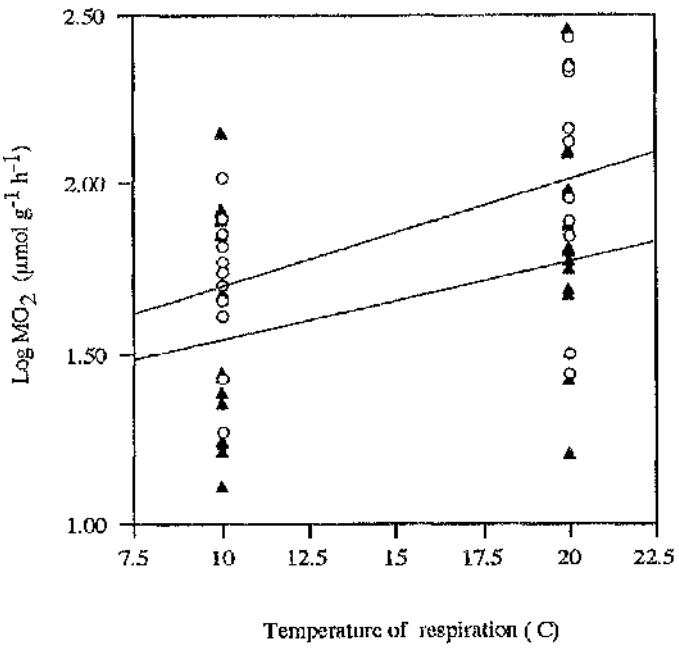
Figure 4.8 Effect of nutritional history on respiration rate of juvenile turbot..



○ Fish acclimated to 10 C $y = 0.090x + 0.502$ $r^2 = 0.237$

△ Fish acclimasted to 20 C $y = 0.033x + 1.264$ $r^2 = 0.074$

Figure 4.9 Effect of temperature on the respiration rate of fish acclimated to 10 C and 20 C for 3 weeks.



○ fish acclimated to 10 C for 10 weeks $y = 0.032x + 1.384$ $r^2 = 0.267$

▲ fish acclimated to 20 C for 10 weeks $y = 0.023x + 1.314$ $r^2 = 0.119$

Figure 4.10 Effect of temperature on respiration rate of fish acclimated to 10 C and 20 C for 10 weeks.

as for Group 1 except acclimated to 20°C). Figure 4.14b. A significant relationship between oxygen consumption and weight was found for both 10°C and 20°C fish with the fish at the higher temperature having a greater oxygen consumption for a given weight. There was no significant difference in the slopes of the two regression lines (ANCOVA $F_{1, 10,} = 3.37$, P = 0.096) but a significant difference in their elevations was observed (ANCOVA $F_{1, 11,} = 16.26$, P = 0.002).

4.3.1.5 Effect of feeding on metabolic rate (SDA)

The standard metabolic rate of a number of turbot was measured for a period of 24-48 h after which the fish were removed from the respiratory chamber and force fed either 3 % or 5 % of their body weight in food and their oxygen consumption followed for another 24-48 h.

The post-prandial increase in oxygen consumption (SDA) following meals of 3 % and 5 % body weight is depicted in Figures 4.11., 4.12., and 4.13. The graphs show an increase of between 9.5 and 18.0 % ($\bar{x}$ = 12.3 % ± 3.0 sd) in the respiration rate of fish fed 3 % of their body weight in food. Fish fed 5 % of their body weight in food show an increase in respiration rate of between 8.5 and 23.8 % ($\bar{x}$ = 14.9 ± 4.8 sd). Generally there was a slight delay of 1-3 hours before the meal had an effect on the respiration rate and in most cases the respiration rate remained elevated for more than 24 h.

From these data and with reference to the data for the effect of body size on the standard metabolic rate of turbot; the energy used per day by a specific sized fish fed a known amount of food can be estimated (see Table 4.9 and Figures 4.26 - 4.29 for details).

4.3.1.6 Investigation to find out if a long-term adaptation in standard metabolic rate occurs at different feeding levels

The relationship between standard metabolic rate and body weight of fish held under different feeding regimes for a period of 10 weeks is shown in Figure 4.14. No significant difference was found in standard metabolic rate between Group 5 (fish at start of experiment) and Group 1 at the end of the feeding experiment (ANCOVA $_{F1, 10,}$ F = 0.02, P = 0.00). Group 1, fed twice daily to satiation had a slightly lower standard metabolic rate for a given weight of fish than Group 3, fed twice daily on a reduced ration. A significant relationship between oxygen consumption and weight was found for both Group 1 and Group 3 with the fish fed on a reduced ration having a greater oxygen consumption for a given weight. There was no significant difference in the slopes of the two regression lines (ANCOVA $F_{1, 10,} = 3.37$, P = 0.096) but there was a significant difference in their elevations (ANCOVA $F_{1, 11,} = 16.26$, P = 0.002). No

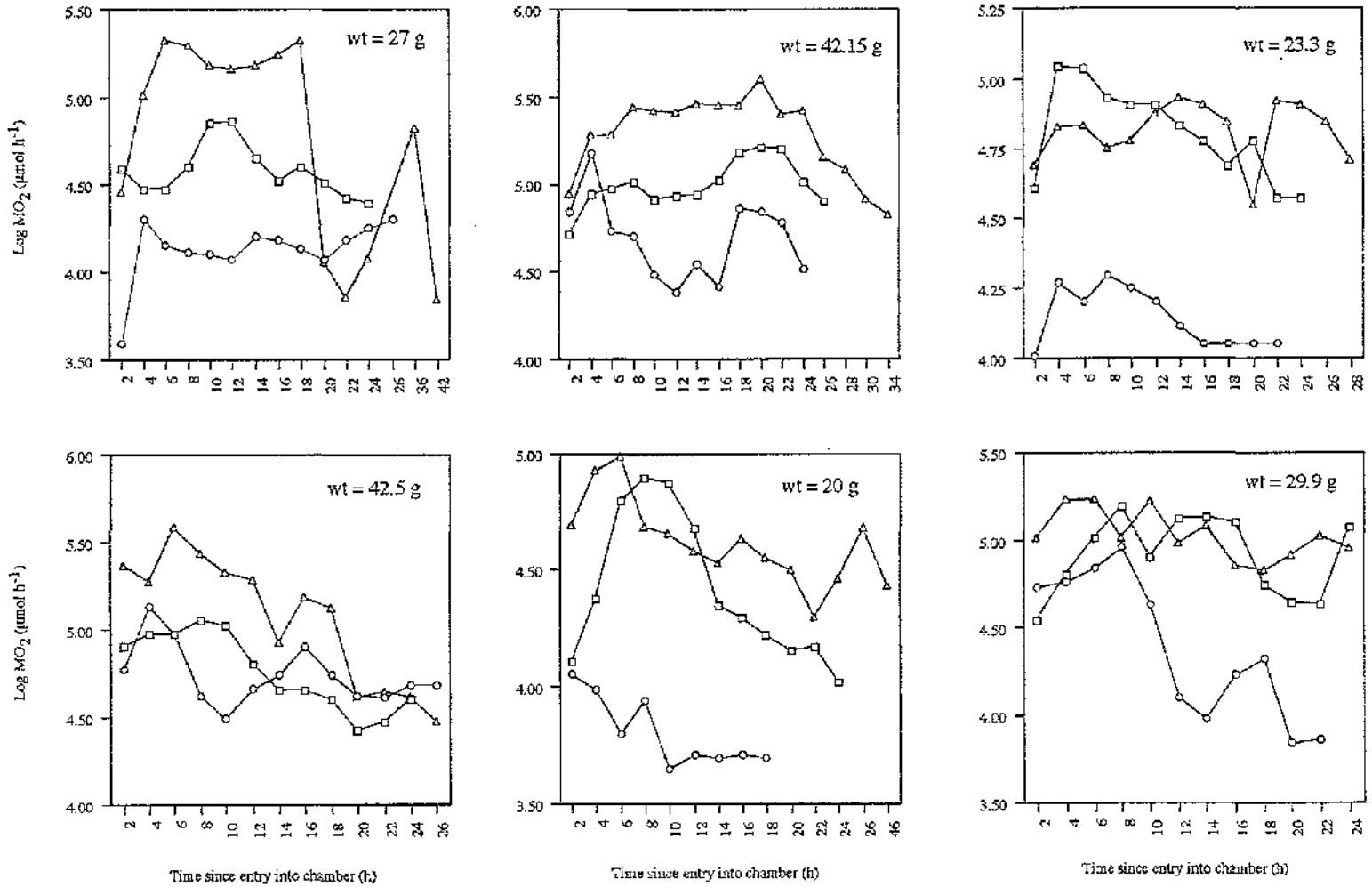


Figure 4.11 Effect on respiration rate of feeding fish 3 % and 5 % body weight in food.

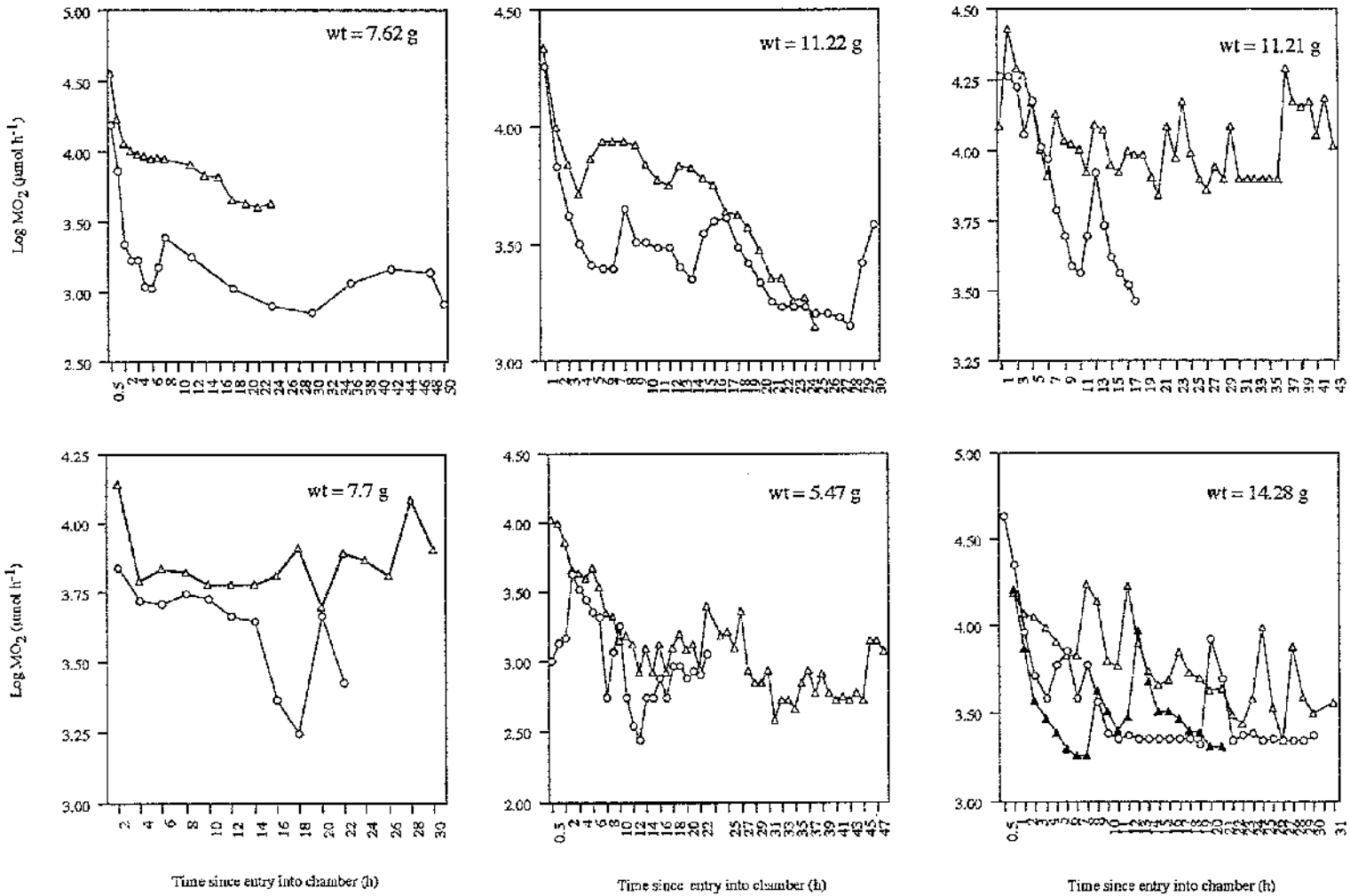


Figure 4.12 Effect on respiration rate of turbot of feeding 5 % body weight in food.

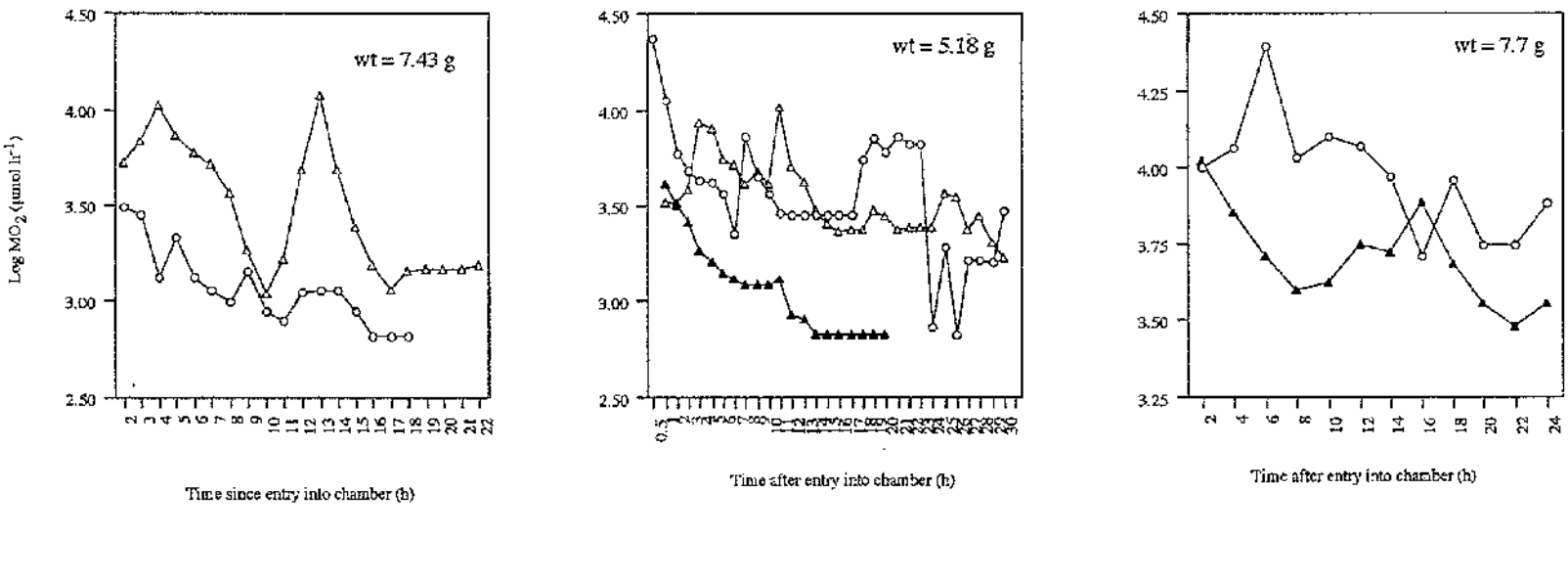
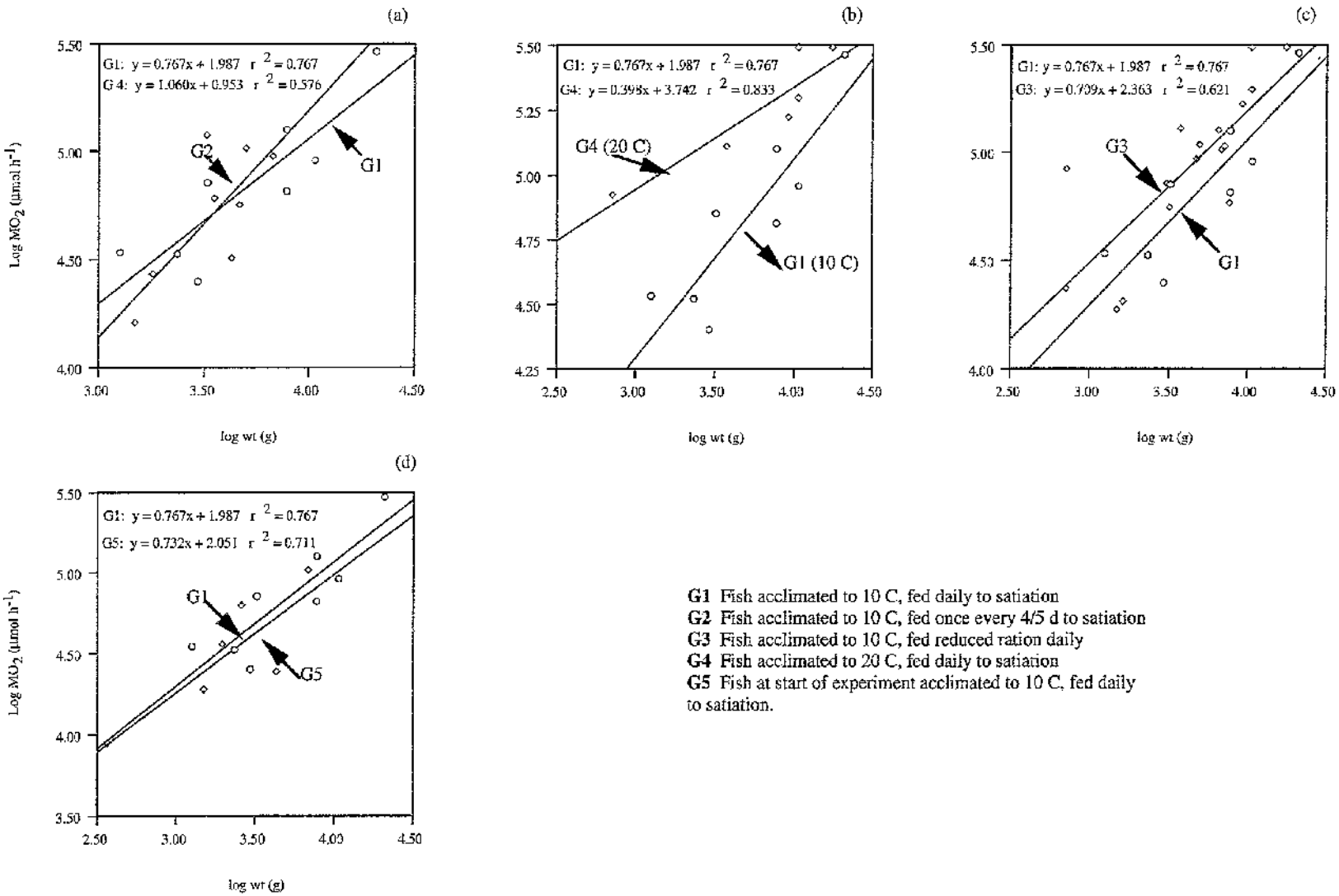
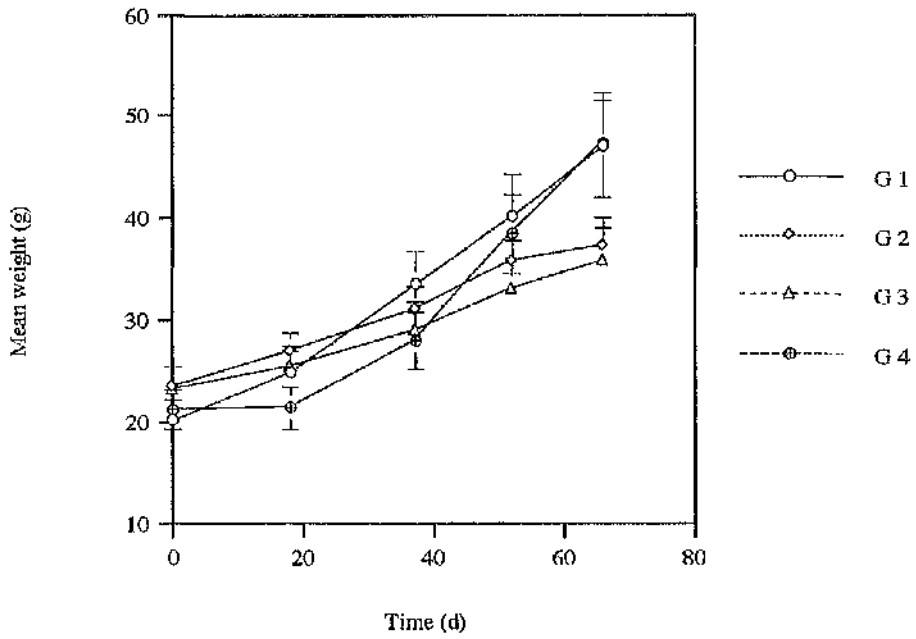


Figure 4.13 Effect on respiration rate of turbot of feeding 5 % body weight in food.



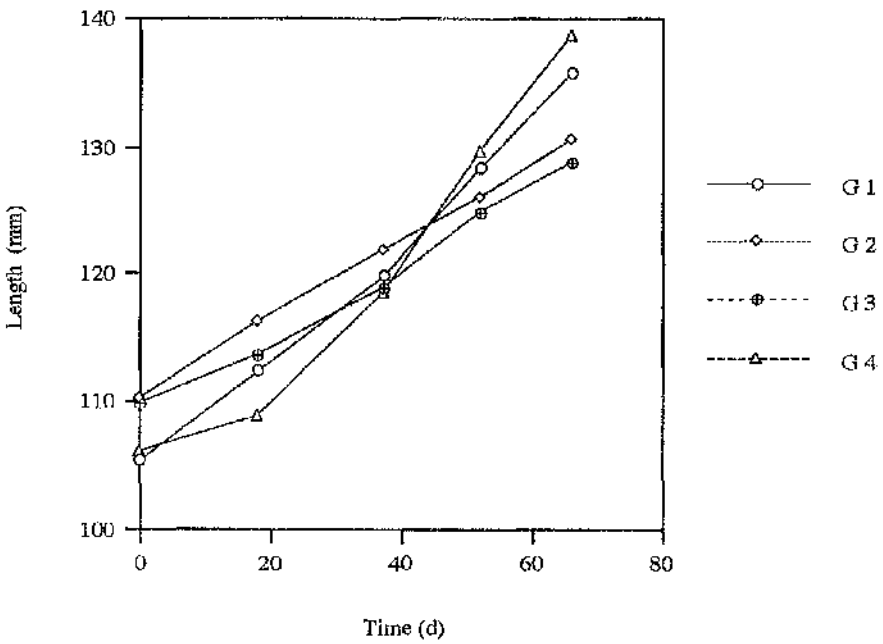
G1 Fish acclimated to 10 C, fed daily to satiation
G2 Fish acclimated to 10 C, fed once every 4/5 d to satiation
G3 Fish acclimated to 10 C, fed reduced ration daily
G4 Fish acclimated to 20 C, fed daily to satiation
G5 Fish at start of experiment acclimated to 10 C, fed daily to satiation.

Figure 4.14 Relationship between standard oxygen consumption and body weight for fish held under different feeding conditions and at different temperatures.



G 1 10 C acclimated fish, fed daily to satiation
G 2 10 C acclimated fish, fed to satiation every 4-5 d
G 3 10 C acclimated fish, fed restricted ration daily
G 4 20 C acclamted fish, fed daily to satiation.

Figure 4.15 Effect of feeding regime and temperature on growth rate of turbot.



Group 1 10 C acclimated fish, fed daily to satiation
Group 2 10 C acclimated fish, fed to satiation every 4-5 d
Group 3 10 C acclimated fish, fed restricted ration daily
Group 4 20 C acclimated fish, fed daily to satiation.

Figure 4.16 Effect of feeding regime and temperature on growth of turbot.

significant difference in standard metabolic rate was observed between Groups 1 and 2 fed on different feeding regimes.

4.3.2 Growth and condition

Four groups of 12 fish were held under different feeding regimes for a 10 week period (see Chapter 3.2 for experimental design). Comparisons were made a) between the growth and condition of fish held on unrestricted and restricted diets, b) between the growth and condition of fish fed to satiation daily and those fed to satiation only once every 4-5 days and c) between the growth and condition of fish held at 10°C and those held at 20°C and both fed to satiation.

4.3.2.1 Effect of feeding regime and temperature on mean specific growth rate

Growth curves of the four experimental groups are depicted in Figures 4.15 and 4.16. Groups 1 and 4 start off with the lowest mean weight and length but finish with the highest mean weight and length, despite a stationary period over the first 18 d for Group 4. Group 4 have the highest mean length at the end of the experiment.

The mean specific growth rates of experimental groups at different time intervals are shown in Table 4.2. Fish held at 10°C and fed twice daily to satiation (Group 1, unrestricted diet) had the highest mean specific rate of growth overall in terms of weight at 1.30 % body weight per day. Fish held at 20°C and fed to satiation twice daily (Group 4) had a slightly lower overall growth rate (1.21 %) than that of Group 1. When the growth rate was examined at

Table 4. 2 Mean specific growth rate (SGR) of turbot

Group	0-18 d	19-37 d	38-52 d	53-66 d	0-66 d
1	1.22 %	1.58 %	1.20 %	1.14 %	1.30 %
2	0.61%	0.74 %	0.93 %	0.29 %	0.69 %
3	0.50 %	0.68 %	0.93 %	0.71 %	0.69 %
4	0.03 %	1.42 %	2.15 %	1.43 %	1.21 %

time intervals throughout the experiment it became evident that Group 4 showed almost no growth over the first 18 d but their growth rate increased in the second time interval and was higher than that for Group 1 for both the third and fourth time interval. (The temperature was

increased slowly to 20°C for Group 4, however, there was possibly still an acute effect on the fish and it may have taken them longer to adapt than expected).

Group 2 (held at 10°C and fed to satiation every 4-5 d) and Group 3 (held at 10°C and daily on restricted ration) had the same overall mean specific rate of growth in terms of weight at 0.69 % and this was almost half that shown in Groups 1 and 4 (Table 4.2). The results suggest that frequency of feeding is very important as Group 2 are unable to accommodate to the less frequent feeding regime than Group 1 even although they are offered food to satiation.

4.3.2.2 Effect of feeding regime and temperature on gross conversion efficiency

When the overall efficiency (Table 4.3) with which each experimental Group of fish converted food into fish flesh was examined in terms of weight gain (g) / food consumed (g) x 100, Groups 1 and 3 were the most efficient at 60.1 % and 59.9 % respectively whilst Group 2 at 56.0 % was less efficient and Group 4 (held at 20°C) at 53.3 %, the least efficient converter (Tables 4. 3 and 4.4).

Table 4. 3 Gross conversion efficiency (weight gain g / food ingested g x 100)

Group	GCE 0-18 d	GCE 19-37 d	GCE 38-52 d	GCE 53-66 d	GCE 0-66 d
1	52.0 %	72.5 %	58.0 %	56.0 %	60.1 %
2	68.0 %	59.0 %	63.0 %	28.0 %	56.0 %
3	43.0 %	55.5 %	73.9 %	66.0 %	59.9 %
4	2.15 %	46.0 %	66.0 %	57.5 %	53.3 %

The results suggest that allowing fish to feed to satiation at frequent intervals is the most efficient method of converting food into fish flesh. Although the 10°C fish had a higher gross conversion efficiency than the 20°C fish the optimum temperature is likely to be somewhere in between these two temperatures and according to published literature is more likely to be around 15-17°C (although this is dependent on fish size).

If the overall gross conversion efficiency is expressed in terms of energy (kJ) again Group 1 appear to be the most efficient in converting food into energy at 17.7 % with Group 3 next at 16.6 %, Group 4 at 16.4 % with Group 2 the least efficient at 15.5 % (Table 4.5). These data are based on the energy values obtained for each Group of fish from bomb calorimetry experiments.

Table 4.4 Cumulative gross conversion efficiency (weight gain g / food ingested g x 100)

Group	0-18 d	0-37 d	0-52 d	0-66 d
1	52.0 %	63.5 %	63.0 %	60.1 %
2	68.0 %	62.7 %	64.2 %	56.0 %
3	42.8 %	49.8 %	57.9 %	59.9 %
4	2.15 %	37.8 %	51.5 %	53. 3 %

Table 4.5 Gross Conversion Efficiency (GCE) (Weight gained kJ /food ingested kJ x 100)

Group	GCE 0-18 d	GCE 19-37 d	GCE 38-52 d	GCE 53-66 d	GCE 0-66 d
1	15.2 %	21.4 %	17.2 %	16.4 %	17.7 %
2	19.5 %	17.0 %	19.2 %	8.3 %	15.5 %
3	11.8 %	15.4%	20.5 %	18.4 %	16.6 %
4	0.66 %	14.4 %	20.7 %	17.7 %	16.4 %

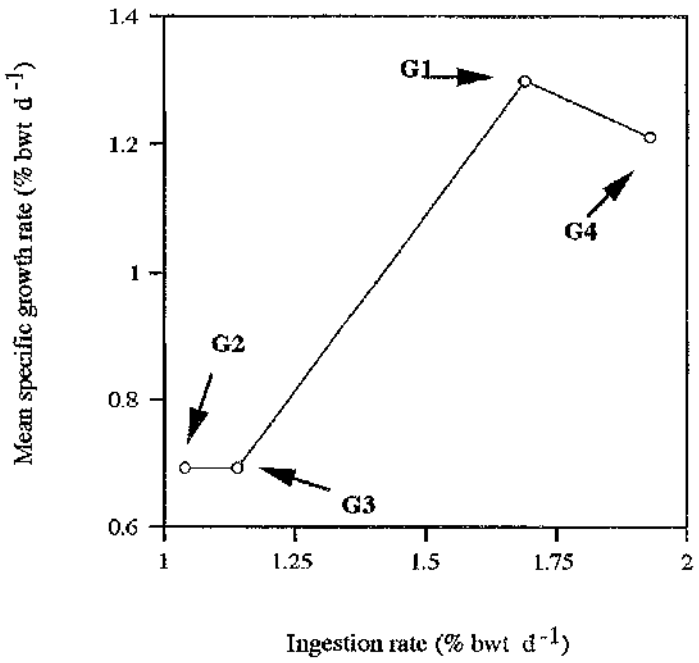
4.3.2.3 Growth-ration curves

The overall relationship between mean specific growth rate and ingestion rate for the four experimental groups are shown in Figure 4.17. Group 4 has the highest ingestion rate but has a slightly lower mean specific growth rate then Group 1. Groups 2 and 3 have the same mean specific growth rate even although Group 3 has a slightly higher ingestion rate.

The relationship between ingestion rate, mean specific growth rate, conversion efficiency and temperature is depicted in Figure 4.18. The ingestion rate of the 20°C acclimated fish is higher than the 10°C acclimated fish but the mean specific growth rate and the conversion efficiency are higher in the cold acclimated fish.

4.3.2.4 The effect of fish body size on the mean specific growth rate

The relationship between mean specific growth rate and body size for fish on restricted and unrestricted diets is shown in Figure 4.19. The larger fish on an unrestricted diet



G1: Fish fed twice daily to satiation and held at 10 C.
G2: Fish fed to satiation once every 4-5 d and held at 10 C.
G3: Fish fed twice daily on reduced ration and held at 10 C.
G4: Fish fed twice daily to satiation and held at 20 C.

Figure 4. 17. Relationship between mean specific growth rate and ingestion rate in fish held under different feeding regimes.

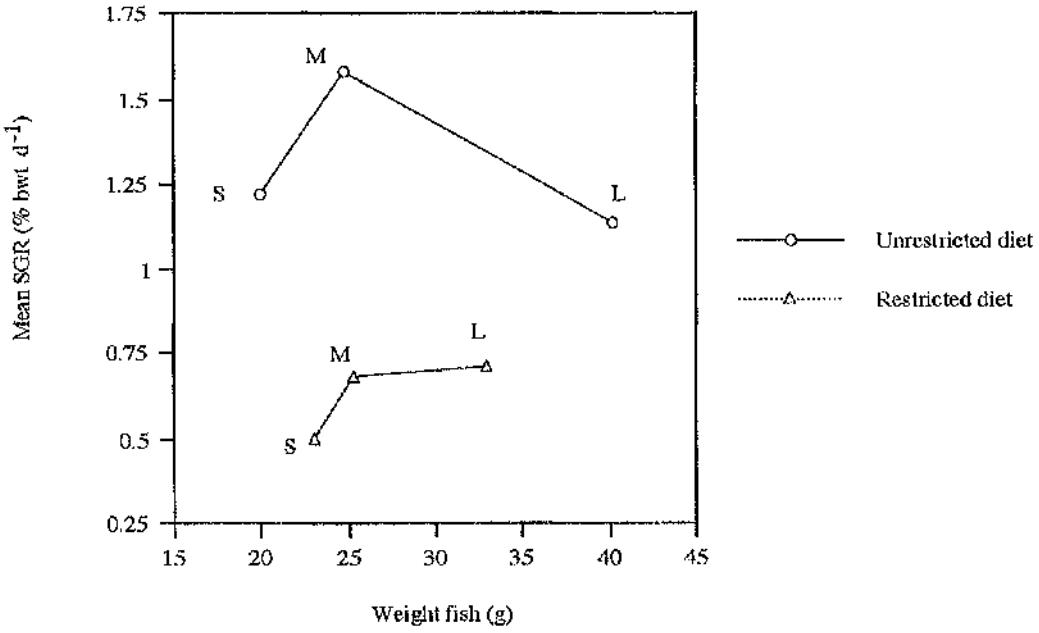


Figure 4.19 Comparison of the relationship between the mean specific growth rate and body size for small, medium and large fish on an unrestricted and those on a restricted diet.

had a lower mean specific growth rate than the small and medium sized fish whilst the larger fish on the restricted diet had a higher specific growth rate than both the small and medium sized fish.

4.3.2.5 The effect of temperature on the relationship between body weight and body length

The effect of temperature on the relationship between body weight and body length at intervals throughout the feeding experiments is shown in Figure 4.20. A significant relationship between weight and length is observed throughout the feeding experiment for both groups of fish. At the start of the trial, no significant difference was observed between the slopes (ANCOVA $F_{1, 21,} = 2.83$, $P = 0.108$) or between the elevations (ANCOVA $_{F1, 22} = 1.80$, $P = 0.194$) of the regression lines of the two groups of fish. However from 37 d and to the end of the trials a significant difference was observed between the elevations of the two lines (ANCOVA $F_{1, 22,} = 4.52$, $P = 0.045$); fish at 20°C were lighter than those at 10°C for a given length of fish.

4.3.2.6 The effect of feeding frequency on the relationship between body weight and body length

The effect of frequency of feeding on the relationship between body weight and body length is shown in Figure 4.21. A significant relationship exists between weight and length for both groups of fish throughout the feeding trials. At the start no significant difference is observed either between the slopes of the regression lines (ANCOVA $F_{1, 21} = 0.57$, $P = 0.459$) nor in their elevations (ANCOVA $F_{1, 22} = 1.37$, $P = 0.255$). After 37 d and to the end of the feeding trials Group 1 fish are heavier for a given length than Group 2 shown by a significant difference in the elevations of their regression lines (ANCOVA $F_{1, 22} = 7.61$, $P = 0.012$). No significant difference was observed between the slopes throughout the trial (ANCOVA $F_{1, 22} = 0.00$, $P = 0.951$).

4.3.2.7 The effect of unrestricted and restricted rations on the relationship between body weight and body length in turbot

The effect of feeding an unrestricted (Group 1) and restricted ration (Group 3) on the relationship between body weight and body length is shown in Figure 4.22. A significant relationship is observed between weight and length throughout the experiment. At the start of the feeding trial no significant difference was found between the slopes of the regression lines (ANCOVA $F_{1, 21} = 3.39$, $P = 0.081$) or in their elevations (ANCOVA $F_{1, 22} = 0.95$, $P = 0.340$). From 37 d to the end of the feeding trials Group 1 were heavier for a given length than Group 3 with elevation (ANCOVA $F_{1, 22,} = 5.16$, $P = 0.034$) and slope (ANCOVA$F_{1, 22} = 3.48$, $P = 0.077$).

4.3.2.8 Comparison of the relationship between body weight and body length in turbot fed on different feeding regimes

The relationship between body weight and body length for Group 2 (fed every 4-5 d to satiation) and Group 3 (fed a reduced ration daily) is shown in Figure 4.23. The amount of food consumed (% body weight per day) by each group over the entire experiment was almost equal. A significant relationship between weight and body length was observed in the two Groups throughout the feeding trial. At the start of the trial no significant difference was observed in the slopes of their regression lines (ANCOVA $F_{1,21,} = 0.39$, $P - 5.38$) or in between the elevations of the regression lines (ANCOVA $F_{1,22} = 0.48$, $P - 0.498$). At the end of the experiment the smaller fish in Group 3 were heavier for a given length than fish of similar length in Group 2 but this is reversed for the larger length of fish where Group 2 are heavier for a given length than Group 3. This is reflected in a significant difference between the slopes of the regression lines at (ANCOVA $F_{1,21} = 12.35$, $P = 0.002$); no significant difference was observed in the elevations of the lines at the end of the experiment (ANCOVA $F_{1,22} = 0.80$, $P = 0.383$).

4.3.2.9 Effect of different feeding regimes and temperature on Condition Factor

At the start of the feeding trials no significant difference was observed in condition factor between any of the four Groups of fish (Mann-Whitney Test). At the end of the feeding trials Group 1 had a significantly higher condition factor than that observed for this Group of fish at the start (Mann-Whitney, $W = 111.5$, $P = 0.0282$); having increased from 1.64 to 1.8. Group 1 also had a significantly higher condition factor at the end the feeding trials than Group 2 (Mann-Whitney, $W = 194.0$, $P = 0.0119$); and was also greater than Group 3 (Mann-Whitney, $W = 128.5$, $P = 0.0192$). No significant difference was observed in condition factor between Group 1 and Group 4 at the end of the feeding trials. See Figure 4.24.

4.3.2.10 Effect of different feeding regimes and temperature on Hepato-somatic index

No significant difference was found in the hepato-somatic index of fish tested at the start of the feeding trials with those at the end of the experiment (Mann-Whitney tests). However, a significant difference was noted in hepato-somatic index when three month old fish were compared with fish at the start of the feeding trials (Mann-Whitney, $W = 71.5$, $P = 0.0075$). See Figure 4.25.

4.3.2.11 Effect of different feeding regimes and temperature on body composition

The gross body composition of turbot prior to, and at the end of the feeding trials is shown in Table 4.6 (see Chapter 2 for methods). The water content was very similar in each

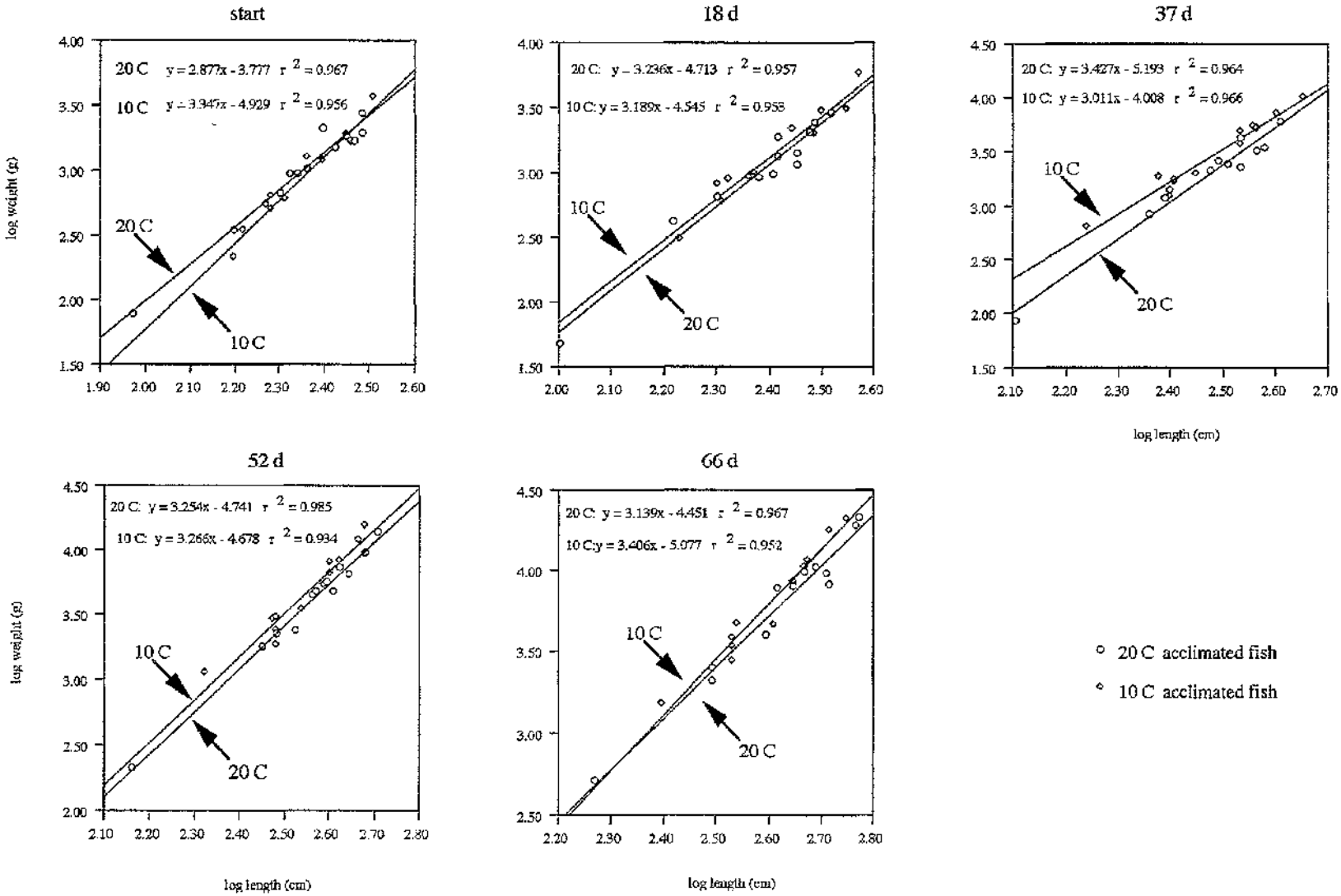


Figure 4.20 The effect of temperature on the relationship between body weight and body length at different time intervals

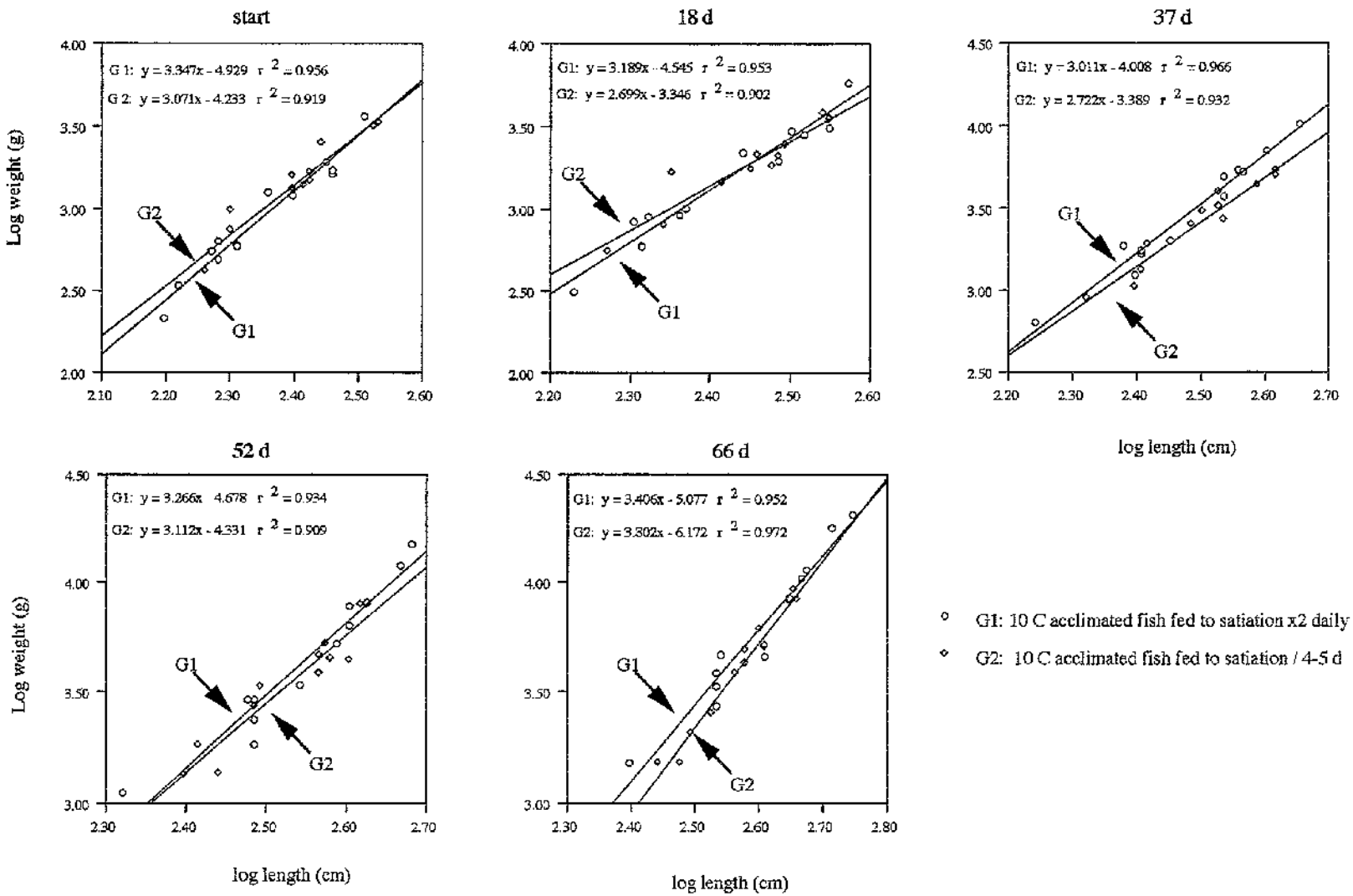


Figure 4.21 Effect of frequency of feeding on relationship between body weight and length in turbot.

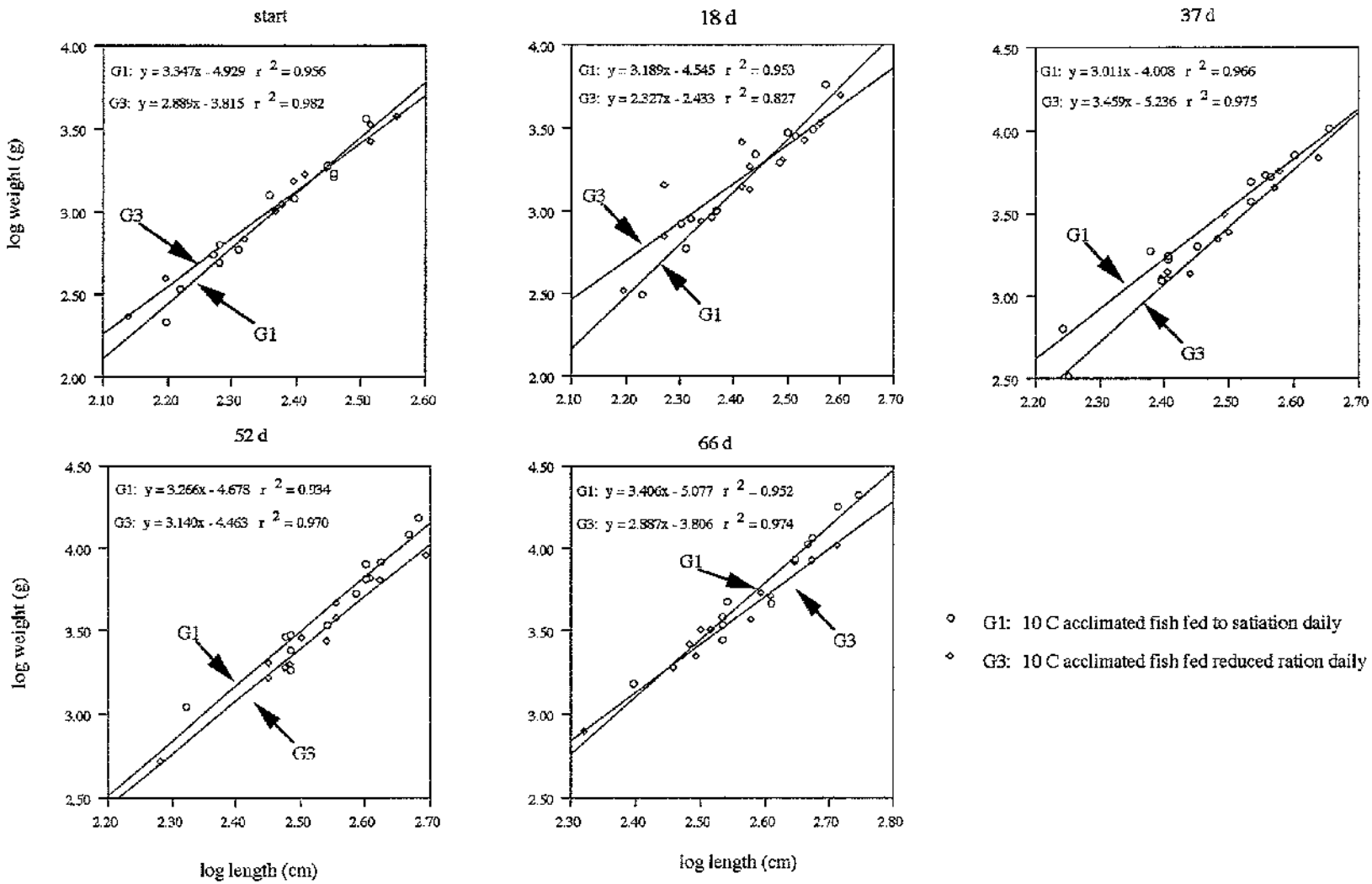


Figure 4.22 Effect of feeding unrestricted and restricted rations on the relationship between body weight and body length in turbot.

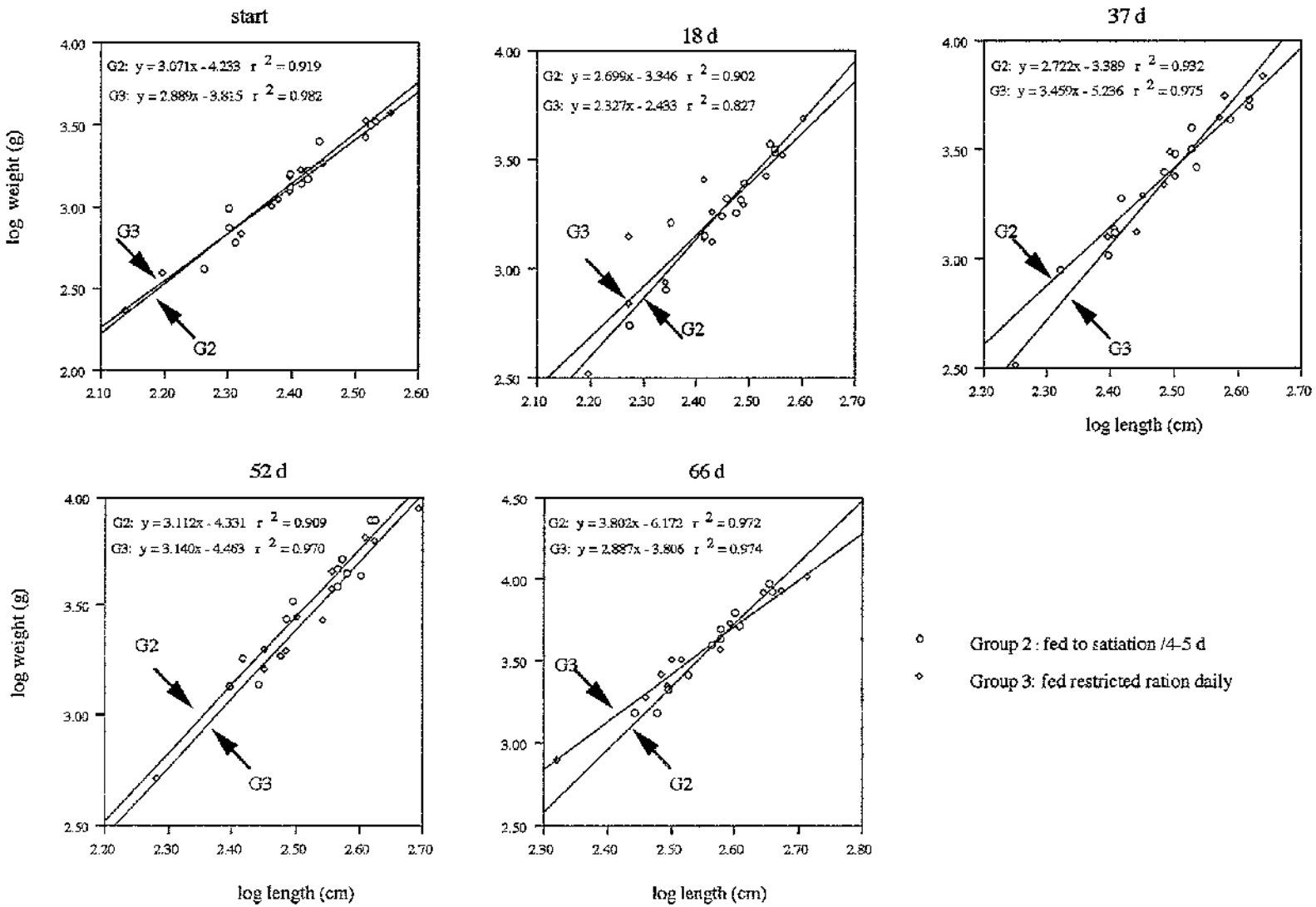
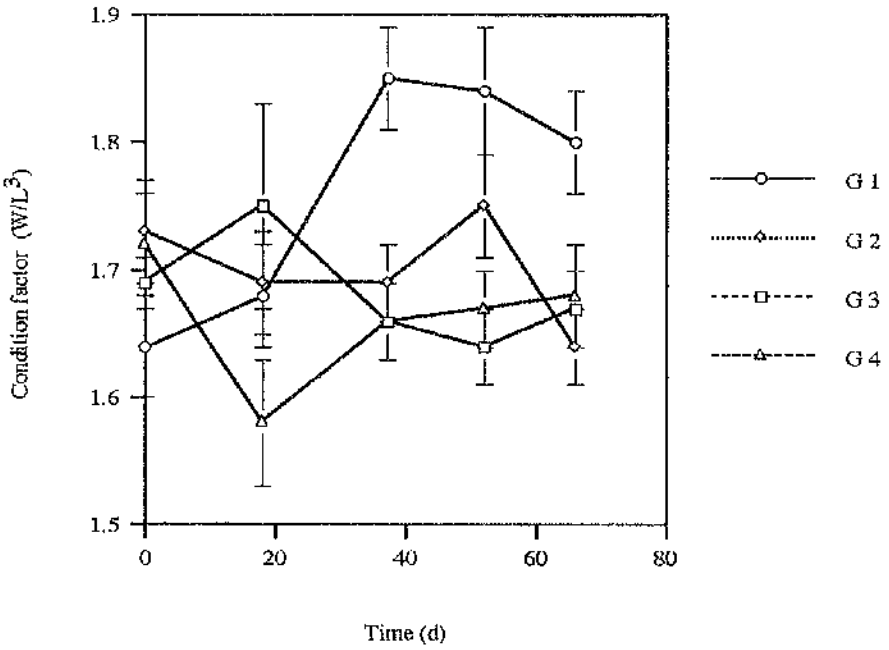
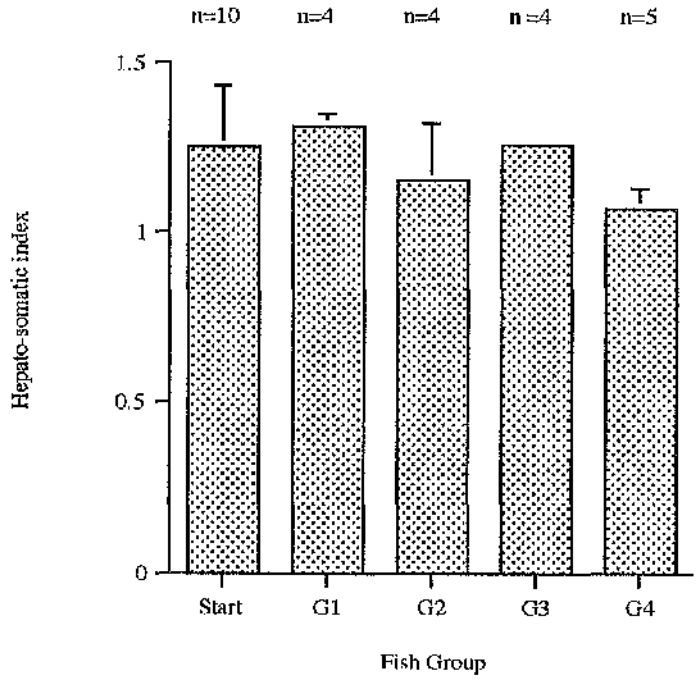


Figure 4.23 Comparison of the relationship between body weight and length in turbot fed on different feeding regimes



G 1 held at 10 C and fed to satiation
G 2 held at 10 C and fed to satiation every 4-5 d
G 3 held at 10 C and fed restricted ration
G 4 held at 20 C and fed daily to satiation.

Figure 4.24 Changes in condition factor with time in turbot held under different feeding regimes. Values given as means + sd.



G 1 Fish held at 10 C and fed daily to satiation
G 2 Fish held at 10 C and fed once every 4-5 d to sation
G 3 Fish held at 10 C and fed reduced ration daily
G 4 Fish held at 20 C and fed daily on reduced ration

Figure 4.25 Hepato-somatic index of fish Groups held under different feeding regimes and at different temperatures.

Group at approximately 80 % except for Group 4 which had a slightly lower water content at 78.9 %. Group 4 had the highest percentage protein at 94.4 % dry weight with Group 3 next at 92.6 % and then Group 1 at 92.2 % and Group 2 at 91.9 %. Group 5 had the lowest percentage protein at 86.8 %. Carcass lipid levels were highest in Group 1 at 14.85 % dry weight with Group 4 at 14.06 %. Groups 2 and 3 had similar lipid levels to those of the fish at the start of the experiment ranging form 11.7 to 12.11 %. Liver lipid levels were very high in Group 4 at 52.9 % with Group 1 at 33.7 % followed by Groups 2 and 3 respectively at 30.7 and 27.25 %. The energy level of the fish carcasses was highest in Group 4 at 3.81 kJ g^{-1} wet weight with Groups 1, 2, 3, and 5 at 3.66, 3.55, 3.43 and 3.68 kJ g^{-1} wet weight, respectively.

4.3.3 Energy budget of turbot held under different feeding regimes and at different temperatures

The standard metabolic rate of juvenile turbot ranging from 3.7 g to 91.7 g and held at 10°C and fed to satiation twice daily was measured (4.3.1.2). The effect of acute temperature changes and short and long-term acclimation to temperature on the respiration rate was recorded (4.3.1.4). The thermic effect (SDA) of feeding fish, 3 % and 5 % of their body weight in food was also evaluated over a 48 h period to assess the extra energy used by fish fed at these levels (4.3.1.5). The assimilation efficiency of fish held at 10°C and at 20°C and under different feeding regimes was assessed (see Chapter 3.3.4.1; Tables 3.7-3.9) as well as the standard metabolic rate under these conditions (see 4.3.1.6; Figure 4.14). Continuous measurements of standard metabolic rate were made over 24 h to assess the total amount of oxygen and therefore the total energy used for maintenance in one day (4.3.1.1). Also, the amount of the host's energy used by the tapeworm, *Bothriocephalus scorpii* in infected turbot, was estimated (see Chapter 6, section 6.3.10 and Table 6.3).

From these data the amount of energy used by infected and non-infected juvenile turbot for maintenance and due to feeding at 10°C and at 20°C, under different feeding regimes was estimated (Tables 4.7, 4.8). An estimate of the energy partitioning in turbot ranging in weight from 10 - 100 g, at 10°C and fed 2 % body weight d^{-1} is given in Table 4.9. The energy budgets of various species of fish is compared with those obtained for turbot held under different feeding conditions in the present study (Table 4.10).

Flow charts showing the energy partitioning for 10 g and 100 g turbot at 10°C and fed 2 % body weight per day and for 100 g turbot held at 20°C and fed 1.85 % and 4 % body weight per day are depicted in Figures 4.26-4-29. (At 100 g fish often have 100 % prevalence of infection).

Table 4.6 Gross body composition of experimental turbot after 10 weeks at different feeding regimes. Values ± sd except when stated otherwise.

Group	Fish wt (g) ± se	T (°C)	Water content	Protein (% wet wt)	Lipid (% wet wt)	Liver lipid (% wet wt)	Ash (% wet wt)	Energy (kJ g^{-1} wet wt)	Protein (% dry wt)	Lipid (% dry wt)	Liver lipid (% dry wt)	Energy (kJ g^{-1} dry wt)
Fish at start	23.7 ± 2.8 (n = 10)	10	79.46 ± 0.24	17.8	2.42		3.91	3.68	86.8	11.77 ± 1.9		17.99
Group 1	46.79 ± 4.53 (n = 12)	10	80.1 ± 0.7	18.36	2.96	6.7	3.43	3.66	92.2	14.85 ± 1.49	33.7	18.37
Group 2	37.22 ± 2.73 (n = 12)	10	80.23 ± 0.24	18.16	2.37	6.1	3.8	3.55	91.9	11.97 ± 0.63	30.7	17.96
Group 3	36.59 ± 3.15 (n = 12)	10	80.53 ± 0.38	18.0	2.36	5.3	3.73	3.43	92.6	12.11 ± 0.34	27.25	17.63
Group 4	47.14 ± 5.1 (n = 12)	20	78.79 ± 0.29	20.0	2.99	11.23	4.03	3.81	94.4	14.06 ± 2.68	52.9	17.94

Group 1 held at 10°C and fed twice daily to satiation; Group 2 held at 10°C and fed to satiation every 4-5 d; Group 3 held at 10°C and fed twice daily on a reduced ration; Group 4 held at 20°C and fed twice daily to satiation.

Table 4. 7 Energy partitioning (expressed in kJ) in turbot held under different feeding conditions.

Group	Energy intake in kJ over 66 d	Assimilated energy in kJ over 66 d (assimilation efficiency %)	Energy in kJ lost in faeces over 66 d (% total energy)	Energy used in kJ for SMR over 66 d (% total energy intake)	*Estimated energy in kJ attributed to SDA over 66 d (% total energy intake)	**Estimated energy in kJ used by tapeworms over 66 d (% total energy at 50 % prevalence)	Estimated energy in kJ used for activity over 66 d (calculated by difference) (% total energy intake)	Estimated energy lost in urine over 66 d (% total energy intake)	Energy gained in kJ by fish over 66 d (Growth) (% total energy intake)
Group 1	6619.8 (100 %)	5441.5 (82.2 %)	1178.3 (17.8 %)	1090.3 (16.5 %)	331.0 (5.0 %)	99.3 (1.5 %)	2281.5 (34.5 %)	463.4 (7 %)	1175.96 (17.8 %)
Group 2	3636.6 (100 %)	2648.2 (72.82 %)	988.4 (27.2 %)	956.4 (26.3 %)	181.8 (5.0 %)	109.1 (3.0 %)	581.7 (18.0 %)	254.6 (7 %)	564.58 (15.5 %)
Group 3	3339.6 (100 %)	2748.5 (82.3 %)	591.1 (17.7 %)	1032.1 (30.9 %)	167.0 (5.0 %)	100.2 (3.0 %)	661.1 (19.8 %)	233.8 (7 %)	554.46 (16.6 %)
Group 4	7220.4 (100 %)	5393.6 (74.7 %)	1826.8 (25.3 %)	1602.9 (22.2 %)	722.0 (10.0 %)	216.6 (3.0 %)	1161.5 (16.1 %)	505.4 (7 %)	1184.1 (16.4 %)

* Estimate of energy in kJ used for feeding metabolism (SDA) for each group based on SDA experiments (4.3).

** Estimate of energy in kJ used by tapeworms based on results in Chapter 6 where the worms were estimated to use 2 % of the assimilated energy intake of the fish at 10° C and prevalences of 50 % were normal. Group 1 held at 10°C and fed twice daily to satiation; Group 2 held at 10°C and fed to satiation every 4/5 d; Group 3 held at 10°C and fed twice daily on a reduced ration; Group 4 held at 20°C and fed twice daily to satiation.

Table 4.8 Partitioning of consumed energy (expressed as a % of the total energy consumed) of turbot held under different feeding regimes.

Group	Faecal loss (% total energy consumed)	R_m (% total energy consumed)	Rsda (% total energy consumed)	Tapeworms (% total energy consumed)	R_a (% total energy consumed)	Urine (% total energy consumed)	Growth (% total energy consumed)	Total
1 Fed to satiation twice daily 10°C	17.8 %	16.5 %	5.0 %	1.0 %	34.5 %	7 %	17.8 %	99.6 %
2 Satiation once every 4/5 d 10°C	27.2 %	26.3 %	5.0 %	1.5 %	18.0 %	7 %	15.5 %	100.5 %
3 Reduced ration twice daily 10°C	17.7 %	30.9 %	5.0 %	1.5 %	19.8 %	7 %	16.6 %	98.5 %
4 Satiation twice daily 20°C	25.3 %	22.2 %	10.0 %	1.5 %	16.1 %	7 %	16.4 %	98.5 %

Table 4.9 Estimated energy partitioning in turbot of different weights (based on fish fed 2 % bwt d^{-1} and held at 10°C).

Wt. fish (g)	Food energy intake kJ d^{-1}	Assimilated energy kJ d^{-1} (assimilation efficiency %)	Energy lost in faeces kJ d^{-1} (% total energy)	Energy for standard metabolism kJ d^{-1} (% total energy)	Energy cost of feeding (SDA) kJ d^{-1} (% total energy)	*Estimated energy used for activity kJ d^{-1} (% total energy)	Energy lost in urine kJ d^{-1} **(% total energy)	Energy used by tapeworm in infected turbot kJ d^{-1} (% total energy)	Energy left over for growth kJ d^{-1} (% total energy)
10	2.48 (100 %)	2.37 (95.8 %)	0.1 (4.0 %)	0.5736 (23 %)	0.12 (5.0 %)	1.03 (41.5 %)	0.17 (7 %)	0.05 (2.0 %)	0.44 (17.7 %)
20	4.95 (100 %)	4.04 (81.7 %)	0.9 (18.2 %)	0.8640 (17.5 %)	0.30 (5.0 %)	1.64 (33.1 %)	0.35 (7 %)	0.08 (1.6 %)	0.87 (17.6 %)
30	7.43 (100 %)	6.1 (82.2 %)	1.3 (17.5 %)	1.0416 (14.0 %)	0.37 (5.0 %)	2.76 (37.2 %)	0.52 (7 %)	0.12 (1.6 %)	1.31 (17.6 %)
40	9.91 (100 %)	8.2 (82.2 %)	1.7 (17.2 %)	1.3536 (13.7 %)	0.49 (5.0 %)	3.77 (38.0 %)	0.7 (7 %)	0.16 (1.6 %)	1.74 (17.6 %)
50	12.39 (100 %)	10.2 (82.2 %)	2.2 (17.7 %)	1.5624 (12.6 %)	0.62 (5.0 %)	4.76 (38.4 %)	0.87 (7 %)	0.2 (1.6 %)	2.18 (17.6 %)
60	14.86 (100 %)	12.2 (82.2 %)	2.7 (18.5 %)	1.7688 (11.9 %)	0.74 (5.0 %)	5.75 (38.7 %)	1.04 (7 %)	0.24 (1.6 %)	2.62 (17.6 %)
70	17.34 (100 %)	14.3 (82.2 %)	3.0 (17.3 %)	2.0832 (12.0 %)	0.89 (5.0 %)	6.85 (39.5 %)	1.2 (7 %)	0.29 (1.6 %)	3.05 (17.6 %)

An oxycalorific coefficient value of 19.4 kJ per litre of oxygen consumed was used in these experiments. This assumes that protein and lipid are the main respiratory substrates and that metabolism occurs aerobically. Energy budget values given in kJ and expressed as percentage of total energy intake. * Energy value for activity is estimated by difference. ** Energy value for non-faecal excretion (urine + gills) is estimated from other authors data.

Table 4.9 Estimated energy partitioning in turbot of different weights (based on fish fed 2 % bwt d^{-1} and held at 10°C).

70	17.34 (100 %)	14.3 (82.2 %)	3.0 (17.3 %)	2.0832 (12.0 %)	0.89 (5.0 %)	6.85 (39.5 %)	1.2 (7 %)	0.29 (1.6 %)	3.05 (17.6 %)
80	19.76 (100 %)	16.2 (82.2 %)	3.6 (18.0 %)	2.2920 (11.6 %)	0.99 (5.0 %)	8.44 (42.7 %)	1.38 (7 %)	0.32 (1.6 %)	3.48 (17.6 %)
90	22.29 (100 %)	18.3 (82.2 %)	4.0 (18.0 %)	2.5008 (11.2 %)	1.11 (5.0 %)	8.76 (39.3 %)	1.56 (7 %)	0.37 (1.6 %)	4.00 (17.9 %)
100	24.77 (100 %)	20.4 (82.2 %)	4.4 (17.8 %)	5.3209 (10.9 %)	1.24 (5.0 %)	9.92 (40.0 %)	1.73 (7 %)	0.41 (1.6 %)	4.36 (17.6 %)

An oxycalorific coefficient value of 19.4 kJ per litre of oxygen consumed was used in these experiments. This assumes that protein and lipid are the main respiratory substrates and that metabolism occurs aerobically. Energy budget values given in kJ and expressed as percentage of total energy intake. * Energy value for activity is estimated by difference. ** Energy value for non-faecal excretion (urine + gills) is estimated from other authors data.

Table 4.10 Energy budgets of various species of fish

Species	T (°C)	Wt start g	Wt end g	Growth rate % d^{-1}	Ingested food per fish kJ d^{-1}	I (%)	R Rm + Rsda + Ra Catabolism (%)	F + U + Gi Faecal + non faecal excretion (%)	P Parasitism (%)	G Growth Anabolism (%)	Reference source
Scophthalmus maximus, turbot	10	20	46.8	1.3	8.4	100	55.9 (16.5 + 5 + 34.4*)	24.8 (17.8 + 7*')	1.5	17.8	Present study (1997)
Scophthalmus maximus, turbot	10	23.5	37.2	0.71	4.6	100	47.3 (26.3 + 5 + 16*)	34.2 (27.2 + 7*')	3.0	15.5	Present study (1997)
Scophthalmus maximus, turbot	10	23.1	36.6	0.70	4.2	100	55.7 (30.9 + 5 + 19.8*)	24.7 (17.7 + 7*')	3.0	16.6	Present study (1997)
Scophthalmus maximus, turbot	20	21.2	47.1	1.21	9.1	100	48.3 (22.2 + 10 + 16.1*)	32.3 (25.3 + 7*')	3.0	16.4	Present study (1997)
Acipenser transmontanus, white sturgeon	18.5	5	-	4.69	2.54	100	60.9*	6.2	-	32.9	Cui *et al.* (1996)
Silurus meridionalis, Southern catfish	18.5	5	-	2.11	2.08	100	34.0*	19.1	-	46.9	Xie and Sun (1990, 1992a,b; 1993), Cui and Wootton (1988a,b,c,d), Cui *et al.* (1996)

Table 4.10 Energy budgets of various species of fish

Species	T (°C)	Wt start g	Wt end g	Growth rate % d^{-1}	Ingested food kJ d^{-1}	I (%)	R Rm + Rsda + Ra Catabolism (%)	F + U + Gi Faecal + non faecal excretion (%)	P Parasitism (%)	G Growth Anabolism (%)	Reference source
Phoxinus phoxinus, European minnow	18.5	5	-	0.98	2.84	100	65.9*	9.9	-	24.2	Xie and Sun (1990, 1992a,b; 1993), Cui and Wootton (1988a,b,c,d) Cui *et al.* (1996)
Sarotherodon mossambicus, tilapia	27	30	51.4	1.67	-	102.2	68.8	20.1 (11.8 + 8.1)	-	13.3	Musisi (1984)
Salmo trutta, brown trout	3.8	50.1	-	-	-	100	74.4*	33 (29 + 4)	-	5	Elliot (1976)
Salmo trutta, brown trout	9.5	50.7	-	-	-	100	62*	31 (24 + 7)	-	33	Elliot (1976)
Salmo trutta, brown trout	15	50.1	-	-	-	100	49*	31 (22 +9)	-	20	Elliot (1976)
Salmo trutta, brown trout	17.8	50.6	-	-	-	100	66*	31 (21 +10)	-	3	Elliot (1976)
Oncorhynchus mykiss, rainbow trout estimated energy budget*[1]	15	100	-	-	-	100	31*[1] (Rm 22 + Rsda 9)	29*[1]	-	40*[1]	Cho and Bureau (1995)
General energy budget for fish fed maximum rations*[1]	-	-	-	-	-	100	60*[1]	-	-	40*[1]	Cui and Lin (1990)

Table 4.10 Energy budgets of various species of fish

Species	T (°C)	Wt start g	Wt end g	Growth rate % d^{-1}	Ingested food kJ d^{-1}	I (%)	R Rm + Rsda + Ra Catabolism (%)	F + U + Gi Faecal + non faecal excretion (%)	P Parasitism (%)	G Growth Anabolism (%)	Reference source
Carnivorous fish - general energy budget*'	-	-	-	-	-	100	44±7*'	27±3*'	-	29±6*'	Brett and Groves (1979)
Herbivorous fish - general energy budget*'	-	-	-	-	-	100	37*'	43*'	-	20*'	Brett and Groves (1979)

* represents energy values estimated by difference.

*' represents estimated energy values based on work by other authors.

The values for the energy budget are in kJ and are expressed as a percentage of the total kJ ingested, I (100 %). R, is the total respiration, or, total metabolism (really catabolism). Rm, is the resting metabolism; Rsda, is the feeding metabolism; Ra, is the active metabolism and is estimated by difference for the present study. F, is the energy lost in the faeces; U, urine and Gi, excretion through gills. Generally U and Gi are pooled together as the non-faecal component of excretion and in the present study these are estimated from data by other authors. P, represents the percentage energy consumed by any parasite harboured by the host but does not include energy loss induced by stress due to parasite. G, is the percentage energy used for growth.

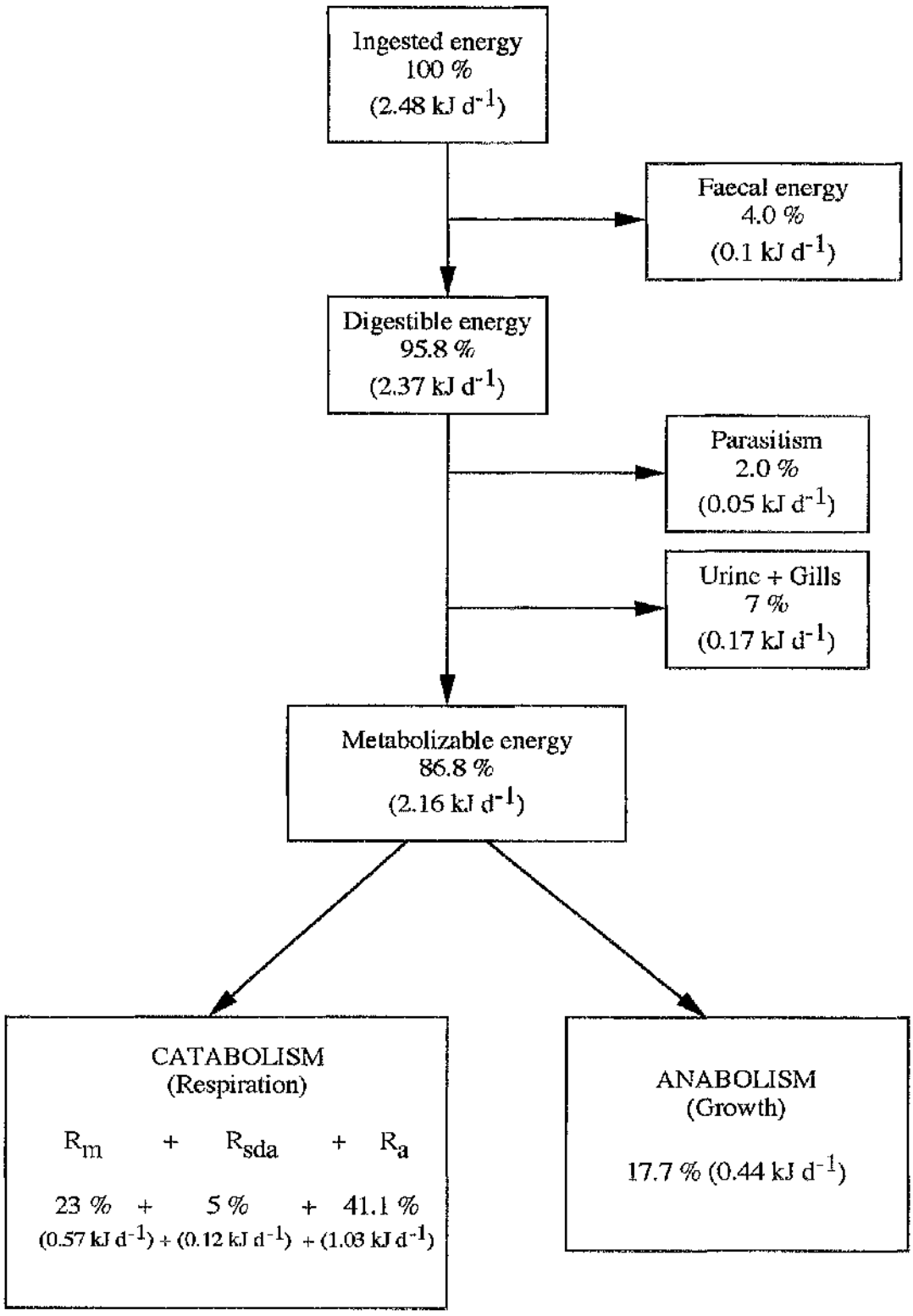


Figure 4.26 Energy budget for 10 g turbot fed 2 % bwt d^{-1} and held at 10 C. All data are estimated from work carried out in the present study apart from Ra which is calculated by difference and also Urine + Gills which is based on an estimate from other authors work. (R_m = standard metabolism; R_{sda}= feeding metabolism; R_a= active metabolism).

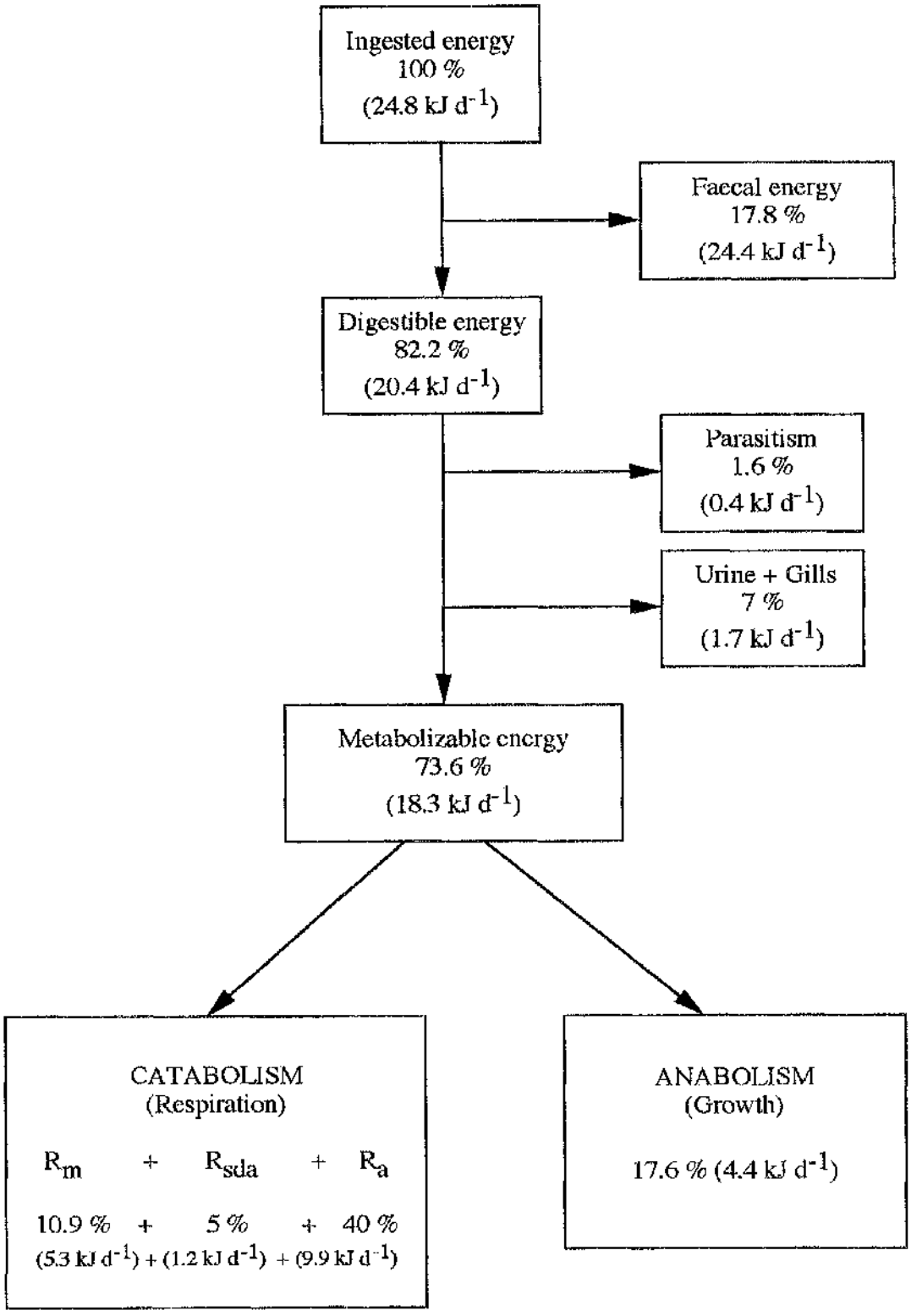


Figure 4.27 Energy budget for 100 g turbot fed 2 % bwt d^{-1} and held at 10 C. All data are estimated from work carried out in the present study apart from Ra which is calculated by difference and also Urine + Gills which is based on an estimate from other authors work. (R_m = standard metabolism; R_{sda}= feeding metabolism; R_a= active metabolism).

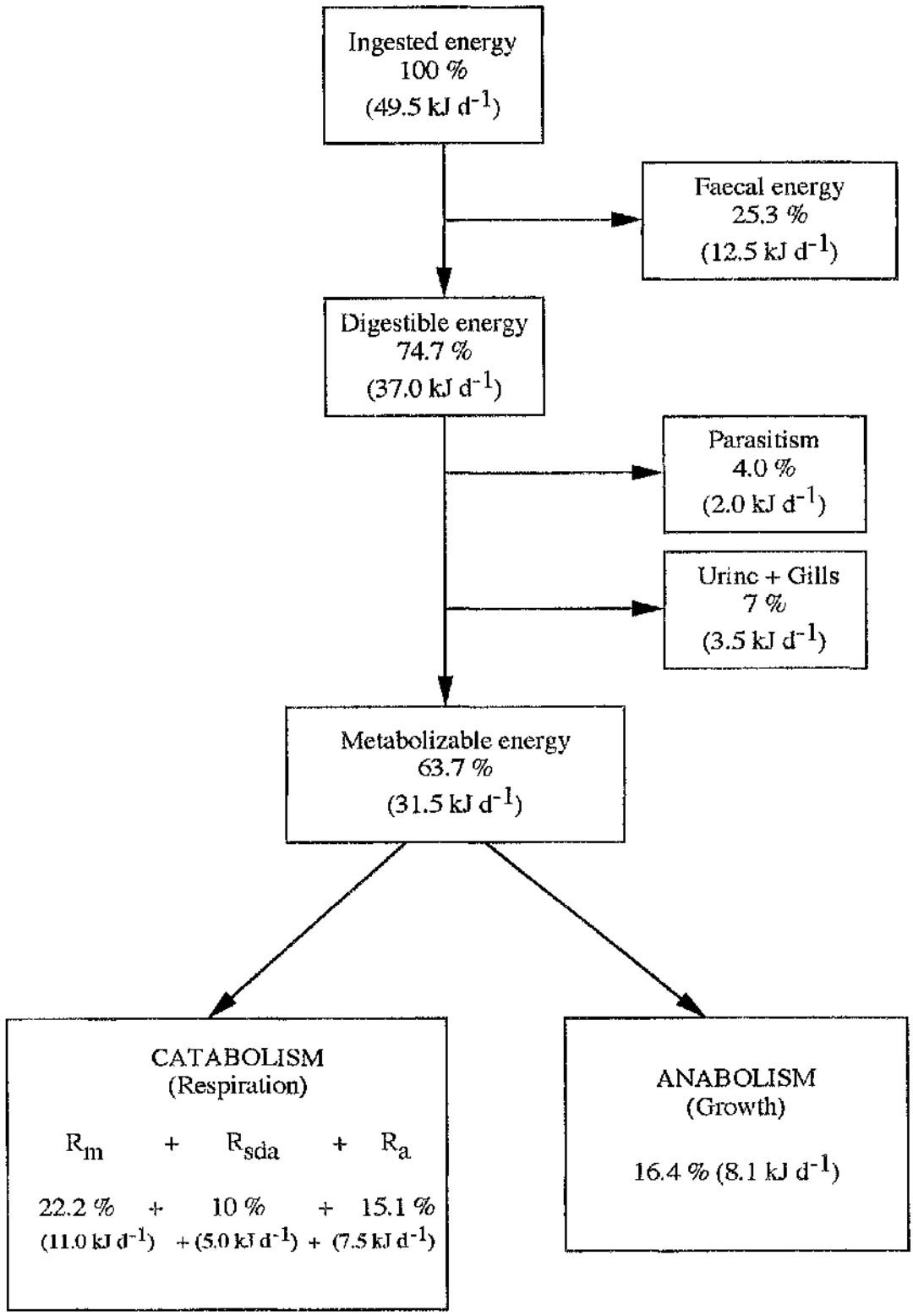


Figure 4.28 Energy budget for 100 g turbot fed 4 % bwt d^{-1} and held at 20 C. All data are estimated from work carried out in the present study apart from Ra which is calculated by difference and also Urine + Gills which is based on an estimate from other authors work. (R_m = standard metabolism; R_{sda}= feeding metabolism; R_a= active metabolism).

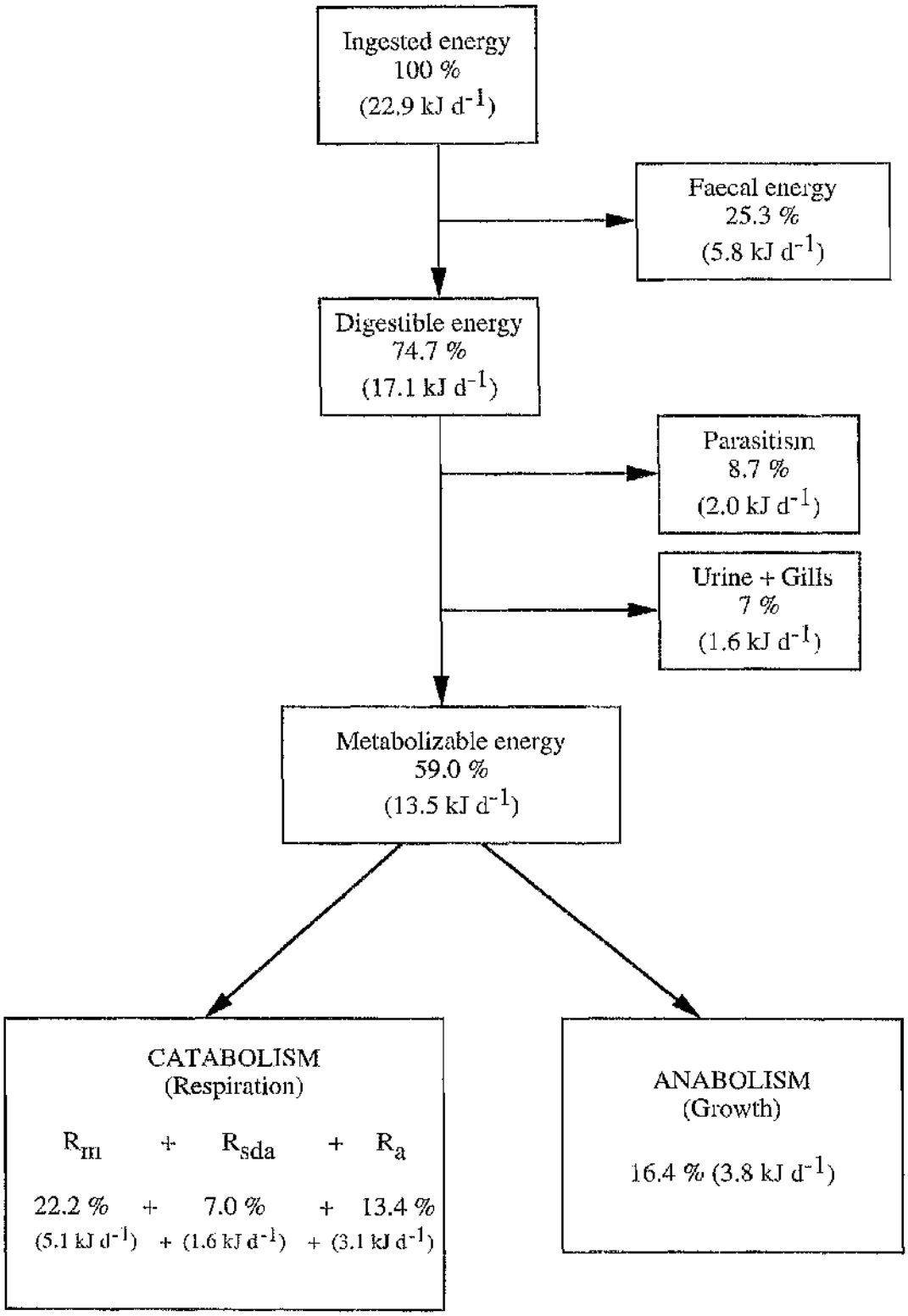


Figure 4.29 Energy budget for 100 g turbot fed restricted ration of 1.85 % bwt d^{-1} and held at 20 C. All data are estimated from work carried out in the present study apart from Ra which is calculated by difference and also Urine + Gills which is based on an estimate from other authors work. (R_m = standard metabolism; R_{sda}= feeding metabolism; R_a= active metabolism).

4.4 DISCUSSION

The main aims of any fish farmer are to maximize the growth and survival of healthy fish stock whilst incurring minimum costs. This is especially difficult to achieve in intensive fish culture systems where the cost of protein-rich foods are very high and reduction in growth and occasionally high mortalities due to disease and other stresses may occur. It is therefore important for a fish farmer to have empirical knowledge of the effects of variations in diet, feeding regimes, temperature, salinity, stocking density and water quality on the metabolism, growth and condition of the fish stock so that the optimum conditions for the rapid growth of healthy fish tissue may be adopted. The use of thermodynamically balanced energy budgets, where all the energy entering and leaving a system is accounted for, can be a very useful tool in the prediction of the effect of dietary or temperature changes on the growth and condition of fish (Beamish *et al.*, 1975; Brett and Groves, 1979; Cho *et al.*, 1982; Calow, 1985; Weatherley and Gill, 1987; Cho and Bureau, 1995; Cui *et al.*, 1996). The more comprehensive the study the more accurate the prediction of any unknown minor components (Jobling, 1983b, Knights, 1985). The respiratory and growth experiments reported in this chapter were designed with the aim of constructing a predictive energy budget for juvenile turbot of different sizes, held under various conditions and also aimed to give an insight into the type of growth occurring (production) under these parameters.

A standardized technique was devised to measure the standard metabolic rate of turbot (4.2.1.1). This procedure was considered suitable for juvenile turbot fed on WFA moist pellets and respired in the apparatus described but might not be suitable for other species or for turbot fed on a more natural diet which would take longer to digest. Continuous measurement of the oxygen consumption of fish at rest over a period of 24-48 h revealed fluctuations around a mean but no distinct daily pattern. Waller (1992) noted a diurnal pattern in the routine oxygen consumption of juvenile turbot held under natural illumination which reflected their normal activity pattern. However, it was assumed that maintaining the fish at constant temperature and under a 12 h light 12 h dark regime overcame daily and seasonal variations in standard metabolism.

The relationship between the standard metabolic rate and body size was established for fish held at 10°C and ranging in size from 3.7 - 91.7 g and at 20°C for fish ranging in size from 4.6 - 70.3 g and was found to be represented by the following equations:

$$10°C: \quad LnY = 0.77LnX + 2.027 \quad (r^2 = 0.89,\ sd = 0.032,\ n = 75,\ p = 0.001)$$

$$20°C: \quad LnY = 0.81LnX + 2.152 \quad (r^2 = 0.86,\ sd = 0.072,\ n = 22,\ p = 0.000)$$

Waller (1992) found the relationship between body size and routine oxygen consumption for turbot to be expressed by the equation:

$$LnY = 0.699LnX - 0.278 \; (r = 0.995, n = 9, p < 0.001)$$

and noted that the exponent for this relationship in roundfish is usually given as 0.76-0.86 (Brett, 1962). He commented that for flatfish the slope is always around 0.7 (Brown *et al.*, 1984; Edwards *et al.*, 1969, 1970; Jobling, 1982) and proposed that a value of 0.7 be used for the weight dependent relationship of metabolic rate. However, on examining these papers, values of 0.724 (Edwards *et al.*, 1970) and 0.721 (Edwards *et al.*, 1969) were found for plaice and 0.75 for turbot at 9-10°C (Brown *et al.*, 1984) and therefore 0.7 may possibly be too low for all flatfish, especially as a value of 0.77 was estimated in the present study. Variations in the weight exponent may possibly be due to differences in the weight range of the fish used for the experiments or, may be due to the conditions and methods employed in measuring the respiration rate. For example Brown *et al.* (1984) found the relationship varied with temperature. Further work on the relationship between standard or routine metabolism and body weight in different species of flatfish should be carried out before a general figure for this exponent in flatfish is or, can be assumed.

Comparisons were made of values for standard oxygen consumption of turbot in the present study with those obtained for routine oxygen consumption by Brown *et al.* (1984) and Waller (1992) and were found to be slightly lower for standard respiration as would be expected. However, again caution should be used when interpreting and comparing results of different studies employing varying techniques.

Temperature acclimation experiments showed that after three weeks acclimation at 10 and 20°C acclimation was partial. This is indicated by the fact that when measured at their respective temperatures the 20°C acclimated fish had a higher rate of respiration than the 10°C acclimated fish. When both groups were measured at 15°C, the 10°C fish had a higher rate of respiration than the 20°C acclimated fish. After 10 weeks acclimation at the respective temperatures the pattern of acclimation was different with translation of the curve whereby the 10 °C fish had a higher rate of respiration than the 20°C fish at all temperatures measured and very little or no change in the slope of the line. Some compensation therefore occurs in standard metabolic rate to accommodate in part for the increase in temperature. This may involve adaptation at the tissue and at the cellular level (such as changes in muscle fibres; number of mitochondria; changes in biochemical pathways and rates of enzyme reactions *e.g.* cytochrome c

oxidase, succinate dehydrogenase and citrate synthase (aerobic energy function) show greatest changes, increasing activity in cold acclimated fish; whilst glycolytic enzymes (phosphofruktokinase, lactate dehydrogenase, pyruvate kinase) show little change during thermal acclimation) (Hochachka and Somero, 1971; Hazel and Prosser, 1974).

Acute temperature changes gave a corresponding increase or decrease in respiration rate. Where possible, temperature changes should be gradual to avoid respiratory stress to the fish and period of acclimation should be quoted when measurements of metabolic rate are given.

The thermic affect of feeding was examined and revealed a definite increase in respiration rate, after an initial delay of 1-3 h, which lasted for a period of approximately 24 h. The size of this increase was positively correlated with the size of the meal ingested. This is in agreement with other work carried out on the effects of feeding on respiration rate of fish (Brett and Groves, 1979; Jobling and Davies, 1980; Jobling, 1981b, 1983a; Knights, 1985; Cho and Bureau, 1995).

Growth-ration curves were constructed from data obtained in feeding experiments, to give some insight into the relationship between mean specific growth rate and ingestion rate, and between ingestion rate, mean specific growth rate, conversion efficiency and temperature for juvenile turbot. These revealed that although turbot held at 20°C had a higher ingestion rate than fish at 10°C their mean specific growth rate was poorer and their conversion efficiency lower than those held at 10°C. This may be slightly misleading as a lag phase occurred in the 20°C Group at the start of the feeding experiments whilst the turbot acclimated to temperature. If the growth-ration curves are adjusted to include only the final 7 weeks of the feeding experiment then the 20°C fish have the highest growth rate although their gross conversion efficiency expressed in terms of energy content is still poorer at 16.4 % for the 20°C acclimated fish compared with 17.7 % for the 10°C acclimated Group fed to satiation. Jones *et al.* (1981) found the temperature for maximum growth for juvenile turbot was 18.9°C but the temperature for maximum food conversion efficiency was 16.2°C. This agrees with more recent work which has shown the growth rate of young turbot (25-74 g) to be greatest between 16-20°C (Brown *et al.*, 1984; Scherrer, 1984; Imsland *et al.*, 1996). The ingestion rate for juvenile turbot in the present study was quite low, possibly due to the size of the tanks the fish were being held in or perhaps the fish were poor feeders since fish were selected by WFA from the small size grade. It has been shown that size rank correlations remain stable in certain species of fish, including turbot, and that this is common under culture conditions (Purdom, 1974; Rosenberg and Haugen, 1982; Economou *et al.*, 1991; Imsland *et al.*, 1996).

That fish in general tend to have a temperature range which is optimal for growth and survival (Brett and Groves, 1979; Weatherley and Gill, 1987; Gadomski and Caddell, 1991; Imsland *et al.*, 1996) and that this range may vary with age and size of the fish since juveniles of

a great many species prefer warmer waters than adults (McAuley and Huggins, 1979; Pedersen and Jobling, 1989; Imsland *et al.*, 1996) is of great importance both to the natural distribution of different life stages of the species and to the optimal rearing of the species under culture conditions (Imsland *et al.*, 1996). The temperature optimum of several fish species has been shown to decrease with increasing size *i.e.* there is an ontogenic shift (Pedersen and Jobling, 1989; Fonds *et al.*, 1992; Hallaråker *et al.*, 1995; Björnsson and Tryggvadóttir, 1996; Imsland *et al.*, 1996). Imsland *et al.* (1996) observed that the temperature optimum producing maximum growth rate in turbot decreased rapidly with increasing size, and for 100 g turbot was between 13-16°C although Waller (1992) suggested that the preferred temperature of 100 g turbot is around 17°C which he found corresponded to the maximum routine oxygen consumption. This may reflect a difference between the populations of turbot sampled or may be due to different techniques employed. According to Person-Le-Ruyet (1990), the optimum environmental requirements for young turbot are still poorly understood as they are often based on data obtained by a variety of techniques and for fish held under different conditions and therefore comparisons are difficult. The data on oxygen consumption of different sized turbot at rest, at specific temperatures, during feeding and for fish held under different feeding regimes for a period of 10 weeks was converted to energy values. In conjunction with data obtained on the assimilation efficiencies, the parasite status, and on the actual growth rates and energy values of the fish held under these different conditions, energy budgets were constructed which revealed patterns of energy partitioning in juvenile turbot ranging in weight from 3-100 g (4. 9). Energy values for standard metabolism in turbot at 10°C and 20°C in the present study, when compared with those obtained for routine metabolism by Brown *et al.* (1984) and Waller (1992) were found to be slightly lower as might be expected. However, direct comparisons are difficult due to differences in techniques used in the three studies, variation in the nutritional and energetic content of the diets used and differences in the length of time allowed after feeding before measurements were taken. Brown *et al.* (1984) took measurements in the field whilst Waller's results are based on laboratory studies. Muller-Feuga *et al.* (1978) demonstrated that large variations may exist between data published on oxygen consumption measured in the laboratory and that measured under farm conditions. In turbot acclimated to 10°C and fed to satiation twice daily, 16.5 % of the ingested energy was used for standard metabolism. The proportion of ingested energy used for standard metabolism increased in turbot fed less frequently (26.3 %), for those fed on a reduced ration (30.9 %) and in those held at 20°C (22.2 %).

It was estimated that in juvenile turbot fed 2 % body weight d^{-1} on WFA pelleted diet and held at 10°C; 5 % of the ingested energy d^{-1} was lost due to the thermic effects of feeding; *i.e.* the increase in heat production associated with amino acid mobilization and the biochemical

processes following digestion (Kleiber, 1975; Emmans, 1994). The energy loss due to feeding turbot held at 20°C was estimated at 10 % of the ingested energy. Priede (1985) suggested that SDA varied between 5 and 20 % of ingested energy depending on the protein content of the diet. Cho and Bureau (1995) attributed 9 % of ingested energy of 100 g rainbow trout, *Oncorhynchus mykiss*, held at 15°C, to feeding metabolism.

Energy budgets were constructed for turbot held at 10 and at 20°C and under different feeding regimes (Table 4.7). These were compared with those obtained by other workers for different species of fish (Table 4.10). In turbot, the proportion of ingested energy used for respiration varied depending on feeding regime and temperature of acclimation with the highest proportion of energy for overall respiration being used by the 10°C acclimated fish fed daily to satiation and those fed daily on a reduced ration. There was a large variation between the amount of food energy used for maintenance metabolism and activity between the two groups with only a slightly greater proportion of the food energy used for growth in the fish fed to satiation. Turbot held at 20°C and fed daily to satiation had a slightly higher proportion of food energy used for maintenance metabolism with an increase in the proportion lost due to feeding and to faecal and non-faecal excretion. There was a substantial decrease in the amount of food energy used for active metabolism leaving only a very slight decrease in the proportion used for growth. These data are perhaps slightly misleading as a large growth check was incurred by the 20°C acclimated fish whilst they acclimatized to temperature at the start of the feeding experiments.

Generally, the proportion of energy used for maintenance metabolism and activity metabolism are very labile depending on temperature of acclimation, the amount of food eaten and the frequency of feeding and size of fish. Results also show that increasing temperature and decreasing feeding frequency result in a large decrease in assimilation efficiency. The amount of food energy used for growth was lowest in the 10°C fish fed to satiation every 4/5 days. The assimilation efficiency was lowest in this group and it was found they were unable to accommodate to the infrequent feeding programme. The results suggest twice-daily feeding to satiation give the optimum conditions for growth in juvenile turbot. The amount of ingested energy consumed by the tapeworm, *Bothriocephalus scorpii*, also varies depending on temperature and amount of food consumed by the fish and this may constitute a considerable cost on a large-scale fish farm. Generally, the proportion of the host's food energy consumed by the tapeworm increases with increasing temperature and decreasing food intake (Chapter 6). Further studies, where the energy partitioning of ingested food in turbot held under a greater range of temperatures, covering the optimum temperature for growth and the optimum temperature for maximum food conversion efficiency over a range of sizes, are necessary to give further insight into the optimum conditions for the growth of healthy fish tissue at minimum cost.

Compared with other data for carnivorous fish (Table 4.10) the proportion of food energy used for growth (17.8 % for turbot compared with 29.6±6, Brett and Groves, 1979 and 40 %, Cui and Liu, 1990) was slightly low but this most likely reflects the fact that 10°C is far from the optimum temperature for growth for turbot in the weight range of 20 - 75 g and at 20°C there was a large growth check at the start of the experiment. Also, the fish in the present study were measured every 2 weeks which may have imposed a further growth check on the fish. Boggs (1991) found the proportion of food energy used for growth and metabolism of adult northern anchovy, *Engraulis mordax,* was about 65 %; this compares with a range of 62.8-73.7 % for turbot in the present study.

Energy budgets for turbot ranging in weight from 10-100 g, fed 2 % body weight d^{-1} and held at 10°C were constructed (Table 4.9). Turbot < 10 g had a very high assimilation efficiency, in terms of energy, at 95.8 % compared with 82 % for turbot in the weight range 11-100 g. The proportion of ingested energy used for standard metabolism decreased from 23 % in fish < 10 g to 10.9 % in fish weighing 90-100 g. Unfortunately the growth rate of fish < 10 g was not established but as the assimilation efficiency was very high it might be expected that there is a correspondingly high growth rate in these small turbot; it would be interesting to see if the proportion of ingested energy used for growth is similar to the larger fish. Also, the preferred temperature of turbot in the 20-75 g range has been established at 16-20°C (Brown *et al.,* 1984; Imsland *et al.*, 1996) with an optimum temperature for growth at 18.5°C (Jones *et al.,* 1981) with an ontogenic shift in the optimum temperature for growth in 100 g turbot to 13-16°C (Imsland *et al.,* 1996). Ideally, growth experiments closer to the preferred optimum temperatures for the different sized fish should be carried out.

Many studies on fish growth simply express the change in magnitude of the weight or length of the fish but neglect to explain what type of growth has occurred. Huxley (1932) discussed the relative, or allometric, growth of different parts of the body; *i.e.* the differences in the relative rates of increase in bulk or volume of different parts of the body which can produce changes in the body proportion. This is clearly of major importance to the fish farmer who is concerned with obtaining a product which is acceptable to the consumer. In the present study, changes in body composition, in energy value, in condition factor and in hepato-somatic index of the turbot held under different feeding regimes and at different temperatures were therefore monitored to give an insight into the type of growth obtained under the specified conditions. In turbot held at 10°C and fed daily to satiation, the condition factor (*i.e.* the 'fatness' or 'well-being' of the fish) increased significantly over the experimental period compared with the 10°C fish fed on reduced rations and with the 20°C acclimated fish where the condition factor decreased over this time. The hepato-somatic index was also greatest in the 10°C acclimated fish fed daily to

satiation and lowest in the 20°C acclimated fish, although the carcass lipid levels of these two groups were similar whereas the liver lipid levels were far greater in the 20°C acclimated fish. The protein and energy contents were slightly higher in the 20°C acclimated fish whilst the water content was slightly lower in these fish compared with the 10°C acclimated fish. These results suggest that condition factor and hepato-somatic index may not be the most accurate indicators of growth in turbot.

Finally, that smaller turbot of 20-75 g have a higher optimum temperature for growth of 16-20°C (Jones *et al.*, 1981; Brown *et al.*, 1984; Imsland *et al.*, 1996) than 13-16°C for turbot of 100 g (Imsland *et al.*, 1996); should be balanced with the fact this may not be the optimum temperature for maximum food conversion efficiency. The frequency of feeding, biochemical, energy content and consistency of food, salinity of water (recent work by Gaumet *et al.* (1995)) has shown that growth of young turbot may be improved by acclimation to salinities closest to the isosmotic point since the larvae of many marine fish have been shown to be good osmoregulators (Alderdice, 1988), health of the fish, stocking density of fish and perhaps most importantly the population of turbot from which the stock are derived may all interplay to produce different optimum conditions for the growth of healthy, fish flesh which is acceptable for the luxury market. Further studies, where the energy partitioning of ingested food in turbot held under a greater range of temperatures, covering the optimum temperature for growth and the optimum temperature for maximum food conversion efficiency at different ontogenic stages, are necessary to give further insight into the optimum conditions for the growth of healthy fish tissue at minimum cost. Also, parasitism by the tapeworm, *Bothriocephalus scorpii*, induces low but nonetheless energetic costs to turbot and it should be noted that under conditions where food is rationed or, if a fish is diseased and consequently, eating less or has a smaller proportion of a nutrient necessary to the parasite, then the proportion of the host's assimilated energy or nutrient consumed by the tapeworm will be increased. The energy drain of the parasite may feasibly account for as much as 10 % of the ingested food (Chapter 6). Further work is necessary to assess the damage and economic cost of this parasite along with the relative cost of anthelminthic treatment compared with other preventative measures.

4.5 SUMMARY

1 Standardization methods, using a continuous flow respirometer, were devised to measure the standard metabolic rate of juvenile turbot. Fish were held under a 12 h light: 12 h dark regime, fasted for 48 h, placed in respirometer and allowed 2 h to settle

before measurements were taken. No distinct daily rhythms in standard metabolic rate were discernible using this procedure.

2 The relationship between body size and standard metabolic rate was represented by the following equations for turbot acclimated to 10°C and 20°C:

10°C: $y = 0.77x + 2.027$ ($r^2 = 0.89$, sd = 0.032, n = 75, p = 0.001)

20°C: $y = 0.81x + 2.152$ ($r^2 = 0.86$, sd = 0.072, n = 22, p = 0.000)

3 Only a slight decline in metabolic rate occurred with increasing time since last meal.

4 Acute temperature changes were followed by a corresponding increase or decrease in standard respiration rate in turbot. Partial acclimation to temperature change occurred over 3 weeks with further compensation taking place over 10 weeks.

5 A post-prandial increase in respiration rate occurred after a meal; the size of increase and the length of time the respiration rate remained elevated, increased with increasing size of meal. A delay of 1-3 h occurred before the meal had an effect on the respiration rate.

6 Long-term adaptation to feeding levels appeared to produce a slight modification in standard metabolic rate.

7 Daily ingestion rates of turbot appeared to be quite low; this may reflect size of tank or possibly turbot selected for experiment were poor growers.

8 A feeding regime where turbot were fed twice daily to satiation achieved the highest mean specific growth rate over a 10 week period in turbot of initial weight of approximately 20 g. Infrequent feeding to satiation did not produce efficient growth rates.

9 Mean specific growth rate was highest in the 20°C acclimated fish if the first two weeks of the growth experiment are discounted where a large growth check occurred whilst the fish acclimated to temperature.

10 The highest food conversion efficiency was found in the 10°C acclimated turbot fed to satiation, even when the first two weeks of the feeding experiments, where a growth check occurred in the 20°C acclimated fish, were discounted.

11 Growth-ration curves showed that although ingestion rate, expressed as % bwt d^{-1}, was higher in 20°C acclimated turbot, their mean specific growth rate and food conversion efficiency was lower than in the 10°C acclimated fish.

12 Fish fed twice daily to satiation and held at 10°C had the highest condition factor *i.e.* they were heaviest for a given length at the end of the 10 week feeding experiment and

also had the highest hepato-somatic index. Analyses of body composition however, revealed the 20°C acclimated fish to have a slightly higher protein content and far greater liver lipid content as well as slightly higher energy content than the 10°C acclimated fish. Protein levels, carcass lipid levels and energy content was lowest in fish fed on a reduced ration and those fed every 4-5 d to satiation.

13 Energy budgets were constructed for juvenile turbot from data obtained on the effects of intrinsic and extrinsic variations on the respiratory physiology, assimilation efficiency and growth of the fish. Generally, the proportion of ingested energy used for standard and active respiration were quite labile, depending on size of fish, and temperature of acclimation. The proportion of ingested energy lost in feeding metabolism was dependent on size of ration and temperature, whilst the proportion of ingested energy used for growth was very similar in all experimental groups with only a slight increase seen in the 10°C fish fed twice daily to satiation.

The proportion of ingested energy used for growth was slightly low when compared with the results of other workers but may reflect the nature of the diet, the holding conditions or possibly the fish, having been selected from the small size grade stock at Ardtoe, were inherently poor growers.

14 It was estimated that the tapeworm, *Bothriocephalus scorpii,* imposed a small, but nonetheless significant energetic cost to its host of between 1.5-10 % of the host's ingested food energy. The extent of the energy drain is dependent on the size and health of the fish, ration size and quality, and the temperature of acclimation. The most significant costs occur in small fish where the parasite index is highest and where fish are on a reduced ration and held at a high temperature (Chapter 6).

15 Constraints in the number of juvenile turbot available for this research combined with limitations in number and size of tanks due to shortage of aquarium space, imposed severe restrictions in experimental design. The lack of a re-circulating sea water system necessitated in the use of an Eheim charcoal filter pump for each tank and involved lengthy daily maintenance procedures (see Chapter 2). Ideally, larger numbers of fish should have been employed for each of the experimental treatments with each treatment being performed in triplicate to overcome any bias in the experimental set up.

16 Much work is yet required on the effects of various intrinsic and extrinsic factors on the energy partitioning of ingested food in turbot to elucidate the optimum conditions for maximum growth of healthy fish tissue at different ontogenic stages and at minimum cost.

CHAPTER 5

REVIEW OF INFECTIOUS DISEASES OF TURBOT

5.1 INTRODUCTION

Infectious diseases of fish may affect the host in a number of ways from suppression of the host's appetite, depriving the host of nutrients and energy, adverse effects on reproduction, to even causing mortality (Ash *et al.,* 1984; Sindermann, 1990; Grabda, 1991). This study was primarily concerned with the energetics of turbot, but it was considered appropriate to include a short review on infectious diseases of turbot in order to stress their importance in intensive fish culture, and as an introduction to Chapter 6 on 'Infection of turbot with the tapeworm *Bothriocephalus scorpii*'.

5.1.1 Importance of disease in intensive fish culture

A major increase in intensive fish farming and the development of aquaculture over the last 20-25 years has been accompanied by a greater appreciation of the problems posed by diseases of fish in nature and under aquaculture conditions. The significance of these diseases to humans and other vertebrates due to parasitic zoonoses, the constraints they impose on the productivity of aquaculture systems, and the need for control of infections are now more widely accepted (Kent and Fournie, 1993; Young, 1994; Amigo *et al.,* 1996).

Conditions in the sea are relatively constant and the transmission and reproductive rate of a fish parasite are often adapted to a relatively low host density. Fish under intensive cultivation, however, may be challenged with fluctuating water quality, crowding, confinement and handling, all of which may cause immunosuppression and thus lower the resistance of the fish to disease or facilitate the spread of disease by increasing the transmission rate (McVicar and MacKenzie, 1977; Fagerlund *et al.,* 1981). Fish diseases may be introduced to a new environment via water, diet or by introduction of a fish species (Kent and Fournie, 1993; Michel, 1995). If the conditions exist for the completion of the life history pattern of a parasite then it will become established in the new situation (Kennedy, 1994).

Particular attention has been given to bacterial infections, such as vibriosis and furunculosis, and viral infections, such as infectious pancreatic necrosis (IPN) and viral haemorrhagic septicaemia (VHS), as these have been responsible for many serious outbreaks of disease and for most fatalities in fish farms (Roberts, 1989; Shepherd and Bromage, 1989). More recently, the potential danger to marine aquaculture and possible threat to human health due to protozoal infections such as *Glugea stephani* and *Tetramicra brevifilum* has been recognized (Figueras *et al.*, 1992; Diamant and Paperna, 1995; Dyková, 1995; Dyková *et al.*,1995; Amigo *et al.,* 1996). Many studies have been conducted on metazoan parasites including ectoparasites such as the sea lice *Lepeophtheirus salmonis* and *Caligus elongatus* (Sharp *et al.*,1994). Helminths such as the monogenean fluke *Gyrodactylus salaris* pose a serious threat to salmon

farmers (Mo, 1994), largely due to having a direct life-history and their ability to produce infections of enormous proportions on fish in confined areas. Larval digeneans such as *Diplostomum* species are also recognized as serious pathogens of captive fish. However, intestinal helminths of fish, including cestode infections, have frequently been considered as less serious and requiring less attention than other diseases.

5.1.2 Diseases of turbot

Investigation into the diseases of turbot has followed a similar trend to that described above for fish in general. An increase in turbot farming in Britain and France in the late nineteen seventies and in Denmark, Norway and north-west Spain in the early nineteen eighties has been accompanied by a rise in the awareness of the pathological problems of turbot (Novoa *et al.* 1992; Toranzo *et al.*, 1993a,b) and so has stimulated a number of studies on the characterization of the aetiological agents (Devesa *et al.*, 1989; Lupiani *et al.*, 1993; Toranzo *et al.*, 1990; Toranzo and Barja, 1990; Bloch *et al.*, 1991; Fouz *et al.*, 1992) and on the prevention and control of the diseases (Santos *et al.*, 1991; Toranzo *et al.*, 1992). Table 5.1 summarizes the infectious diseases of turbot.

Bacterial and viral infections have received a great deal of attention as outbreaks often cause very heavy mortalities in turbot stocks. Outbreaks of vibriosis caused by the bacterium *Vibrio anguillarium* have occurred in cultured turbot over many years. Fatalities due to infection with this organism were in fact a problem at the beginning of the present study when turbot were obtained from Hunterston. These were overcome partly by improvements in husbandry techniques and also with reference to the existing knowledge on control of the disease. Many studies into the cause of the disease and to methods of prevention and control have been undertaken (Horne *et al.*, 1977; Santos *et al.*, 1991; Vigneulle and Laurencin, 1991; Westerdahl *et al.*, 1991; Olsson *et al.*, 1992; Skiftesvik and Bergh, 1993; Toranzo, *et al.*, 1993a,b; Munro *et al.*, 1995). Generally, losses due to *Vibrio anguillarium* can be controlled by chemotherapy (Toranzo *et al.*, 1993b). More recently, other biotypes have also been shown to be responsible for outbreaks of vibriosis *e.g. Vibrio damsela* (Fouz *et al.*, 1992); *Vibrio alginolyticus* (Austin *et al.*, 1993); *Vibrio pelagius* (Toranzo *et al.*, 1993b); *Vibrio splendidus* (Angulo *et al.*, 1994). *V. alginolyticus* is frequently associated with mortalities of larval stage turbot (Toranzo *et al.*, 1993b).

A rare oxidase-negative strain of the bacterium *Aeromonas salmonicida* was reported to be the causative agent of two disease outbreaks leading to 100 % mortality in turbot in a fish farm in Denmark (Pedersen *et al.*, 1994). A simultaneous report of the isolation of a rare oxidase-negative strain of *Aeromonas salmonicida* from the ulcers of flounders, *Pleuronectes*

Table 5.1 Infectious diseases of turbot

Group	Name of disease	Causative agent	Literature Sources
Viruses	Infectious Pancreatic Necrosis Virus (IPNV)	Birnovirus	Roberts (1989); Shepherd and Bromage (1989); Castric *et al.* (1987); Mortensen *et al.* (1993); Novoa *et al.* (1993b)
	Viral Hemorrhagic Septicemia (VHS)	Rhabdovirus	Castric *et al.* (1984)
	Turbot herpesvirus disease	*Herpesvirus scophthalmi*	Buchanan and Madeley (1978); Buchanan *et al.* (1978); Plumb, (1993)
	Encephalomyelitis	Picornavirus or enterovirus	Bloch *et al.* (1991)
		Iridovirus-like agent	Bloch and Larsen (1993)
		Birnavirus	Novoa *et al.* (1993a)
	Turbot aquareovirus (TRV)	Aquareovirus	Lupiani *et al.* (1993)
Bacteria	Vibriosis	*Vibrio anguillarium*	Horne *et al.* (1977); Santos *et al.* (1991); Vigneulle and Laurencin (1991); Westerdahl *et al.* (1991); Olsson *et al.* (1992); Skiftesvik and Bergh (1993); Toranzo *et al.* (1993); Estevez *et al.* (1994); Munro *et al.* (1995)
	Vibriosis	*Vibrio damsela*	Fouz *et al.* (1992)
	Vibriosis (Gill disease)	*Vibrio alginolyticus*	Austin *et al.* (1993)
	Vibriosis	*Vibrio splendidus*	Angulo *et al.* (1994)
	Vibriosis	*Vibrio pelagius*	Novoa *et al.* (1992)
	Furunculosis	*Aeromonas salmonicida*	Pedersen *et al.* (1994)
	Furunculosis (experimental)	*Aeromonas hydrophila*	Gatesoupe (1991)
	Systemic disease	*Flavobacterium scophthalmum*	Mudarris *et al.* (1994)
		Flexibacter maritimus	Toranzo *et al.* (1993b)
		Pseudomonas spp.	Novoa *et al.* (1992); Toranzo *et al.* (1993a,b)
		Streptococcus	Novoa *et al.* (1992); Toranzo *et al.* (1993a)
		Staphylococcus	Novoa *et al.* (1992); Toranzo *et al.* (1993a)
Protozoa		*Trichodina* sp. (ciliate)	Novoa *et al.* (1992)
		Cryptocaryon sp. (ciliate)	Novoa *et al.* (1992)

Table 5.1 Infectious diseases of turbot

Group	Name of disease	Causative agent	Literature Sources
Protozoa		Histophagous ciliate (Hymenostomata)	Dykova and Figueras (1994)
		Costia. sp. (flagellate)	Novoa *et al.* (1992)
		Tetramicra brevifilum (myxosporean)	Estevez *et al.* (1992); Figueras *et al.* (1992); Novoa *et al.* (1992); Toranzo *et al.* (1993a,b); Dykova and Figueras (1994); Lom and Bouix (1995)
	Myeloid leucosis	*Haemogregarina sachai* (apicomplexa)	Ferguson and Roberts (1975); Kirmse and Ferguson (1976); Kirmse (1978, 1980) Anderson *et al.* (1976)
		Myxidium incurvatum (myxosporean)	Anderson *et al.* (1976)
		Rhabdospora thelohani *	Anderson *et al.* (1976)
	Amoebic gill disease	Amoebae (not identified)	Dyková *et al.* (1995); Lom and Bouix (1995)
Metazoa Helminths		*Bothriocephalus scorpii* (cestode)	Rees (1958); Jones (1975); Davey and Peachey (1968); de Groot (1971); McKinnon and Featherston (1982); Robert *et al.* (1988, 1990); Duran *et al.* (1989); Renaud *et al.* (1990); Williams and Jones, (1994)
Molluscs		*Hemibdella* sp. (leech)	Roberts (1989)

* see Chapter 6

flesus, in the Baltic Sea (Wiklund and Bylund, 1991) suggests the existence of a formerly unrecognized but potentially important group of fish pathogenic bacteria (Pedersen *et al.*, 1994). The possibility of links between disease of wild and captive stocks also stresses the need for widespread epidemiological knowledge of the area surrounding fish farms. Systemic disease in turbot in Scotland was found to be caused by a previously unrecognized cytophagan bacterium, *Flavobacterium scophthalmum* sp. nov. (Mudarris *et al.*, 1994). A specific infection of the lateral line by cytophagan bacteria in turbot held at cold water temperatures has also been observed (Roberts, 1989). In recent years there has been an increase in the number of cases of ulcerative disease due to the filamentous gliding bactetria, *Flexibacter maritimus*, which, although it does not cause direct mortality, is believed to facilitate the infection of turbot by other pathogens (Toranzo *et al.*, 1993b).

An outbreak of encephalomyelitis resulting in 100 % mortality in larval turbot was caused by a virus belonging to the Picornaviridae or possibly an enterovirus (Bloch *et al.*, 1991). A Danish rearing unit attributed 70 % mortalities in turbot fry due to an iridivirus-like agent (Bloch and Larsen, 1993). Light but persistent mortalities in a turbot farm in Galicia, northwesten Spain, were due to a Birnavirus; infectivity trials found the virus only produced mortalities in small turbot (2 g) (Novoa *et al.*, 1993a,b). Infectious pancreatic necrosis virus (IPNV) has been described in turbot fry (Castric *et al.*, 1987; Mortensen *et al.*, 1993; Novoa *et al.*, 1993a,b). Viral haemorrhagic septicaemia virus (VHSV) causing dark skin, haemorrhagic head, eyes, fins, liver and abdominal wall as well as spleen and kidney necrosis was observed in turbot by Castric and Dekinkelin (1984).

Herpesvirus disease is an infection of turbot caused by *Herpesvirus scophthalmi* and characterized by giant cells on the skin and gill epithelium of young fish (Buchanan and Madeley, 1978; Buchanan *et al.*, 1978; Plumb, 1993). The disease has been shown to occur in both wild and intensively reared fish in Scotland where heavy mortalities have been recorded in fish farms (Buchanan *et al.*, 1978; Plumb, 1993).

The understanding of the pathogenicity of some parasitic protozoa and the dangers posed to marine fish farming, including turbot farming, has improved in recent years (Diamant and Paperna, 1995; Dyková, 1995). McVicar and MacKenzie (1977) discussed the influence of intensive monoculture systems on the transmission of parasites with direct life-cycles such as the ciliate protozoans. They noted infections with the ciliate *Trichodina* sp. were common amongst flatfish. Infections with *Trichodina* sp. and *Cryptocaryon* sp. have been reported in turbot farms in Galicia, north-western Spain (Novoa *et al.*, 1992) and a histophagous ciliate causing mortalities in turbot was observed by Dyková and Figueras (1994). Endoparasitic protozoans such as microsporidians have become increasingly abundant and are gaining importance as fish

pathogens in the Mediterrancan. High mortalities in turbot due to the microsporidian *Tetramicra brevifilum* have been recorded over the last few years (Estevez *et al.*, 1992; Figueras *et al.*, 1992; Novoa *et al.*, 1992; Dyková and Figueras, 1994; Lom and Bouix, 1995). Figueras *et al.* (1992) noted losses of 11.5 % in a stock of 30000 fish infected with *Tetramicra brevifilum* in one grow-out plant in Galicia along with a reduction in growth rate of 50 % in infected fish. Economic losses are high because the parasite causes high morbidity, a reduction in fish growth, is difficult to control and necessitates the destruction of all affected stocks (Toranzo *et al.*, 1993b). Anderson *et al.* (1976) reported the presence of the myxosporean *Myxidium incurvatum* and another parasite, *Rhabdospora thelohani*, in turbot suffering from the hepato-renal syndrome. They suggested the parasites played an opportunistic role in the diseased fish. *Rhabdospora thelohani* was observed in turbot in the present study, however its status as a protozoan parasite or as a type of host cell remains in question and this problem is addressed in Chapter 6. Infection rates of up to 6 % by the hemogregarine, *Haemogregarina sachai* causing myeloid leucosis in cultured turbot were recorded in Scotland (Ferguson and Roberts, 1975; Kirmse 1978, 1980; Sindermann, 1986). The hemogregarines are common blood parasites of fish and are normally benign, however intensive culture conditions, such as overcrowding and involving other stressors, may lead them to become pathogenic.

Although lesions due to amoebic infections are relatively rare in fish, a recent serious outbreak of amoebic infection affecting the gills of turbot has been observed in turbot farms in Spain (Lom and Bouix, 1995); the parasite species is as yet unidentified. According to Diamant and Paperna (1995), there is an epizootiological link between protozoan infections of wild and captive fish which is responsible for the appearance of previously unknown species. They recommended studies on protozoonoses of cultured fish should be associated with simultaneous research of wild fish.

Of the parasitic metazoans, leeches of *Hemibdella* sp. have been shown to be important ectoparasites of cultured turbot (Roberts, 1989). Interest and research into intestinal helminths of turbot has not received the same amount of attention as viral, bacterial and protozoan infections despite the fact that infection with the tapeworm, *Bothriocephalus scorpii*, is widespread in turbot and may cause mortality through intestinal blockage and reduction in growth rate of the infected fish (Durán, *et al.*, 1989; Sindermann, 1990).

A relatively high prevalence of infection with the tapeworm *Bothriocephalus scorpii* was observed in turbot during the present study. As one of the objectives of this project was to examine the partitioning of energy consumed by the fish for maintenance, activity and growth, it seemed important to study any possible effect the parasite may have on its host. The biology and epidemiology of the infection in turbot are investigated in Chapter 6.

5.2 DISCUSSION AND CONCLUSIONS

The study of infectious diseases of fish is of great importance both to fish farmers and fisheries scientists from a biological and, ultimately, an economic point of view. Detailed epidemiological surveys are required to gain accurate knowledge of the incidence, intensity, and mortality rates of diseases in wild and captive stocks (Anderson, 1978, 1979; Anderson and Gordon, 1982; Munro *et al.*, 1983; Sindermann, 1986, 1990). It is important to be aware of the possibility of introducing pathogens which may then spread to wild stocks when fish species are translocated from one area to another for aquacultural purposes (Sindermann, 1990). Knowledge of the biology and the response of fish to pathogens would help give an understanding of the pathological effects of disease on the health and, consequently, on the growth rate of cultivated fish stock. Little is known of the bioenergetic cost of disease in fish; this is an area that has only recently begun to draw research interest (Beamish *et al.*, 1996). An accurate knowledge of the aetiological factors contributing to disease, such as pollution levels and other forms of stress, is required to further the understanding of, and hence the prevention of, such diseases. An understanding of these relationships could contribute to the use of fish diseases as indicators of environmental pollution levels (Möller, 1986). The study of the relationships between infectious diseases of fish and human health is of prime importance as fish are such an important source of protein for humans. Diseased fish are often rejected by the public and reduced demands may ensue, especially if a risk to public health is perceived (Amigo *et al.*, 1996).

The control of fish diseases in intensive fish farming is critical and depends on extensive knowledge of the aetiology and the epidemiology of the disease, accurate diagnostic techniques, management of stress, prophylactic measures such as immunization and chemotherapy, and chemotherapeutic treatment. Chemotherapeutic techniques are standard practice on many fish farms but the problem of possible drug-resistant strains developing should be considered as well as the any negative effect the chemicals may have on the on the food chain or on the environment (Young, 1994).

Although the study of infectious diseases of turbot has increased in recent years it is an area still very much in its infancy. The importance of bacterial and viral infections has received most attention whilst the pathogenicity of some parasitic protozoa is becoming increasingly evident (Sindermann, 1986, 1990; Diamant and Paperna, 1995; Dyková, 1995). Research on intestinal helminths of turbot is sparse, largely because they are considered to have little, or no, pathogenic effect. However, intestinal helminths derive their nutritional energy from their host and must therefore effect a nutritional drain and hence cost to the aquaculture industry.

Further research into the infectious diseases of turbot is essential if efficient methods of control are to be enforced where necessary and maximum sustained yields are to be realized.

Mawdesley-Thomas (1972) stated that " .... disease *per se*, is not an entity in itself. Disease is the end result of an interaction between a noxious stimulus and a biological system and to understand disease is to understand all aspects of that system" (Snieszko, 1974).

5.3 SUMMARY

1 A major increase in intensive culture of turbot has led to a concomitant rise in the pathological problems of turbot; most of the recent research has focussed on bacterial infections (vibriosis and furunculosis) and on viral infections (infectious pancreatic necrosis {IPNV}, viral haemorrhagic septicaemia {VHS}).

2 The pathogenicity of some parasitic protozoa, especially the myxosporeans and microsporidians, and their threat to turbot stocks has become evident and has led to recent research in this area.

3 Some infectious agents which are normally benign may become pathogenic under conditions of stress, such as those encountered in intensive fish culture (*e.g. Haemogregarina sachai*).

4 Accurate knowledge of the effect of disease on wild and cultivated turbot population numbers is necessary. Quantitative knowledge of the effect of disease on the growth rate of fish is required.

5 An accurate knowledge of the actiological factors contributing to the disease and an understanding of the defence mechanisms of turbot are important in the prevention and control of disease.

6 Research into the infection of turbot with the pseudophyllidean tapeworm, *Bothriocephalus scorpii*, has been largely overlooked due to the belief that it has little, or no, pathogenic effects. It could, however, have important effects on energy availability as discussed in Chapter 6.

CHAPTER 6

INFECTION OF TURBOT WITH THE PSEUDOPHYLLIDEAN TAPEWORM, *BOTHRIOCEPHALUS SCORPII*

6.1 INTRODUCTION

6.1.1 Importance of cestode infections in fish

The importance of disease in intensive fish farming and the infectious diseases of turbot are reviewed in brief in Chapter 5. Research in this area has focused mainly on bacterial and viral infections, although recently the potential threat to aquaculture by protozoal infections has received attention (Figueras *et al.*, 1992; Diamant and Paperna, 1995; Dyková, 1995; Dyková *et al.*, 1995). Intestinal helminths of fish, including cestodes, have generally been considered to be a less serious problem than other diseases and consequently less research has been carried out on this topic. There remains something of a controversy over the pathogenicity of tapeworms.

Dick and Choudhury (1995) stated "Anyone who has necropsied a fish is immediately aware that tapeworms and fish are an important biological duet". Despite the fact that very heavy cestode infections are relatively common in fish some scientists consider this group of parasites is of little importance in fish health, partly because few have been shown to cause mortality in fish (Rees, 1967). Other scientists, however, along with fish farmers and anglers, believe the massive tapeworm burdens carried by some fish must have an adverse effect on the host, either by causing fish mortality due to intestinal blockage or by reducing the growth rate of fish (Williams, 1963; Durán *et al.*, 1989; Sindermann, 1990; Hoole, 1994).

A relatively high prevalence of infection with the tapeworm *Bothriocephalus scorpii* was observed in turbot during the present study. As one of the objectives of the project was to examine the partitioning of energy consumed by the fish for maintenance, activity and growth, it seemed important to study any possible effect the parasite may have on its host. To do this, it was first necessary to gain an understanding of the general biology of the tapeworm and of the epidemiology of the infection.

6.1.2 Biology of *Bothriocephalus scorpii*

6.1.2.1 Background and systematics

Bothriocephalus scorpii (Mueller, 1776) Cooper, 1917 (Eucestoda: Pseudophyllidea) is an intestinal parasite of marine fish belonging to the family Bothriocephalidae. Cooper (1918) reported *B. scorpii* from over 50 species of marine teleosts but recent chemotaxonomic techniques have shown some species believed to infect a wide range of hosts are really species complexes which may show strict host specificity (Williams and Jones, 1994). For example, enzymatic polymorphic analysis of *Bothriocephalus* from turbot and brill from the Mediterranean revealed a complete mating isolation of populations from the host species

and led the authors to conclude each parasite population should be considered a distinct species (Renaud *et al.*, 1983, 1986; Robert *et al.*, 1990). Renaud *et al.* (1983) named the parasite of turbot as *Bothriocephalus gregarius*. However, in the present work the name *Bothriocephalus scorpii* will be retained as this is the name most frequently used in the literature.

Systematics of *Bothriocephalus scorpii*:

The following system of classification is based on that used by Schmidt (1986).

PHYLUM: Platyhelminthes

CLASS: Eucestoda

ORDER: Pseudophyllidea

FAMILY: Bothriocephalidae Blanchard 1849

GENUS: *Bothriocephalus* Rudolphi 1808

SPECIES: *B. scorpii* (Mueller, 1776) Rudolphi 1808

6.1.2.2 Life history pattern

The life history of *Bothriocephalus scorpii* was first described by Markowski (1935) who believed that two intermediate hosts were necessary; firstly, a cyclopoid copepod in which the procercoid stage develops and then a species of fish (goby) in which the plerocercoid stage develops. However, Davey and Peachey (1968) demonstrated that '0' group turbot at a stage where their diet consists almost exclusively of polychaetes and crustaceans do harbour plerocercoid worms. This indicated that a second intermediate host was not required. This situation was further elucidated by Robert *et al.* (1988) who noted that young turbot became infected by eating copepods containing proplerocercoids (short cycle with an obligatory host, see Figure 6.1) and older turbot became infected by eating infected gobies (long cycle where goby is paratenic host). The authors considered the goby to be a paratenic host even though the parasite undergoes some development from a proplerocercoid to a strobilating plerocercoid within them as they are not necessary for the maturation of the cestode to the adult stage. They also speculated on the hypothesis described by Euzet and Combes (1980) where the maturation of worms in what was once a paratenic host could possibly be a mechanism for sympatric speciation in the *B. scorpii* complex and may explain the explosion of species belonging to the *B. scorpii* complex seen in the Atlantic (Robert *et al.*, 1988; Renaud *et al.*, 1990). Figure 6.1 depicts the life history of *Bothriocephalus scorpii*.

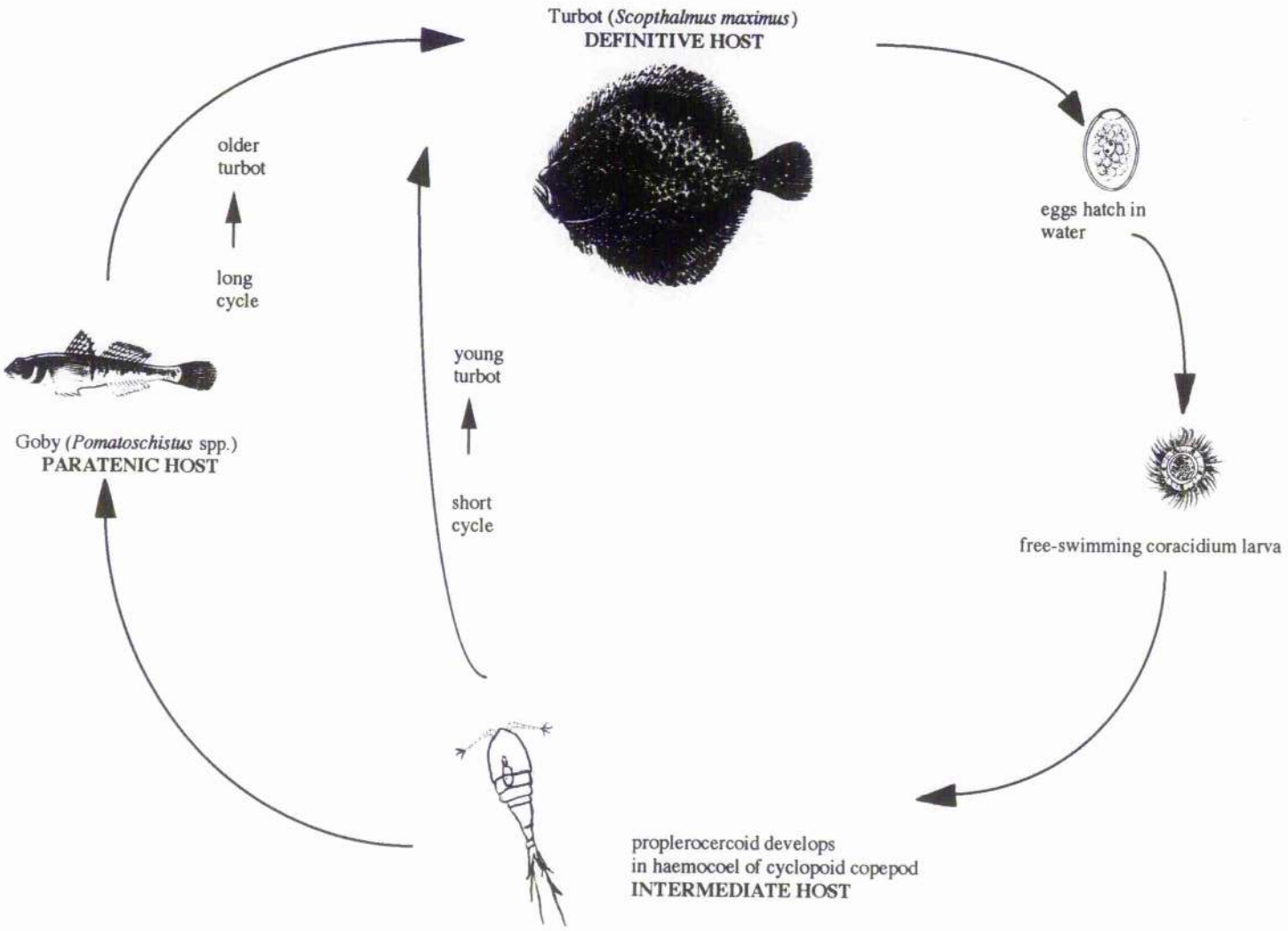


Figure 6.1 Life history pattern of *Bothriocephalus scorpii* depicting short cycle where turbot are infected directly from infected copepods and the long cycle involving the paratenic host.

6.1.3 The epidemiology and pathology of *Bothriocephalus scorpii* infection in turbot

In turbot, the prevalence and intensity of *Bothriocephalus scorpii* is observed to increase with host size (Davey and Peachey, 1968; Robert *et al.*, 1990). Prevalences have been recorded ranging from 30-36 % in young turbot of length <100 mm from the North Sea (Davey and Peachey, 1968; de Groot, 1971) to 54 % in turbot in the 110-200 mm length group (de Groot, 1971) and to 100 % prevalence in larger fish from the North Sea, Atlantic and Mediterranean (Davey and Peachey, 1968; de Groot, 1971; Robert *et al.*, 1990). According to Davey and Peachey (1968) initial infection occurs very early, in turbot of 32 mm in length. Intensity of infection was found to be lower in fish less than 210 mm in length than in those over 210 mm in length (Davey and Peachey, 1968; Robert *et al.*, 1990). Increasing prevalence and intensity in fish greater than 120 mm in length is correlated with a change in diet from crustaceans and molluscs to almost exclusively fish (gobies), the paratenic host. When turbot reach 300 mm in length the prevalence and intensity of infection no longer increases. The fish are then three years old and the life expectancy of the parasite is 3 years. This stability in parasitism has been linked to the death of the adult cestodes and migration of the fish away from the coast (Robert *et al.*, 1990). The role of the paratenic host (goby) in the transmission of the parasite has been discussed by Robert *et al.* (1988).

The distribution pattern of *B. scorpii* in turbot corresponds to a lognormal curve (intensities of > 10 worms per fish, which is rarer than a negative binomial distribution). Davey and Peachey (1968) found adult turbot to be infected with between 7-194 worms per fish, with a mean of 44.5, and o-group to have mainly 1-3 worms per fish, although a few of these had many worms (4-66) resulting in a mean of 13 worms per fish.

Many workers consider that the adult cestode causes little or no damage to the host and that any pathological effects are usually the result of mechanical damage by the attachment organs, with an inflammatory response at the site of attachment (Williams and Jones, 1994). This has been the view held by some authors for infection of turbot with the cestode, *Bothriocephalus scorpii*. "The presence of tapeworm does not appear to cause any ill-effect in either turbot or brill" (de Groot, 1971). On describing the site of attachment of the scolex in turbot, Jones (1975) observed mucosal cell damage and an increase in the connective tissue underlying the epithelium adjacent to the scolex. Davey and Peachey (1968) stated "the host tissue reaction appears to be restricted to a reduction in the size of the epithelial cells and in the number of goblet cells" . This agrees with Rees (1958) who observed "the effect on the mucosa is slight, due to the absence of very powerful muscles and of other specialized adhesive structure" although this was based on a sample of one. According to Durán *et al.* (1989), however, *Bothriocephalus scorpii* may cause mortality and economic loss due to intestinal blockage and

spoilage of host substances and decreased growth rate of the infected fish. This view was also held by Williams (1963) who thought turbot harbouring massive infections of *B. scorpii* must be deprived of a considerable quantity of nutrients. Person-Le-Ruyet (1990) noted that wild turbot were often infested with *B. scorpii* and massive infestations caused apathy and progressive physical degeneration.

Although no overt pathology was visible in turbot during the present work, an organism of such a size inhabiting the restricted habitat in the intestine must have some effect on its host. Tapeworms must obtain all their nutrients and energy from their host's food and digestion products, or, more commonly, from assimilated food substances obtained from their host's tissues and metabolites (Crompton, 1991). It would seem reasonable to assume that they are depriving their host of a certain quantity of its consumed energy and nutrients. Studies on the effect of low level *Eubothrium salvelini* infection in various salmonids have shown parasitism to have a deleterious effect on growth and survival of the fish (Boyce, 1979; Hoffmann *et al.*, 1986; Bristow and Berland, 1991). It was therefore decided to examine the prevalence and intensity of *Bothriocephalus scorpii* infection and the corresponding distribution pattern in farmed juvenile turbot and establish the parasite index (proportion of the host-parasite relationship representing the tapeworm) for different sized fish. Histological investigations were carried out to find the site of attachment and discover if any associated tissue pathology was induced by the parasite. Investigations were also carried out to find out if parasitism had any effect on the condition factor or the hepato-somatic index of the fish as these are commonly used as indices of growth and condition in fish. Finally, estimations were made of the amount of energy the tapeworms consume in a specific size of fish under various conditions.

6.1.4 Research aims

1 To find out the prevalence and intensity of infection and examine the frequency distribution pattern of the tapeworm, *Bothriocephalus scorpii* in juvenile turbot obtained from Hunterston and Ardtoe and compare these data with information for natural populations of turbot. These statistics may provide an insight into the possible mortality and morbidity due to the infection and also give information on the potential transmission patterns of the parasite and hence the regulation of parasite numbers within the host population in different environments.

2 To measure the proportion of the host-parasite relationship which is attributed to the parasite (Parasite index) and examine this in different size classes and age groups of turbot.

3 To carry out a histological investigation of the site of attachment and observe if there are any signs of local tissue pathology and compare this with information from other sources.

4 To study the effect of the parasite on the condition factor of turbot.

5 To study the effect of the parasite on the hepato-somatic index of turbot.

6 To estimate the amount of energy consumed by the parasite per day in a specific weight of fish held under a specific food regime and at a specified temperature.

6.2 MATERIALS AND METHODS

The source of fish and preparation of material for the experiments described in this chapter were described previously in Chapter 2. Methods specific to this chapter are described below:

6.2.1 Epidemiology of *Bothriocephalus scorpii* infection in turbot

Epidemiology of parasitic infection is the study of all the ecological aspects to explain its transmission, distribution, prevalence and incidence within a host population (Schmidt and Roberts, 1989) Descriptive statistics are usually employed to quantify the levels of parasitic infection within the host. Prevalence and intensity of infection, the frequency distribution of parasite numbers within the host (with associated pattern of distribution), and parasite index were used to determine the level of *Bothriocephalus scorpii* infection in '0' group turbot.

Prevalence of infection

Prevalence (P) of infection is the proportion (most frequently expressed as %) of the host population infected with a parasite at a given time.

$$\text{Prevalence} = \frac{\text{number of infected individuals}}{\text{number of hosts sampled}} \times 100$$

Intensity of infection

The intensity of a parasitic infection is usually defined as the number parasites per infected host.

$$\text{Intensity} = \text{number of parasites per infected host}$$

Parasite index

The intensity of infection does not always reflect the true parasite burden and so the weight of worm burden per weight of host, sometimes expressed as the parasitization index (Arme and Owen, 1967; Pennycuick, 1971a,b,c,d), is often used. According to Pennycuick (1971d), the weight of the parasite burden is an important determinant of pathology.

$$\text{Parasite index} = \frac{\text{weight of } \textit{Bothriocephalus scorpii}}{\text{weight of turbot} + \text{weight of } \textit{B. scorpii}} \times 100$$

Frequency distribution of numbers of worms per host

Parasite prevalence and mean abundance are statistics of the probability or frequency distribution of parasite numbers per host. The form of this distribution determines the relationship between prevalence and intensity (Anderson, 1993). These statistics are often related to the mortality and morbidity of the infection and the pattern of parasite aggregation may also have important implications in the regulation of parasite numbers within the host population. Basically, there are three patterns of dispersion which the distribution of parasite numbers per host can adopt (Anderson and Gordon, 1982). A simple measure of the degree of dispersion is the variance to mean ratio of the parasite numbers per host denoted by the expression $s^2 / \bar{x}$, where s^2 is the sampling variance and $\bar{x}$ is the mean of the sample. In an under-dispersed distribution pattern, most of the hosts have a similar numbers of parasites and in this case the variance to mean ratio is less than one. The distribution of the parasites may be random where the variance to mean ratio is approximately equal to one. Finally, the distribution pattern of the parasites may be overdispersed within their host population where most hosts have few parasites and a few hosts harbour most of the parasite population. In this case the variance to mean ration is greater than one.

Helminth parasites generally tend to be overdispersed where many hosts have few parasites and a few hosts have many parasites. This variability is due to heterogeneity in host susceptibility which may be caused by differences in host behaviour, distribution of infective stages or differences in host immunocompetance. Anderson and Gordon (1982) noted the factors which lead to under- or overdispersion of parasite populations within their host (Figure 6.2)

6.2.2 Condition factor

The body condition factor (CF) is a standard variable used by fish biologists as an indirect measure of the energy status of a fish. It is used to compare the 'fatness' or 'well-being' of a fish and is based on the hypothesis that the heavier a fish is for a specified length the greater

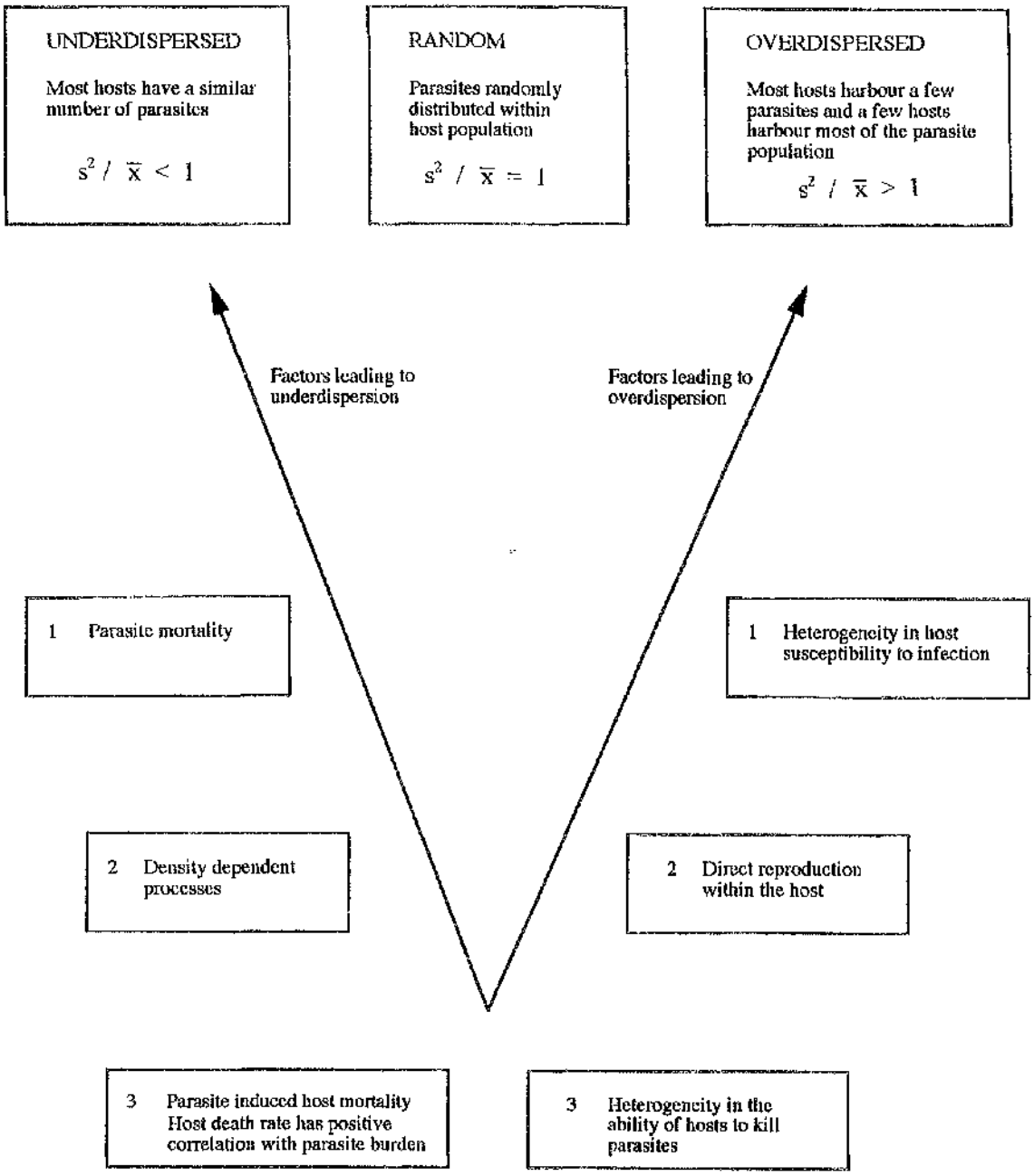


Figure 6. 2 Factors leading to under- or overdispersion in parasite populations (adapted from Anderson and Gordon, 1982)

the condition of the fish (Weatherly and Gill, 1987). Many workers use the following simple formula for the determination of body condition where the weight of the fish is measured in grams and the length refers to the fork length of the fish measured in cm.

$$\text{Condition factor (CF)} = \frac{\text{weight of fish}}{\text{length of fish}^3} \times 10^2$$

6.2.3 Hepato-somatic Index

The hepato-somatic index represents the weight of the liver expressed as a percentage of the total body weight and is often used by fish biologists as an indication of condition in fish. The liver is a major energy store, especially in non-fatty fish (Chellappa *et al.*, 1995), and whenever dissection is possible the HSI is often used as an indication of the energy status of the fish (Wooten, *et al.*, 1978; Campbell and Love, 1978; Chellappa *et al.*, 1995).

$$\text{Hepatosomatic Index} = \frac{\text{weight of liver}}{\text{weight of fish}} \times 100$$

The weight of the liver and of the fish is measured in grams.

6.3 RESULTS

6.3.1 Identification, location and means of attachment of *Bothriocephalus scorpii*

Detailed morphological features of *Bothriocephalus scorpii* from the turbot, *Scopthalmus maximus* (L.), have been described by Hilmy (1929), Rees (1958) and Jones (1975). In the present study, identification of the tapeworms recovered from juvenile turbot was made by comparison with the details described by the earlier authors.

Specimens of *Bothriocephalus scorpii* were observed in the intestine of juvenile turbot with their scoleces attached either in the pyloric caeca or in the anterior intestine immediately behind the pyloric caeca. The worms were tightly attached by the scolex to the host tissues. Histological examination revealed the scolex to be quite muscular and the bothria well formed suggesting they are important as means of attachment. The scolex was deeply embedded in the primary folds of the intestine with the bothria acting as pincers, gripping the secondary folds of the intestinal mucosa (Figures 6.3, 6.4).

6.3.2 Local tissue pathology associated with *Bothriocephalus scorpii* infection

The area of intestinal tissue next to the scolex showed some signs of damage. There was a proliferation of fibrous connective tissue in this region and in some parts the mucosal epithelial cells appeared to be squashed together. There also appeared to be clumping or flattening of the microvilli in this region of the intestine adjacent to the scolex (Figure 6.4).

6.3.3 Prevalence of *Bothriocephalus scorpii* in turbot

From a sample of 53 'o' group turbot ranging in length from 50-152 mm, 25 were found to be infected with the tapeworm *Bothriocephalus scorpii* giving an overall prevalence of 47.2 %. The first two samples of turbot were obtained from Hunterston and these were found to have a prevalence of 54.6 % and 60.0 % for three and four month old turbot respectively (all of the 21 fish sampled were under 100 mm in length). All further samples were obtained from Ardtoe, and for these the prevalence was found to be 40 % for 6 month old fish in the length range 96 - 136 mm. Figure 6.5 illustrates the prevalence of *B. scorpii* in 'o' group turbot according to age groups. Figure 6.6 shows the prevalence of infection in different length classes of turbot in the present study and also includes data obtained by Davey and Peachey (1968) for adult turbot from British coastal waters.

A higher prevalence of infection with the tapeworm was found in 'o' group turbot which had died early in the study due to infection with the bacterium *Vibrio anguillarium*. Of thirty-six fish autopsied, twenty-eight were infected with the tapeworm giving a prevalence of 77.8 %.

6.3.4 Intensity of *Bothriocephalus scorpii* in turbot

The intensity of infection in farmed 'o' group turbot was found to be very low with most fish harbouring only one tapeworm and a few harbouring two or three worms. Fish under 100 mm in length had only single infections whilst data from Davey and Peachey (1968) showed adult turbot from the North Sea had a mean intensity of infection of 44.5 worms per fish (Figure 6.7).

6.3.5 Frequency distribution of numbers of worms per fish

The frequency distribution of parasite numbers per fish for 'o' group turbot sampled from Hunterston and Ardtoe was random (variance to mean ratio of 0.9). This is very close to one and suggests a random distribution pattern (Figure 6.8). Data from Davey and Peachey (1968) was analysed to find the frequency distribution of parasite numbers for adult wild caught turbot. A variance to mean ratio of 22.7 indicates the parasite population was overdispersed and showed a

Source	Number of fish	Length class (mm)	Number infected	Prevalence = % infected	Total parasite number	Mean abundance	SE	SD	Variance: mean ratio $s^2 / \bar{x}$
Hunterston + Ardtoe	53	0-200 mm	25	47.0 %	30	0.56	0.97	0.71	0.9
Hunterston	26	0.100 mm	14	53.8 %	14	0.54	0.87	0.50	0.47
Ardtoe	27	101-200 mm	11	40.7 %	16	0.53	0.15	0.87	1.27
North Sea (Davey and Peachey, 1968)	45	200+ mm	45	100 %	2002+	44.5	4.9	32.7	23.7

Table 6.1 Prevalence, mean intensity and variance to mean ratio of *Bothrioceaphalus scorpii* in different length classes of farmed (Ardtoe and Hunterston) and wild (North Sea) turbot.

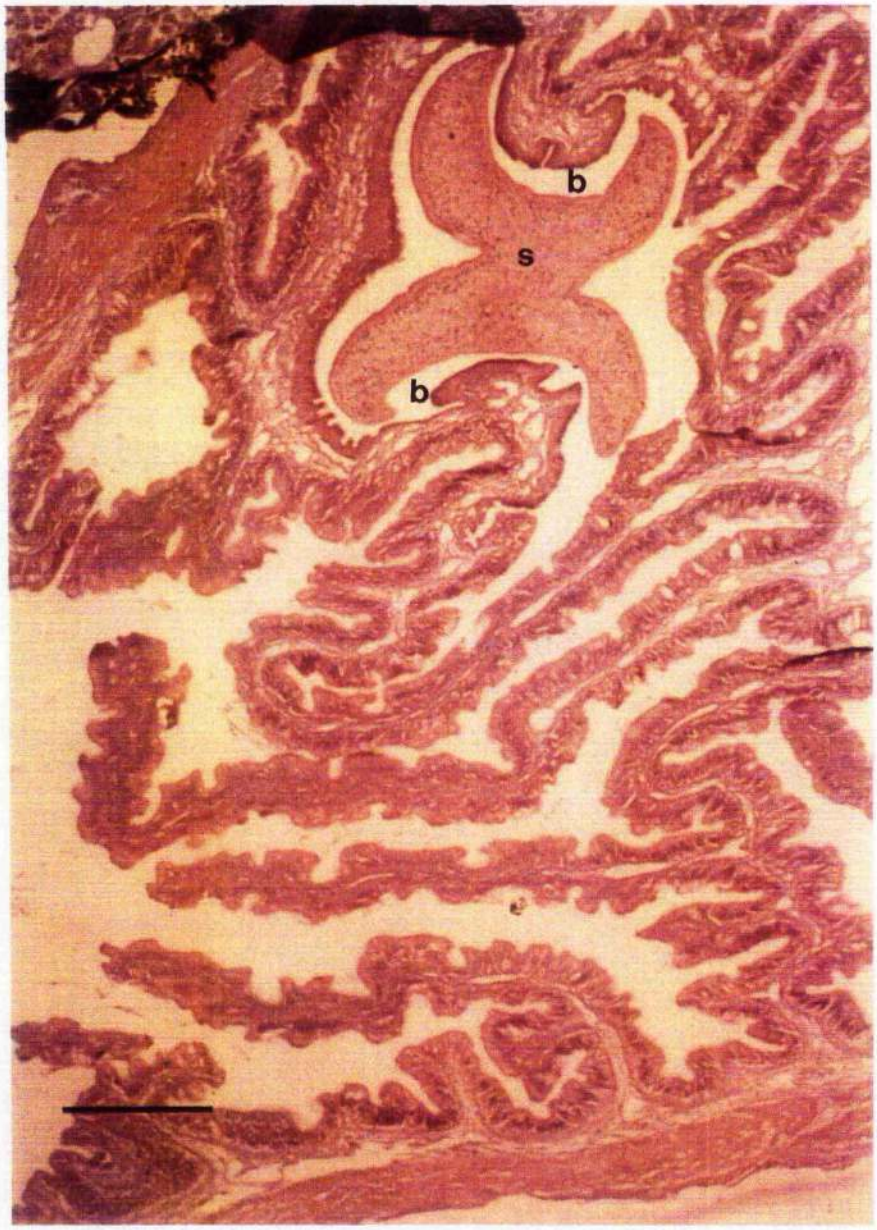


Figure 6.3 Transverse section through anterior intestine of turbot showing scolex (s) of *Bothriocephalus scorpii* deeply embedded in primary folds with the bothria (b) acting as pincers, gripping the secondary folds. Scale bar: 100 μm.

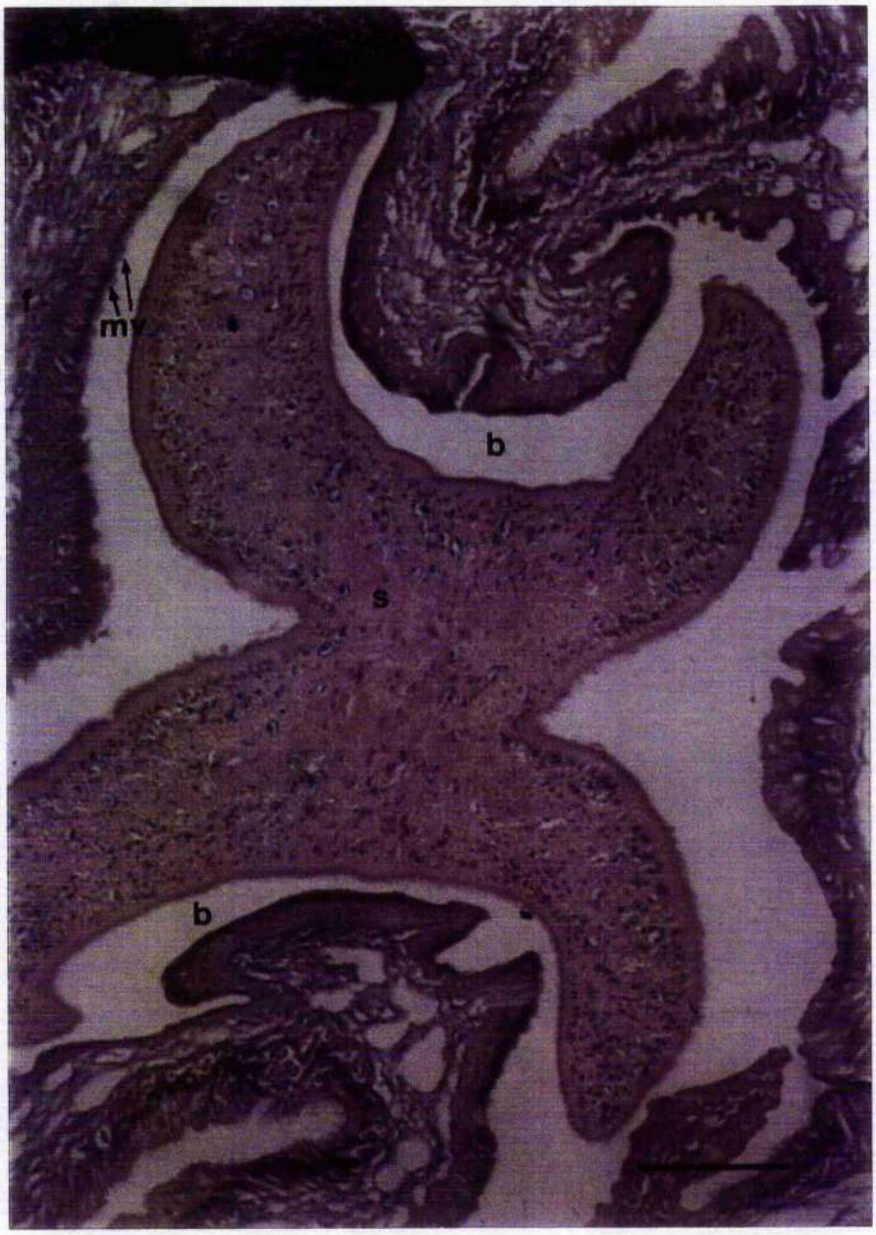


Figure 6.4 Transverse section through scolex of *Bothriocephalus scorii* embedded in the primary folds of the anterior intestine of turbot. Shows muscular nature of scolex (s) and bothria (b) acting like pincers on secondary folds of intestine. Also shows proliferation of fibrous connective tissue (f) in the region of intestine adjacent to the scolex and some clumping or flattening of the microvilli. Scale bar: 25 μm.

negative binomial relationship. Table 6.1 shows the prevalence, intensity and variance to mean ratios of *Bothriocephalus scorpii* infection in turbot.

6.3.6 Parasite index

The median parasite index was examined for different age and length groups of '0' group turbot. Three month old turbot of length range 50-70 mm had a parasite index of 0.97 %, 4 month old turbot in the length range 55-84 mm had a parasite index of 1.94 %, 6 month old turbot had a parasite index of 0.35 % and 8 month old turbot had a parasite index of 0.51 % (Figure 6.9). There was a statistically significant difference between the parasite index of the 4 age groups (Kruskal-Wallis Test, H = 11.77, df = 3, p = 0.008). When this was examined in more detail there was a statistically significant difference between the parasite index of 3 month old turbot and 4 month old turbot (Mann-Whitney U-Test at 0.03), 4 month and 6 month (Mann-Whitney U-Test at 0.014) and between 4 month and 8 month old turbot (Mann-Whitney U-Test at 0.013).

When the parasite index is related to length classes, fish in the 0-100 mm length class are found to have a parasite index of 1.46 % whilst the 100-200 mm length class have a parasite index of 0.45 %. There was a statistically significant difference between these two groups (Mann-Whitney U-Test at 0.0041). Examination of raw data obtained by Davey and Peachey (1968) for adult turbot in the length class 240-760 mm revealed a parasite index was of 0.14 % (Figure 6.10).

6.3.7 Effect of infection with *Bothriocephalus scorpii* on the condition factor of turbot

The condition factor, that is the relationship between weight and length in turbot expressed as $CF = W/L^3 \times 100$, was examined for uninfected and infected juvenile turbot (Table 6.2).

Table 6.2 Comparison of condition factor (CF) and hepato-somatic index (HSI) of infected and uninfected turbot.

	HSI ($\bar{x}$±se)	CF ($\bar{x}$±se)
Uninfected fish	1.56±0.14	1.61±0.03
Infected fish	1.50±0.11	1.59±0.02

The mean condition factor for uninfected fish was 1.61 ± SE 0.03 and that for infected fish was 1.59 ± SE 0.02. There was no significant difference between the condition factor of infected and uninfected fish (Mann-Whitney U-test at 0.05). When the turbot are divided into two different length classes, the mean condition factors for the uninfected and infected 0-100 mm length class and for the 101-200 mm length class are all the same at 1.61 ± SE 0.03. There was no statistically significant difference between any of the groups (Mann-Whitney U-test at 0.05). Figure 6.11 shows the relationship between condition factor and length of fish for infected and uninfected fish.

6.3.8 Effect of infection with *Bothriocephalus scorpii* on the hepato-somatic index of turbot

The hepato-somatic index (HSI), that is the weight of fish liver expressed as a percentage of the total body weight of the fish, was calculated for uninfected and infected fish (Table 6.2). The mean HSI for uninfected fish was 1.56 ± SE 0.14 and that for infected fish was 1.50 ± SE 0.11. There was no significant difference between the HSI of uninfected and infected fish (Mann Whitney U-test at 0.05). When the mean HSI figures are re-examined for two different length classes of turbot, 0-100 mm and 101-200 mm, there was no statistically significant difference between uninfected and infected HSI at either length class (Mann-Whitney U-test at 0.05) Figure 6.12 shows the relationship between the HSI and body length in infected and uninfected fish.

6.3.9 Effect of infection with *Bothriocephalus scorpii* on the relationship between body weight and body length in turbot

There was no significant difference in the relationship of body weight and body length between infected and uninfected turbot. Figure 6.13 shows the log-linear relationship between the weight and length of uninfected and infected turbot.

6.3.10 Estimated amount of energy consumed by *Bothriocephalus scorpii* in turbot of specified weight and held under various conditions

It was possible to calculate from feeding experiments carried out in the present study, the amount of energy consumed daily by a 10 g turbot held at 10°C and feeding on WFA pellets. The average weight of *Bothriocephalus scorpii* infecting a 10 g fish was also established from the present work. The proportion of the daily energy consumed by a 10 g fish which was utilized by the tapeworm was then estimated from these data. To do this, an assumption was made that the metabolic rate of the tapeworm is roughly equivalent to that of its host when adjusted for

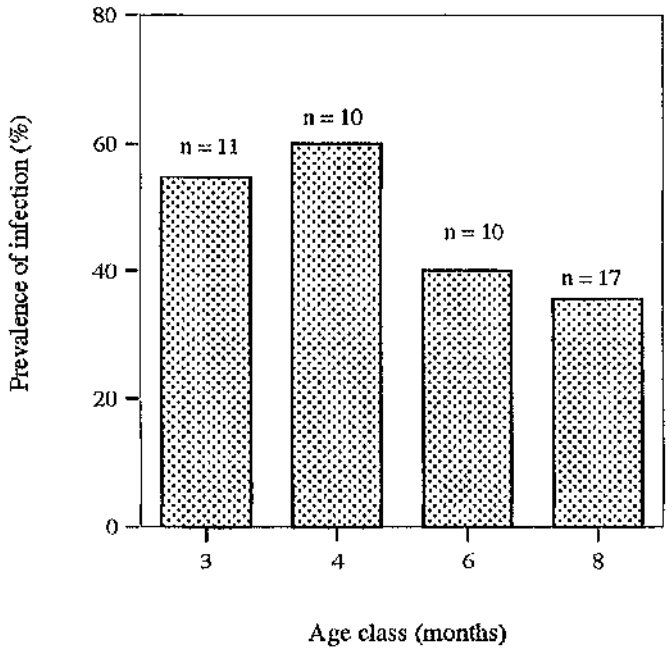


Figure 6.5 Prevalence of *Bothriocephalus scorpii* infection in different age classes of turbot

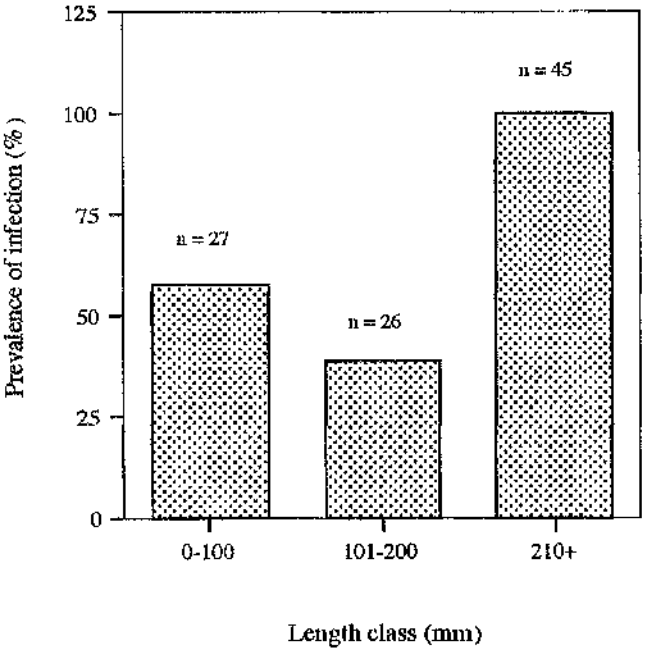


Figure 6.6 Prevalence of *Bothriocephalus scorpii* infection in different length classes of turbot. Data for adult turbot from Davey and Peachey (1968)

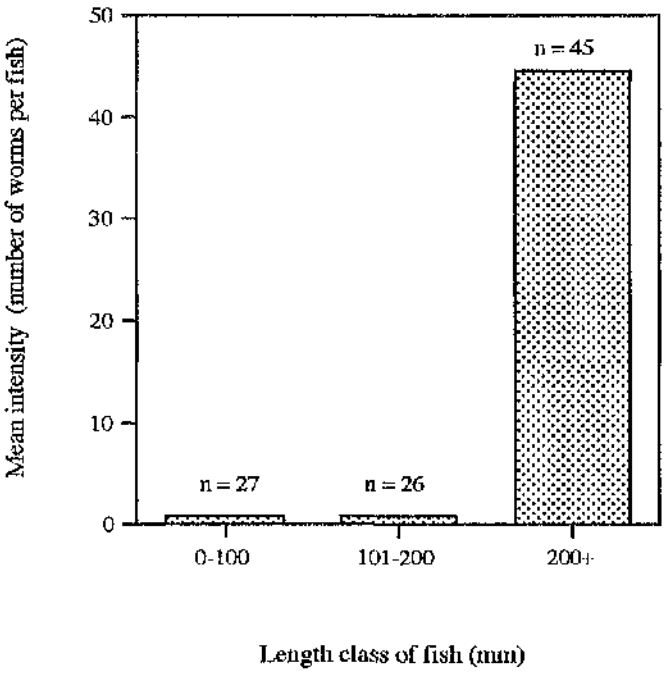


Figure 6.7 Mean number of worms per fish in different length classes of farmed turbot (0-200 mm). Data for adult turbot (200+ mm) from Davey and Peachey (1968)

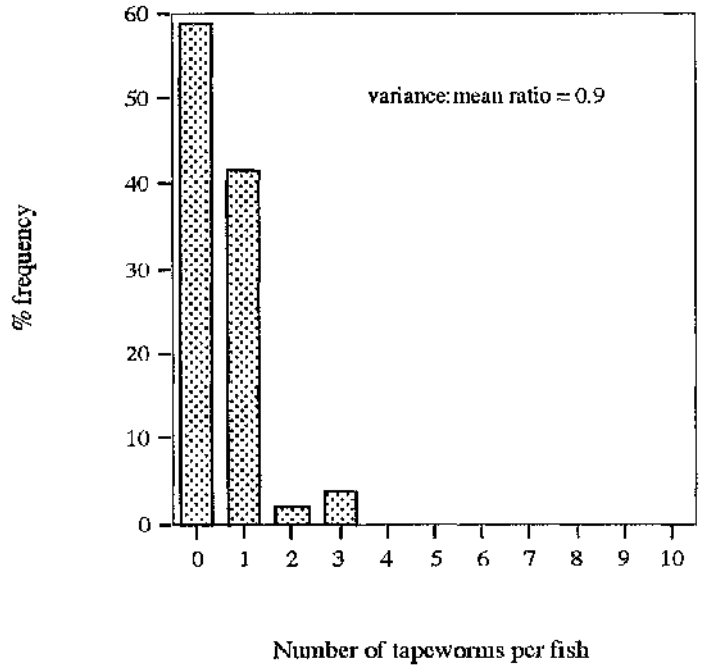


Figure 6.8 Frequency distribution diagram of numbers of *Bothriocephalus scorpii* infection in turbot (n=53)

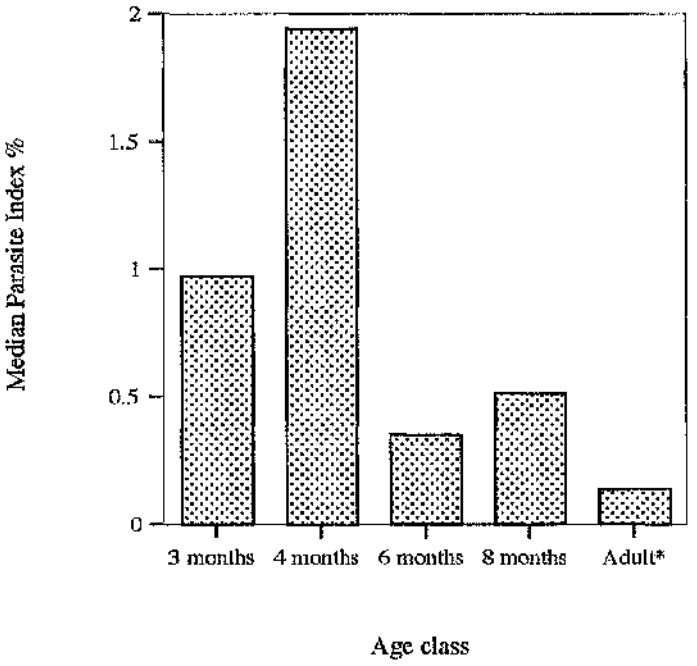


Figure 6.9 Median parasite index in different age classes of turbot. Data for adult turbot from Davey and Peachey (1968).

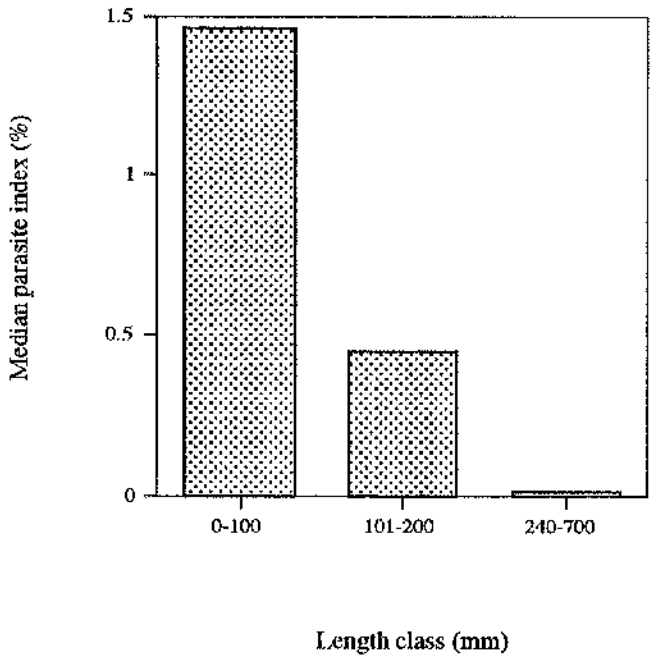
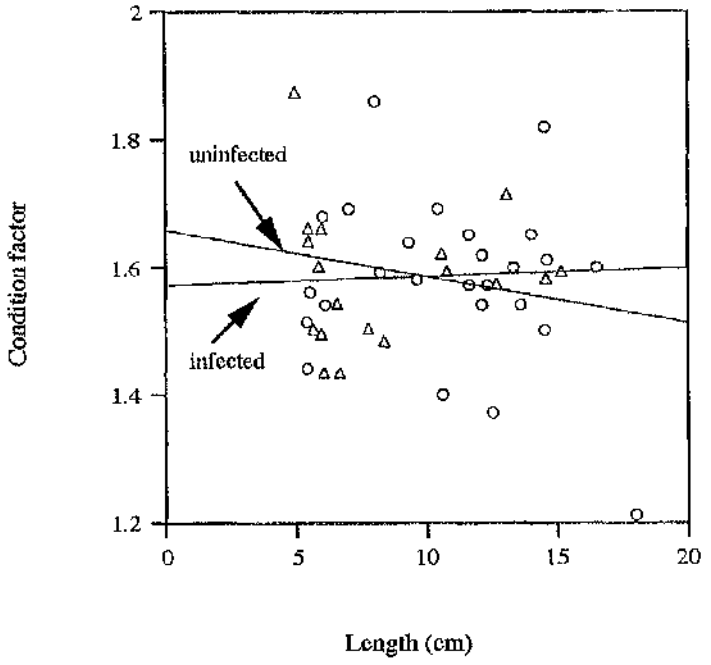


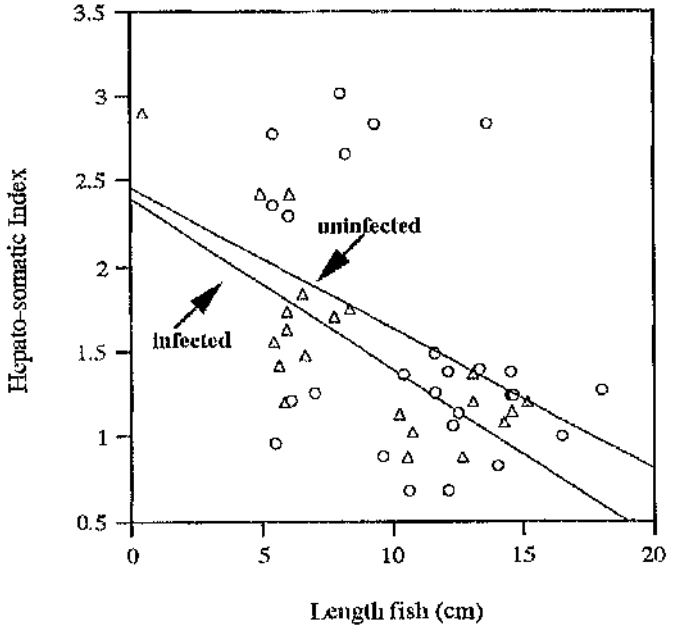
Figure 6.10 Median parasite index in different length classes of turbot. Data for adult turbot (240-700 mm) from Davey and Peachey (1968).



o Condition factor of uninfected fish $y = -0.007x + 1.658$ $r^2 = 0.040$

Δ Condition factor of infected fish $y = 0.001x + 1.570$ $r^2 = 0.002$

Figure 6.11 Relationship between condition factor and body length in infected and uninfected fish.



○ Hepato-somatic Index (uninfected) $y = -0.083x + 2.455$ $r^2 = 0.164$

△ Hepato-somatic Index (infected) $y = -0.100x + 2.403$ $r^2 = 0.576$

Figure 6.12 Comparison of the relationship between hepato-somatic index and body length in infected and uninfected turbot

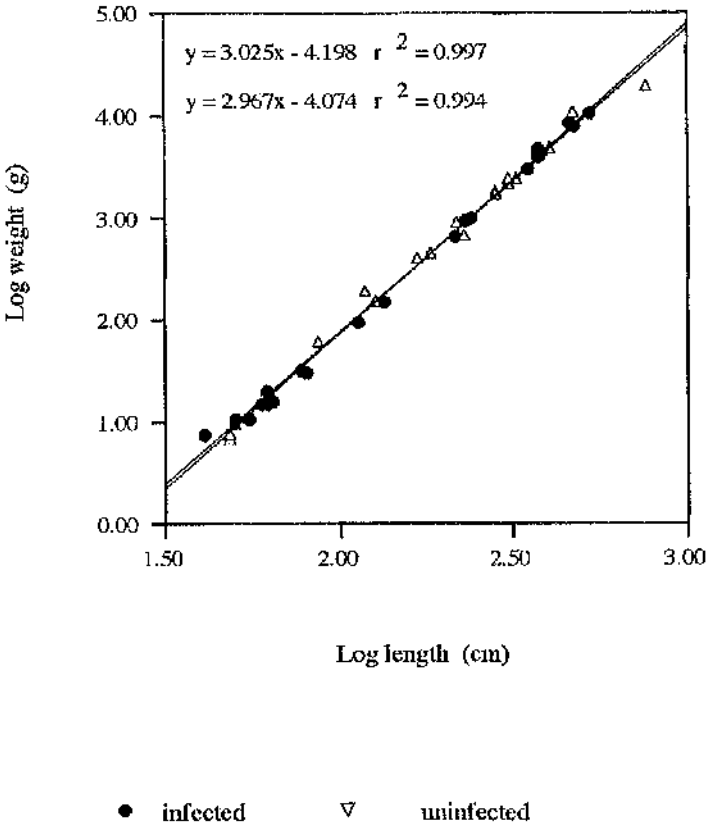


Figure 6.13 Log-linear relationship between the weight and length of uninfected and infected turbot.

Table 6.3 Estimate of the proportion of the food intake of turbot used by *Bothriocephalus scorpii* under different conditions

Weight of fish (g)	Weight of worm (g)	Number of fish	Temperature of acclimation	Percentage of host's food intake consumed by tapeworm	Prevalence of infection	Amount of food consumed by fish per day (g)	Amount food used by worms per day (g)	Energy intake of fish per day (kj)	Energy requirement of worms per day (kj)
10 g	0.2 g	10000	10°C	2.0 %	50 %	2500 g (satiation)	25 g	31000 kj	310 kj
10 g	0.2 g	10000	10°C	4.4 %	50 %	1150 g (reduced intake)	25 g	14260 kj	310 kj
100 g	1.0 g	10000	10°C	1.0 %	100 %	25000 g (satiation)	250 g	310000 kj	3100 kj
100 g	1.0 g	10000	10°C	2.2 %	100 %	11500 g (reduced intake)	250 g	142600 kj	3100 kj
10 g	0.2 g	10000	20°C	4.0 %	50 %	4000 g (satiation)	80 g	49360 kj	987 kj
10 g	0.2 g	10000	20°C	8.6 %	50 %	1850 g (reduced intake)	80 g	22940 kj	987 kj
100 g	1.0 g	10000	20°C	2.0 %	100 %	40000 g (satiation)	800 g	493600 kj	9870 kj
100 g	1.0 g	10000	20°C	4.3 %	100 %	18500 g (reduced intake)	800 g	229400 kj	9870 kj

weight. In a 10 g turbot harbouring a tapeworm of 0.2 g, we can assume the tapeworm has a metabolic demand of approximately 2 % of the host and therefore will require 2 % of the host's energy intake. This is likely to be an underestimate for the following reasons. Firstly, smaller animals tend to have a higher metabolic rate per gram of tissue than larger ones. Secondly the tapeworm may be producing vast quantities of eggs which requires substantial amounts of energy and thirdly, the tapeworm is likely to be anaerobic and will consume more food per energy gained.

If the food intake of a 10 g turbot held at 10°C and fed to satiation on WFA pellets is 2.5 % body weight per day and the WFA pellets contain 12.4 kJ / g food (wet weight), then the fish is consuming 0.25 g food (or 3.1 kJ) per day. The tapeworm, consuming 2 % of its host's food intake, will therefore use 0.005 g per day (or 0.06 kJ) per day. In a fish farm holding 10000 such fish with a 50 % prevalence of *Bothriocephalus scorpii*, the fish will consume 2.5 kg food per day or 31000 kJ of which the tapeworms will use 25 g (or 310 kj) per day.

If the food intake of the hypothetical 10 g fish is decreased for some reason, such as infection with a more pathogenic parasite or if the bulk of the tapeworm in the intestine decreases food intake or food is simply being rationed to save costs, the proportion of the food intake of the fish which is consumed by the tapeworm will be increased. Table 6.3 gives estimates of the proportion of the host's food consumed by the tapeworms under different conditions.

6.4 DISCUSSION

A considerable increase in turbot culture since approximately 1975 in Britain and France and since the early eighties in Norway, Denmark and Galicia (north-west Spain) has resulted in a concomitant rise in pathological problems in this species (Novoa *et al.*, 1992; Toranzo *et al.*, 1993a). Bacteria, viruses and more recently, protozoan diseases as well as those helminths which have direct life histories have received most attention due to the ease and rapidity with which they can spread under systems of monoculture and also because of their pathogenicity (Estevez *et al.*, 1992, 1994; Bloch and Larsen, 1993; Novoa *et al.*, 1993a, b; Dyková and Figueras, 1994; Mudarris *et al.*, 1994; Pedersen *et al.*, 1994; Lom and Bouix, 1995; Munro *et al.*, 1995).

Intestinal helminths of turbot have received much less attention partly because they have been regarded as less of a threat, owing to their complex life histories and the associated difficulties in completing these under a monoculture situation. Also, there is a belief that they have little or no pathogenic effect and few have been shown to cause mortality in fish (de Groot, 1971; Jones, 1975). *Bothriocephalus scorpii*, unlike many of the diphyllobothriid worms which have a complex life history requiring two intermediate hosts (usually a copepod and a species of

fish), can be transmitted to the final host via a single intermediate copepod host. A paratenic host, the goby, is also involved in transmission of the parasite to turbot of lengths where fish is the main food source. It is common in parasites with complicated life histories that the stage that infects the final host is blocked from the food cycle and this often reduces the risk of infection in captive fish. The use of a paratenic host may provide a solution to this obstruction both in nature and in captive fish and may explain the very high prevalence and intensity of infection of *Bothriocephalus scorpii* found in adult turbot even when the copepod is no longer accessible (Robert *et al.*, 1988).

Despite the fact that the tapeworm *Bothriocephalus scorpii* is widespread in turbot, little attention has been given to the prevalence and intensity of infection in farmed fish and even less to the possible effects of the parasite on various physiological parameters and even on the possible cause of mortality in farmed fish due to intestinal blockage.

Results from the present study showed turbot from Hunterston had a prevalence of infection with the tapeworm *Bothriocephalus scorpii* of 54.6 % and 60 % in 3- and 4-month-old fish respectively and for 6 month old fish obtained from Ardtoe a prevalence of 40 % was recorded. When divided into length classes, the infection rate for turbot in the length class 0-100 mm was 54.5 % and for the 101-200 mm length class was 38.5 %. These data differ from those of de Groot (1971) and Davey and Peachey (1968) for wild caught 'o' group turbot where lower prevalences of 36.5 % and 29 %, respectively were found in the 0-100 mm length classes. A prevalence of 53.6 % was obtained for the 101-200 mm length class (de Groot, 1971). Data from Robert *et al.* (1990) for the 0-100 mm length class revealed prevalences of infection of 4.8 % and 20 % for populations of turbot from the Atlantic (coasts of Brittany) and Mediterranean (Gulf of Lions), respectively. Prevalences of infection of 100 % for fish > 200 mm in length have commonly been reported (Davey and Peachey, 1968; de Groot, 1971; Robert *et al.*, 1990). However, these are natural populations and data are lacking on prevalences of infection in adult captive fish.

Only single infections were found in the 0-100 mm length class in the present study. Mostly single infections were found in the 101-200 mm length class but one fish harboured two worms and two harboured three worms. This differs from data obtained by Davey and Peachey (1968) where most of the infected 'o' group turbot had between 1-3 worms but 12 had from 4-66 resulting in a mean of 13 worms per fish. Adult fish in the same study had a 100 % prevalence and a mean intensity of 44.5 worms per fish.

The differences in prevalence and intensity observed in various studies for fish of the same size class may reflect variations in diet or in environmental conditions between the fish from these sites. For example, the higher prevalences found in turbot of the 0-100 mm size class from

Hunterston compared with those from Ardtoe and the wild caught fish in other studies could possibly reflect the higher water temperatures of the fish held at Hunterston. Temperature variations have been shown to be a predominant factor in infection of carp *Cyprinus carpio*, mosquito fish *Gambusia affinis*, red shiners and fathead minnows *Phoxinus phoxinus* with *Bothriocephalus acheilognathi* (Granath and Esch, 1983; Hoole, 1994) as well as in infection of sockeye salmon with *Eubothrium salvelini* (Smith, 1973; Esch and Fernandez, 1993). The lower prevalences found in small turbot by Robert *et al.* (1990) from the Atlantic could reflect the more hostile environment which may decrease the number of host-parasite encounters thus resulting in a lower prevalence and intensity of infection (Horn and Gibson, 1988). This also stresses the importance of obtaining epidemiological information pertaining to all the parameters for every population, whether wild or captive.

The frequency distribution pattern of the number of parasites per turbot in the present study followed a random dispersion pattern having a variance to mean ratio of approximately one, *i.e.* the parasites were randomly distributed within the host population. This is quite rare for macroparasites, particularly helminths, and may reflect the conditions under captivity, or, possibly is a result of the small sample size. Anderson (1993) commented on the high degree of variability in parasite numbers per host and stressed the need for large samples. Anderson and Gordon (1982) discussed the factors leading to underdispersion of a parasite population within its host and one possible explanation may be parasite-induced host mortality where the host death rate has a positive correlation with parasite burden. This would be a serious situation in a fish farm and could possibly reflect mortality of fish due to intestinal blockage by worms. The data provided by Davey and Peachey (1968) showed an overdispersed pattern for both 'o' group and adult turbot. Robert *et al.*, (1990) found *B. scorpii* (*B. gregarius*) were overdispersed within their host populations and had a mean intensity of 62.3 *B. scorpii* per fish in the Mediterranean and 14.6 in the Atlantic. However, they found the distribution frequency of *B. scorpii* corresponded to a lognormal curve. The origin and significance of the lognormal distribution has been discussed by Pennycuick (1971c) for *Schistocephalus solidus*, where the distribution pattern is attributed to the death of highly parasitized hosts and to the uncertainties of the environment. This might also explain the lognormal distribution found by Robert *et al.* (1990) for natural populations of turbot where fish over 100 mm in length rapidly obtain prevalences of infection of 100 % accompanied by an increase in intensity of infection. This is related to a change in infection pattern from the short cycle involving only the intermediate copepod host to the long cycle involving the copepod and a paratenic host (goby). At 300 mm in length the prevalence and intensity of infection stabilize and this is attributed to the death of the adult cestodes whose life span is approximately three years (Robert *et al.*, 1988) and to migration away

from the coast. Anderson and Gordon (1982) discussed the dynamic nature of parasite frequency distributions and suggested the degree of aggregation changes with time. The variation in the epidemiology of *Bothriocephalus scorpii* infection in different populations of turbot shows the importance of studying the specific parameters pertaining to the infection in each situation.

The proportion of the host-parasite relationship due to the tapeworm (parasite index) was examined and found to be greater in the length class 0-100 mm (1.46 %) than in the 101-200 mm length class (0.45 %) for turbot in the present study. Data from Davey and Peachey (1968) was analysed to obtain the parasite index of turbot in the >200 mm length class. This was found to be 0.15 % and therefore showed a general decrease in parasite index with increase in fish size. From these data, one might expect any effect the parasite may have on the host's physiology might be greater in the smaller sized fish as these are carrying the heavier parasite burden. That the weight of the parasite burden might be more important than the intensity of infection was suggested by Pennycuick (1971a) for *Schistocephalus solidus* infection in the stickleback, *Gasterosteus aculeatus*.

Intestinal parasites may adversely affect their host in a number of ways. Firstly, the parasite receives all its energy and nutrient requirements for growth, development and the maintenance of physiological functions from the host's intestine and must therefore effect a nutritional drain on its host. This may have little effect in a well nourished and healthy host with unlimited amounts of food but if infection is combined with other environmental stressors (physical, chemical, dietary or other disease) which have a limiting effect on the host's food intake then the proportion of the host's food intake used by the parasite will increase. Pascoe and Mattey (1977) reported that among a sample of three-spined sticklebacks placed on a restricted diet, those infected with *Schistocephalus solidus* died before non-infected fish. Pascoe and Cram (1977) also demonstrated that in the same host-parasite system parasitized fish were less tolerant of cadmium toxicity. Parasitic infections appear to cause the host to eat less food, resulting in the parasite consuming a greater proportion of the assimilated food (Ash *et al.*, 1984). There have been numerous reports of fish infected with fresh water bothriocaphalid worms (*B. acheilognathi*) becoming sluggish and emaciated, ceasing to feed and swimming closer to the surface (Hoole, 1994). Current evidence suggests that species of fresh water bothriocephalids affect host intestinal enzymes, for example, trypsin, chymotrypsin, phosphatases and amylase (Matskási, 1984; Kurovskaya and Kititsyna, 1986) and therefore probably effect host nutrition. Certain host respiratory enzymes (succinate dehydrogenase, lactate dehydrogenase, peptidases and hydrolase) may also be affected by the worms, although recent evidence suggests they do not affect the oxygen consumption of infected fish (Kititsyna and Nikitenko, 1986). Attempts were not made to compare the oxygen uptake of infected and uninfected fish in the present study. This

was partly because the respiratory equipment available would most likely not have been accurate enough to discern such small differences. Also, this would have necessitated destructive sampling and the numbers of fish available were severely restricted.

The presence of the parasite in the intestinal tract may alter the gastric evacuation rate by impeding the passage of food through the gut and therefore may decrease the host's food intake by impeding digestion and absorption of nutrients by the intestinal lining (Ash *et al.*, 1984). This would have the effect of increasing the proportion of the host's food intake consumed by the worm as the worm's nutrient and energy intake remain the same. Generally, the greater the degree of pathogenicity invoked by a parasite the greater the decrease in food intake of the host. This could have a profound effect when the fish is suffering from a second infection caused by a more highly pathogenic organism such as *Vibrio anguillarium*. This would decrease the food intake of the host and consequently increase the proportion of the host's food intake consumed by the tapeworm. The tapeworm will have a more substantial effect on its immunocompromised host and may tip the balance between survival and death of its host. This may explain the higher prevalence of infection with *B. scorpii* observed in turbot dying from vibriosis than in the other fish sampled.

Most pathology due to tapeworms occurs at the site of attachment. The pathology induced by *Bothriocephalus scorpii* in turbot has been reported as 'slight' (Rees,1958); 'damage to the epithelial cells and proliferation of connective tissue underneath the epithelium adjacent to the scolex' (Jones, 1975); and 'reduction in the size of the epithelial cells, in the number of goblet cells and hypertrophy of the connective tissue' (Davey and Peachey, 1968). Histological observations on the site of attachment of the scolex in the present study revealed damage had occurred to the epithelial cells. This included clumping of the microvilli, reduction in size of epithelial cells and a proliferation of fibrous connective tissue in the area underlying the epithelium, next to the scolex. Such damage must have an energetic cost to the fish host in terms of reduction in absorptive area of the intestine and in the cost of repair of damaged tissue.

A local inflammatory response has been recorded at the site of attachment of *B. gowkongensis* in cyprinid fish (Scott and Grizzle, 1979). It has not been established whether *B. scorpii* is responsible for any chronic inflammatory reaction in infected turbot (Fletcher *et al.*,1980). The production of C-reactive protein is documented in turbot but its functional significance is not yet known in fish (Williams and Jones, 1994).

Many intestinal tapeworms are positioned in the intestine behind the bile duct indicating that the host's bile is in some way necessary in rendering the host's food suitable for absorption by the worm. *Bothriocephalus scorpii* was located in the anterior intestine, posterior to the bile duct opening in juvenile turbot in the present study. This is in agreement with previous studies

(Rees, 1958; Davey and Peachey, 1968; Jones, 1975). Some tapeworms have been shown to actively move up and down the intestine, possibly searching for optimum concentrations of amino acids and other molecules. Such movements may be the worm's way of compensating for the competitive edge that the host's mucosa may have in terms of absorption (Chandler, 1939; Bråten and Hopkins, 1969; Cannon and Mettrick, 1970, Crompton, 1973, 1991; Holmes, 1973; Mettrick and Podesta, 1974; Kennedy,1983; Ash *et al.,* 1984) or they may also be induced by immune reactions of the host (Threadgold and Hopkins, 1981; Kennedy, 1983). McKinnon and Featherston (1982) discovered a linear relationship between the length of *B. scorpii* and the site of attachment along the gut of *P. bacchus* which they related to the nutrient availability in different regions of the gut. These observations suggest some level of competition between the worm and the host and imply the worm may decrease the host's digestive efficiency possibly even its growth rate. Parasitism of sockeye salmon, *Oncorhynchus nerka* by *Eubothrium salvelini* had a deleterious effect on the growth, survival and swimming performance of the fish (Boyce, 1979). A 10 % loss of growth was recorded in farmed salmon with low level infections of *Eubothrium* species and this was more pronounced in males than in female fish (Bristow and Berland, 1991) . They estimated a direct loss of millions of dollars per year in Norway due to the infection as well as noting the loss of food and the possible increased susceptibility to disease in infected fish.

The parasite index for *B. scorpii* infection in juvenile turbot is fairly low ranging from 1.0 to 2.0 %. It was assumed that the worm would therefore require approximately 1-2.0 % of its host's food intake for growth, development and maintenance of physiological functions. The amount of food and the energy equivalent required by the worm and the relative proportion was estimated and shown in Table 6.3. It can be seen that the amount of the host's food intake consumed by the tapeworm is relatively low in healthy fish fed to satiation and may go unnoticed by fish farmers but in fish with a decreased food intake for any reason, whether due to physical, chemical or biological stress then the proportion of the host's food intake consumed by the worm is increased. If 10 % of the host's food intake is consumed by the tapeworm then this constitutes a significant nutritional drain to the host as well as being a financial drain to the fish farmer.

Despite the observed pathology and the nutritional and energy drain in the fish due to infection with *B. scorpii,* no significant difference was found between the condition factor or in the hepato-somatic index of uninfected and infected juvenile turbot. This is probably because all the fish used in the feeding experiments in the present work were relatively well fed and healthy, even those on a reduced ration. Possibly if the fish were held on maintenance rations or if a secondary infection had been acquired during the study, then a difference in these gross parameters may have been detected. Many whole organism studies show parasitized animals

grow normally and show few, if any, signs of clinical disease, but evidence exists that physiological changes do occur (Mettrick, 1980; Arme *et al.*, 1983). The fact that such changes result in few gross effects may be due to a long evolutionary relationship between host and parasite and/or a compensatory effect of alternative physiological mechanisms being employed by the parasitized animal (Mettrick, 1980; Arme *et al.*, 1983).

The view that cestode species of vertebrates induce no, or minor, morphological and pathophysiological changes in the intestine of their hosts may persist because very few studies have examined the effect of parasites on their hosts using more sophisticated techniques (Eckert, 1991). Recent work has demonstrated pathomorphological changes in biopsied jejunal samples in 80 % of patients infected with *Taenia saginata;* pronounced proliferation of enterocytes and mucosa infiltration with lymphocytes, mast cells, and eosinophils was observed. This cellular infiltration was described as the host reaction to parasite antigens (Kociecka, 1987). Also, a reduction in parietal cells in the gastric mucosa of 30 patients infected with *T. saginata* and which was reversible in 66 % of patients after elimination of the tapeworm was noted. This suggests that tapeworm pathogenicity should be re-examined for its immunological and biochemical aspects (Eckert, 1991). The pathogenicity of *Diphyllobothrium latum* infection in humans is well documented due to the absorbtion of Vitamin B_{12} by the worm causing megaloblastic anaemia in approximately 2 % of patients (Von Bonsdorff, 1977).

Durán *et al.* (1989) stated that "infection with *B. scorpii* creates a significant economic problem for turbot aquaculture because of spoilage of host substances which decreases the growth rate of infected fish and mortality due to intestinal blockage". This statement needs to be substantiated with more sophisticated investigations into the effect of *B. scorpii* infection on its host and to establish the economic importance of the infection.

Different strategies are employed to control tapeworm infections and these usually reflect their perceived economic importance, the availability of control measures and whether the parasite is an established one or recently introduced (Hoole, 1994). The anthelminthic efficacy of four compounds for the treatment of *B. scorpii* infection in turbot was examined by Durán *et al.* (1989). The use of drugs to control tapeworm infections has several potential problems. The cost of the drugs makes them suitable only for luxury fish, brood stocks or fish prior to transport and there may be a problem with toxicity to the fish and/or to invertebrates (Hoole, 1994). Control of tapeworm infections is perhaps best achieved by disruption of the life history pattern, for example by using filters to remove infected copepods from intensive fish rearing facilities and in this way preventing infection of young turbot. It is also important to consider the attraction of possible disease-carrying paratenic hosts to caged turbot (McVicar and MacKenzie, 1977).

The importance of accurate epidemiological studies of fish farms and the natural environment in contributing to the understanding and prevention of parasitic infections in captive fish was stressed by Euzet and Raibaut (1985). This has been reinforced more recently by Lom and Bouix (1995) who recognized an epizootiological link between protozoan infections of wild and cultured fish, which is responsible for the appearance of previously unknown species. Kennedy (1994) recognized the potential hazards of introduced fish species and the parasites introduced along with them. The number of introduced species of fish in Britain has increased greatly in recent years, in part due to increasing ease of transportation. In this way, natural barriers to dispersal are broken down and parasites may be brought into contact with a new host species or with a new strain of its preferred host and into a situation where its numbers may increase rapidly. One such parasite is the tapeworm *Bothriocephalus acheilognathi* which is indigenous to Japan and China but which has recently been introduced to Britain and is currently disseminating in a variety of freshwater fish (Hoole, 1994). The infection has been associated with a reduction in body weight of the fish and is therefore a problem to fish farmers.

Finally, it is perhaps worth remembering a comment made by Mueller (1968) where he pointed out that "pseudophyllidean cestodes appear to be intimately involved with the physiology of their hosts". He also noted that parasitism by *Diphyllobothrium latum, Spirometra mansonoides* and *Ligula intestinalis* is "considerably more than a mechanical affair". Arme and Walkey (1970) commented that much work on the host-parasite relationship of this group of cestodes was necessary. Twenty-five years later our knowledge and understanding of the host-parasite relationship of pseudophyllidean tapeworms is still incomplete. This is particularly true of the host-parasite relationship between *Bothriocephalus scorpii* and the turbot, *Scopthalmus maximus* (L.). Fish farming is a business and as with any business it must be cost effective. Knowledge of the amount of damage caused by and the amount of energy used by the worm, in other words the cost of helminth infections, is very important. This would give the fish farmer an informed basis on which to decide whether it is cost effective to use anthelminthic drugs or to carry out any other preventative measures to avoid or treat the infection.

6.5 SUMMARY

1 Juvenile turbot obtained from Ardtoe and Hunterston were found to be infected with the tapeworm, *Bothriocephalus scorpii.*

2 The prevalence of infection was recorded at 47 % in fish ranging in length from 50-152 mm. Fish from Hunterston (< 100 mm) had a prevalence of infection ranging from 55- 60 % whilst turbot from Ardtoe (96 - 136 mm) had a prevalence of 40 %.

3 The intensity of infection in juvenile turbot in the present study was very low with most fish harbouring only one tapeworm.

4 The frequency distribution pattern of parasite numbers per fish was underdispersed with a variance to mean ratio of 0.9. This is rare for helminth infections and differed from data obtained for natural populations of turbot infected with *B. scorpii* where the distribution pattern was found to be overdispersed. This may reflect the unnatural conditions of fish in captivity and also the small number of experimental animals.

5 A parasite index (proportion of host-parasite relationship due to parasite) of 0.97 for three month old turbot (50-70 mm), 1.94 for four month old turbot (55-84 mm), 0.35 for 6 month old turbot (96-136 mm) and 0.51 for 8 month old fish (116-180 mm) was recorded. When related to length class, fish of 0-100 mm had a parasite index of 1.46 whilst those in the 101-200 mm length class had a parasite index of 0.45. Data on adult turbot from Davey and Peachey 1968) was analysed and a parasite index of 0.14 was obtained. This indicates that parasite index decreases as the fish get larger and any pathology observed may be more pronounced in younger fish. The effect of having a greater parasite index in young fish could be compounded by the fact their immune system is not fully evolved. Further research using greater numbers of fish is necessary to establish parasite indices for different length classes of turbot held under various conditions.

6 Parasitism did not appear to have any statistically significant effect on the condition factor or on the hepato-somatic index of juvenile turbot although greater numbers of infected and uninfected fish would be necessary to establish this.

7 The estimated amount of the host's food intake consumed by the tapeworms was calculated for different conditions. This may have little effect on the growth rates of healthy fish fed to satiation but if the fish is nutritionally challenged and/or is immunocompromised for any reason the parasite will exact a greater nutritional and energetic drain on the fish and consequently will be a greater financial drain to the fish farmer.

8 Further work using uninfected and experimentally infected fish at different ontogenic stages and held under various environmental conditions could help to establish the bioenergetic cost of bothriocephaliasis in turbot.

CHAPTER 7

RODLET CELLS

7. 1 INTRODUCTION

7. 1. 1 Historical background

It is now more than a century since Thélohan (1892a,b) first described the rodlet cell as an extracellular sporozoan parasite, probably a coccidian. Rodlet cells have since been found in many epithelial and endothelial tissues from various teleost groups. Thélohan based his conclusion largely on the 'cyst' wall surrounding the cell and granular inclusions (rodlets) within the cell which he thought were sporozoites. He reported the cell, which Laguessé (1895) later named *Rhabdospora thelohani,* in cyclostomes, elasmobranchs, freshwater and marine teleosts and hyalid frogs. Plehn (1906a,b) interpreted the rodlet cell as an endogenous gland cell suggesting the inclusions were secretory material and Duthie (1939) believed the cells were coarse granulocytes. Since then, rodlet cells have been reported from various tissues in many fish species and controversy has ensued to this date over their puzzling status and origin.

Those supporting the parasitic nature of the cells include Thélohan (1892a,b), Laguessé (1895), Dawe *et al.* (1964), Hale (1965), Bannister (1965; 1966), Iwai (1968c), Anderson *et al.* (1976), Mayberry *et al.* (1979), Viehberger and Bielek (1982), Barber and Westermann (1983), Bielek and Viehberger (1983), Richards *et al.* (1994), and Wayne and Mayberry (1994). The view that the cells are an endogenous component of fish tissue is held by: a) Catton (1951), Weinreb and Bilstad (1955) and Smith *et al.* (1995) who, like Duthie (1939), believed the cell may be a kind of granular leukocyte; b) Leknes (1986), Balabanova and Matey (1987) and Imagawa *et al.* (1990) who suggested that the rodlet cell may play a role in the defence system of the fish; and c) Al-Hussaini (1949a,b), Vickers (1962), Fernhead and Fabian (1971), Leino (1974, 1982), Desser and Lester (1975), Morrison and Odense (1978), Mattey *et al.* (1979), Paterson and Desser (1981), Leknes (1986), and Smith *et al.* (1995) who, like Plehn (1906a,b), have attributed various secretory functions to the cell such as pH control, osmoregulation, reduction of friction, ionic regulation, antibacterial effect and production of a substance similar to that of mucous cells.

7. 1. 2 Morphological structure of rodlet cells

Generally speaking, there has been more consensus on the morphological structure of rodlet cells than on their enigmatic nature, although those of different species do differ in size and in some details (Barber and Westermann, 1983). They are widely dispersed and have been found from almost every fish tissue but are commonly associated with epithelial and endothelial tissues where the apex of the cell borders on the free surface and where the contents of the cell, especially the rodlets, appear to be excreted. Excluding Dawe *et al.* (1964), all workers have

reported rodlet cells to be intercellular. They are commonly described as pear- or oval-shaped cells measuring approximately 3-10 μm by 7-20 μm and bound by a trilaminar membrane.

Figure 7.1 gives a diagrammatic representation of a rodlet cell. Underlying the outer membrane is a thick fibrillar border (sometimes referred to as the cyst or capsule wall) approximately 0.5 μm wide and composed of microfilaments arranged in parallel around the circumference of the cell. Regularly spaced electron dense bands are located beneath the plasma membrane and run parallel to the microfilaments. Some authors believe the capsule wall has contractile properties (Leino, 1974; Desser and Lester, 1975; Flood *et al.* 1975; Morrison and Odense, 1978; Mattey *et al.* 1979; Mayberry *et al.* 1979 and Barber and Westermann, 1983). The fibrillar border is absent from the apex of the cell when the cell reaches the epithelial surface. A stoma of approximately 1 μm in diameter and bound only by the plasma membrane is found in this region. The basal portion of the cell sometimes has a cytoplasmic extension projecting beyond the fibrillar border (Barber *et al.* 1979). The cell contains a basal nucleus which may be round, oval or u-shaped. Leino (1974) held that the shape of the nucleus was dependent on the species of fish while Anderson *et al.* (1976) believed they were found in different stages of the parasite. Within the cytoplasm, positioned above the basal nucleus, lie a number of refractile bodies, the rodlets. These are club or rod-like in shape with rounded basal ends (1-1.5 μm in diameter), a pointed apex and are arranged in a cone-shaped format with the tips of the rodlets at the apex of the cell. They are bound by a single, trilaminar membrane and are composed of three main layers - an electron dense central core surrounded by a moderately dense medullary part and a thin cortical layer. Tubular mitochondria are found clustered in the apical cytoplasm whilst a moderate amount of endoplasmic reticulum is scattered throughout the remaining cytoplasm along with ribosomes, vesicles and large vacuoles. Some ribosomes and vacuoles are also found within the fibrillar border.

7. 1. 3 Approaches used to elucidate the nature of rodlet cells

Various approaches and techniques have been used in attempts to elucidate the nature of rodlet cells. Secretory cells would be expected to develop from the germinal epithelium whilst the occurrence of cells in a wide variety of tissues in variable numbers could be indicative of a type of blood cell. Cells of an exogenous nature might be expected to cause some pathology or be isolated in some way from the surrounding tissue. The presence of DNA different from that of the host would be conclusive of either the parasitic nature or parasitic origin of the cell. Recent studies have addressed this issue.

Light and electron microscopy combined with cytochemical techniques have been used to examine the fine structure with a view to producing insights into the possible function or nature

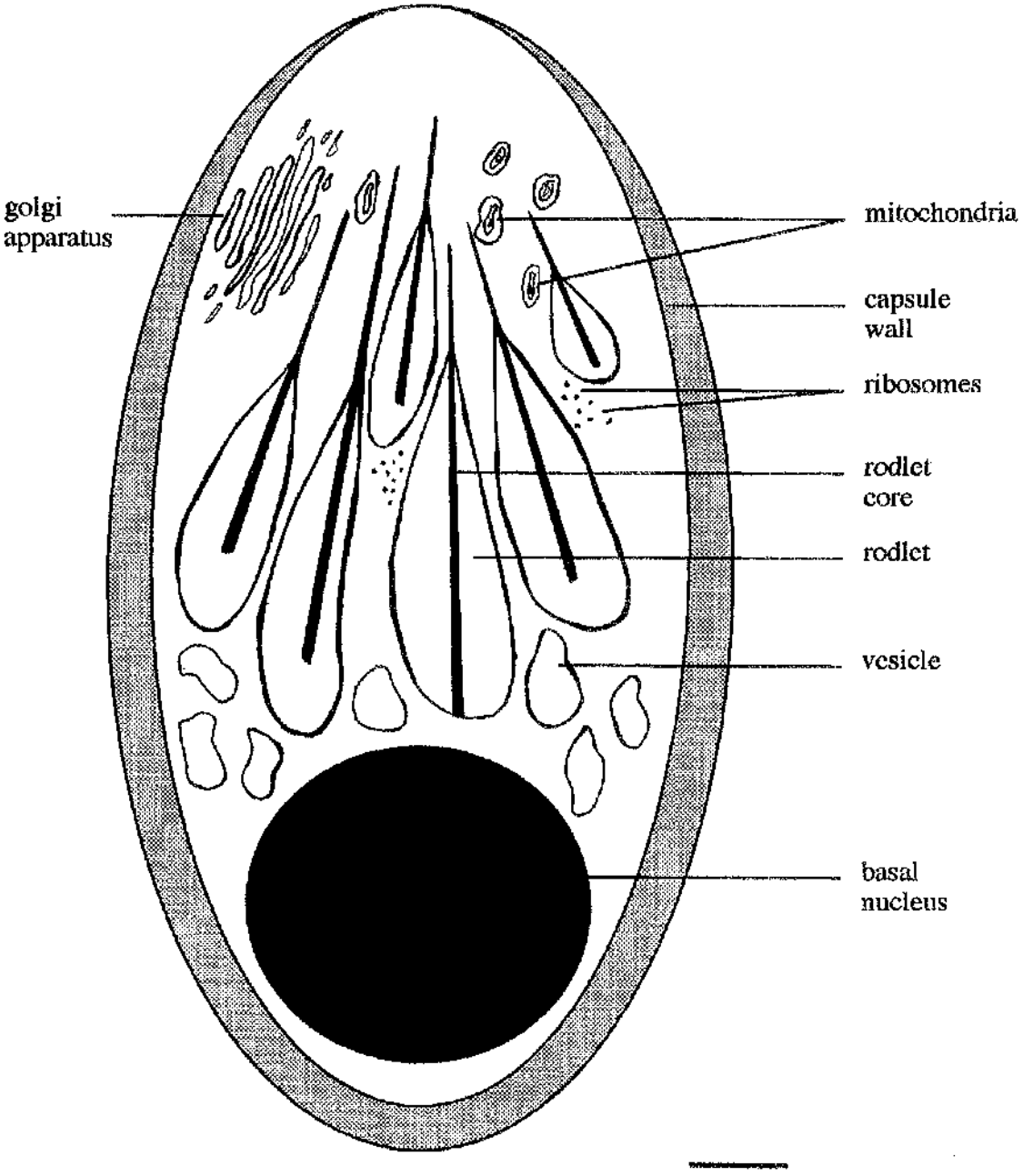


Figure 7.1 A schematic representation of a 'mature' rodlet cell. (Scale bar: 1.0 μm)

of rodlet cells. Much of the research has centred on the granular inclusions or rodlets. Most agree on the nature of the cortical layer of the rodlet but not on the core material. Bannister (1966) demonstrated the rodlets to be Feulgen-negative whilst the rodlet cortices were positive for periodic acid schiff (PAS), a test for polysaccharide. Morrison and Odense (1978), Mattey *et al.* (1979) and Leino (1982) all found the rodlets were positive for PAS and suggested that rodlet cells may have functions similar to mucous cells. Mattey *et al.* (1979) noted the rodlet sacs, excluding the rodlet cores, were positive for a stain selective for glycoprotein but did not stain with alcian blue, a test for acid mucopolysaccharides. Morrison and Odense (1978) also demonstrated the rodlets were negative for alcian blue but noted that staining with PAS was variable, suggesting a variation in the functional state of the cell. Leino (1982) further demonstrated the rodlet cores were rich in protein but poor in carbohydrate whilst the peripheral region of the rodlets were rich in both protein and carbohydrate, possibly in the form of neutral glycoprotein.

Barber and Westermann (1985,1986a), using microdensitometry and DNA hybridization techniques, demonstrated the nucleus of the rodlet cell to contain a similar quantity of DNA to other cell nuclei from the same fish species and concluded that the rodlet cell itself was probably of teleost origin. Further evidence from DNase1-gold and RNase-gold preparations led Barber and Westermann (1986b) to conclude that the dense rodlet cores contained DNA in a conformation not familiar in the eukaryote nucleus and since previous experiments had demonstrated the rodlet cell nucleus to contain the same amount of DNA as nuclei of teleost cells they proposed the theory that the rodlets were invasive structures of unknown phylogeny which converted the metabolism of the teleost cell to rodlet production. Viehberger and Bielek (1982) and Bielek and Viehberger (1983) using the Bernhard method of DNase digestion also demonstrated the rodlet core to be positive for DNA of an 'extraordinary configuration' and like previous workers (Bannister, 1966 and Barber and Westermann, 1975), they found the rodlet cores to be negative for the Feulgen reaction for DNA. They noted that some organisms such as amoeba, insects and flowering plants, are known to contain Feulgen negative DNA and concluded that the rodlets were possibly transport units of genetic material. They also observed phagocytosis of discharged rodlets by macrophages and by heterophilic granulocytes. Flood *et al.* (1975) and Barber *et al.* (1979) also supported the idea that the rodlet cell behaves like foreign or invasive material but were inconclusive as to the nature of the cells.

Leino (1974, 1982), Desser and Lester (1975), Barber *et al.* (1979), and Mattey *et al.* (1979) described developmental stages of the rodlet cell, with a gradual appearance of capsule wall and rodlets and a transient increase in granular endoplasmic reticulum and Golgi aparatus and took this as proof of an endogenous origin. Flood *et al.* (1975) also noted various stages but

believed the cell forms observed were possibly phases in the life history of the parasite. Using ultrastructural studies they tried to establish the relationship of the rodlet cell with the surrounding tissue in the bulbus arteriosus of the goldfish *Carassius auratus* and to correlate the structure with physiological activities. They described pre-encased, semi-encased (the 'immature' cells of Desser and Lester, 1975) and encased cells ('mature cells'). The semi-encased cell with prominent Golgi apparatus and extensive endoplasmic reticulum was considered to be physiologically 'active' and typical of a secretory phase. The encased, or mature, cell was interpreted as 'inactive' as there were fewer ribosomes, little endoplasmic reticulum and the mitochondria were tubular with thickened membranes and were found in the apical region of the cell where junctional complexes with the endothelial cells were formed.

Vickers (1962) noted an increase in density of rodlet cells, similar to that of goblet cells, with increasing concentration of cobalt salts and suggested there may be some type of relationship between the two cell types. Fearnhead and Fabian (1971) demonstrated changes in both numbers and appearance of rodlet cells in response to salinity changes and concluded the cell might be involved with host osmoregulation, which could explain the wide variation in rodlet cell numbers.

Mayberry *et al.* (1986) used an epidemiological approach to examine the nature of rodlet cells, surveying carp from fish farms and comparing the incidence of rodlet cells in fish of varying sizes with that in laboratory-reared fry of 10 and 60 days old. Their results suggested a migration of rodlet cells from anterior loose connective tissue through the ventral aorta wall to the bulbus arteriosus and they proposed that this implied, but did not conclusively prove, the cells were not parasites. However, Wayne and Mayberry (1994) found rodlet cells were absent from 5 and 10 day old carp fry which had been fertilized and reared in the laboratory and concluded the cells were probably of an exogenous nature.

More recently, Smith *et al.* (1995) demonstrated large numbers of rodlet cells in aggregation around the inner wall of the post-orbital blood vessel and in the extravascular space of angelfish *Pterophyllum scalare*. Mayberry *et al.* (1986) also showed aggregation of rodlet cells at the periphery of the ventral aorta. The rodlet cells appeared to be traversing the vessel endothelium in a manner similar to mammalian neutrophils and Smith *et al.* (1995) concluded that the rodlet cell most closely resembled a form of granulocyte in its morphology, distribution and behaviour.

The lack of a pathological tissue reaction around rodlet cells has frequently been used as an argument against their parasitic status, but not all reports support this conclusion. According to Schaperclaus (1979), sporozoans often cause no significant defence mechanisms in their host. Bielek and Viehberger (1983) observed phagocytosis of released rodlets by macrophages and

heterophilic granulocytes and also isolation of the cells from the adjoining tissue, whilst Richards *et al.* (1994) observed phagocytosis of both whole rodlet cells and liberated rodlets by macrophages and neutrophils. Mayberry *et al.* (1986) used the term "rhabdosporosis" to describe the heavy invasion in the bulbar intima and media of *Cyprinius carpio* by large numbers of "rhabdosporans" because they believed the cell was disrupting the integrity of the intima. Morrison and Odense (1978) observed rodlet cells remained intact in trypsinized fish tissue and Anderson *et al.* (1976) noted rodlet cells were separated from surrounding epithelial tissue by a space and survived in autolysing cell debris in both the kidney and liver of the turbot *Scophthalmus maximus* (L).

7. 1. 4 Research objectives

It was decided to examine the distribution, abundance and morphology of the rodlet cells throughout the intestine of the turbot *Scophthalmus maximus* (L.) and search for any alteration in the tissues or any other features which may help to explain the nature of these cells. In particular the following questions were addressed.

1 Is there a pathology associated with the rodlet cell? If the rodlet cell is a parasite some pathology might be expected, though not all parasites cause discernible damage to their host. If there is associated tissue damage, what effect might this have on the host's nutrition and, hence, body condition?

2 Do all fish examined have rodlet cells? A parasite would not normally be expected to infect every individual except perhaps under intensive rearing conditions. Is there a variation in the distribution and abundance of the cell in different individuals? Intensity of infection of a parasite usually varies between individuals.

3 Is there a variation in morphology of the rodlet cells in the different regions of the gut? It is unusual for a parasite to inhabit such diverse sites and so many different species. Is there more than one species of rodlet cell or are they different stages in the life history?

4 Is there a change in the distribution and abundance of the rodlet cell with variation in temperature, amount of food fed to fish or with feeding regime?

5 If the rodlet cell is a host cell, what is its function? Is there a site-specific function?

7. 2 MATERIALS AND METHODS

7. 2. 1 Light microscopy

Maintenance procedures and history of the fish were described in Chapter 2. Samples for light microscopy were taken from fish at the end of feeding experiments (details of these experiments are described in Chapter 3). At the end of 10 week experiments, four or five fish were sampled from each group held under the following feeding regimes:

Group 1: Turbot held at 10°C and fed daily to satiation for 10 weeks.

Group 2: Turbot held at 10°C and fed once every 4 or 5 days to satiation for 10 weeks.

Group 3: Turbot held at 10°C and fed daily on a reduced ration for 10 weeks.

Group 4: Turbot held at 20°C and fed daily to satiation for 10 weeks.

Group 5: Turbot prior to experiment, held at 10°C and fed daily to satiation for 3 weeks.

Food was withheld for 24 h then fish were killed and placed on a tray of crushed ice to slow down lysis of tissues. The alimentary tract was dissected out and placed in 10 % formal saline. The inside of the gut was infused with fixative and the gut was then cut into the following sections: oesophagus and stomach; pyloric caeca and anterior intestine; posterior intestine and rectum.

The sections were left overnight in fixative and processed according to the procedure described in section 2.9.1. Processed sections were stained with Ehrlich's haematoxylin and eosin for general staining and alcian blue for acid mucopolysaccharides.

7. 2. 2. Transmission Electron Microscopy

Juvenile turbot were obtained from same original source as for light microscopy experiments and held in a constant temperature aquarium (10°C). Eight fish (body weight 10-15 g) were fed daily to satiation on WFA7 pellets for 16 weeks. Five had food withheld for 20 h before they were sampled (the fed group), whilst three had food withheld for two weeks prior to sampling (the unfed group). Another five fish (body weight 12-20 g) were fed below maintenance level (< 1 % body weight in food) once every week for 17 weeks and were sampled one week after the last meal (the nutritionally-deprived group). One yearling turbot fed on live sprats *Sprattus sprattus* and held in sea cages for over 9 months was sampled to compare features of the alimentary tract of juvenile with yearling turbot (body weight 360 g). The fish were killed by spinal severing and the alimentary tracts were dissected out in fish saline. Fish were sampled and preserved in sequence. The procedure was carried out over a container of crushed ice to keep the tissues cool and prevent lysis of tissue. Small sections of the alimentary tract measuring less than 1 mm were cut from each of the following regions: oesophagus; stomach; anterior intestine; posterior intestine and rectum (3-4 sections from each region). The tissues were then processed and stained with uranyl acetate and lead citrate as described in section 2.9.2.

7. 3 RESULTS

7. 3. 1 Light Microscopy

Rodlet cells were observed in all samples from each group of fish investigated using light microscopy. The cells were positive for haematoxylin and eosin stain. The basal nucleus stained dark purple and the rodlets ranged from pale pink to purple in colour whilst the capsule wall stained a pale pink shade. Alcian blue was not taken up by rodlet cells.

7. 3. 1. 1 Morphology of rodlet cells

The basic morphology of rodlet cells was found to be similar throughout the various regions of the alimentary tract investigated and agreed with the description of 'mature' rodlet cells of previous workers (Leino, 1974, 1982; Mayberry *et al*, 1979; Morrison and Odense, 1978). However, a slight variation in shape was observed between the rodlet cells in the intestine and those in the stomach. In general, the rodlet cells were ovoid in shape (3-10 μm x 10-20 μm) with a large spherical, basal nucleus, large rod-like inclusions and thickened 'capsule wall'. Rodlet cells in the intestine and rectum appeared to be longer and narrower (mean width (± sd), 4.0 ± 1.3 μm; mean length, 18.71 ± 2.6 μm; n = 50) than those in the stomach, which appeared more ovoid in shape (mean width, 5.6 ± 0.3 μm; mean length, 12.11 ± 2.1 μm; n = 50). The large, basal nucleus stained dark blue in colour whilst the capsule wall and other cytoplasmic inclusions stained pink with Erlich's haematoxylin and eosin stain. The cells were orientated with their apical surface situated next to the lumen and with their basal nucleus furthest away. In some rodlet cells, only the capsule wall and the large nucleus were visible. In these cells, a stoma opened into the lumen giving the appearance that the rodlet inclusions had been discharged (Figure 7.2).

7. 3. 1. 2 Distribution of rodlet cells

Rodlet cells were found in the mucosal epithelium of every region of gut investigated, namely, the oesophagus, the stomach, the anterior and posterior intestine and the rectum. However, their distribution and abundance varied in each section of the alimentary tract and between fish. Generally, the abundance of rodlet cells per mm^3 of epithelial layer was greater in the stomach (mean number of rodlet cells per mm^3 = 67803 ± 9381 s.e.) than for all other regions of the alimentary tract; oesophagus (mean number of rodlet cells per mm^3 = 18089 ± 5890 s.e.); anterior and posterior intestine (mean number of rodlet cells per mm^3 = 49131 ±

12911 s.e.); rectum (mean number of rodlet cells per mm^3 = 30246 ± 17479 s.e.). Mean number of cells per mm^3 for all other areas, 24367 ± 2891 s.e. The abundance of rodlet cells in the different regions of the alimentary tract is shown in Figure 7.3. There was no observable difference between the distribution or abundance of rodlet cells in fish held at different temperatures or under different feeding regimes. Rodlet cells were frequently observed in aggregations or clumps at, or close to, the surface epithelium of the stomach, pyloric caeca, anterior intestine, posterior intestine and the rectum. Occasionally only a few rodlet cells were observed at a particular region of the gut. The greatest abundance of rodlet cells appeared to be situated at the lateral edges and apices of the stomach and intestinal mucosal folds. In regions where large numbers of mucous cells were present few, or no, rodlet cells were observed.

7. 3. 1. 3 Pathology associated with rodlet cells

In all sections observed, rodlet cells appeared to be surrounded by a vacuole, giving the appearance that the cells were infact in isolation from the surrounding tissue (Figure 7.2). In poorly fixed sections, where the tissues were badly disrupted, rodlet cells appeared intact.

7. 3. 2 Electron microscopy

Many rodlet cells were observed in the alimentary tract of the juvenile turbot held at below maintenance rations for 17 weeks. No rodlet cells were observed in the sections from fish fed 16 h before sampling or from the yearling turbot, although this does not necessarily mean that no rodlet cells were present elsewhere in the fish. Large vacuoles, containing rod-like structures in some cases, were found in the fish starved for two weeks; these may have been disintegrating rodlet cells.

The fine granular area and the central core of the rodlet sacs took up lead stain strongly but the core stained more heavily. The fibrillar 'capsule' wall stained lightly whereas both the 'capsule' wall and the rodlets varied in the intensity of staining. Generally, the staining was most intense when the rodlet cells were nearest to the epithelial surface. The basal nucleus of the rodlet cell stained in a similar fashion to normal host cell nuclei.

7. 3. 2. 1. Morphology of 'mature' rodlet cells

General features

The basic morphological features of 'mature' rodlet cells were fairly consistent throughout the various regions of the gut, however the detailed structure depended on the cell's position within the mucosal epithelium and whether or not they were in the process of 'secreting' their contents. Rodlet cells situated at the surface of the epithelium were elongate and oval in shape, with a large, spherical basal nucleus, a number of large, electron dense bodies (rodlets),

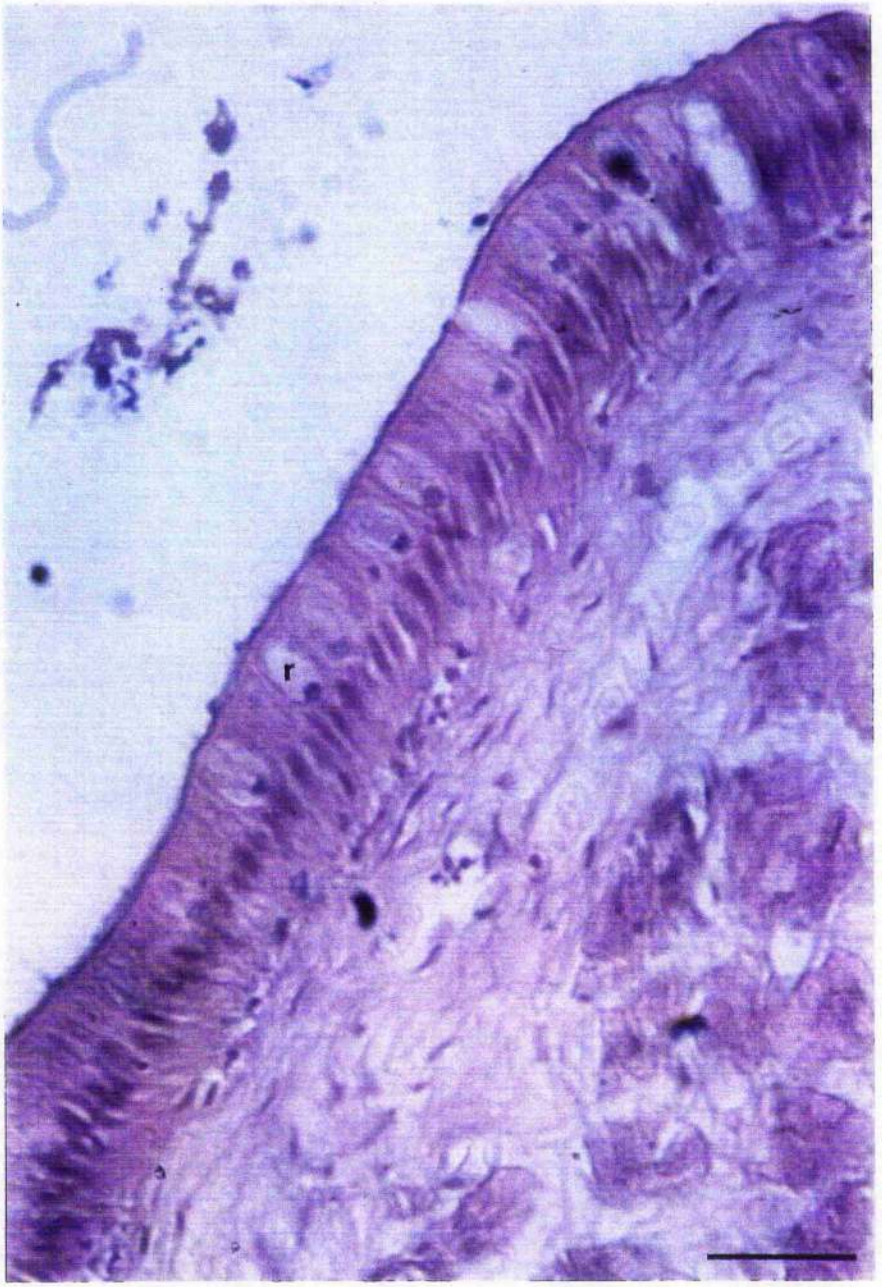
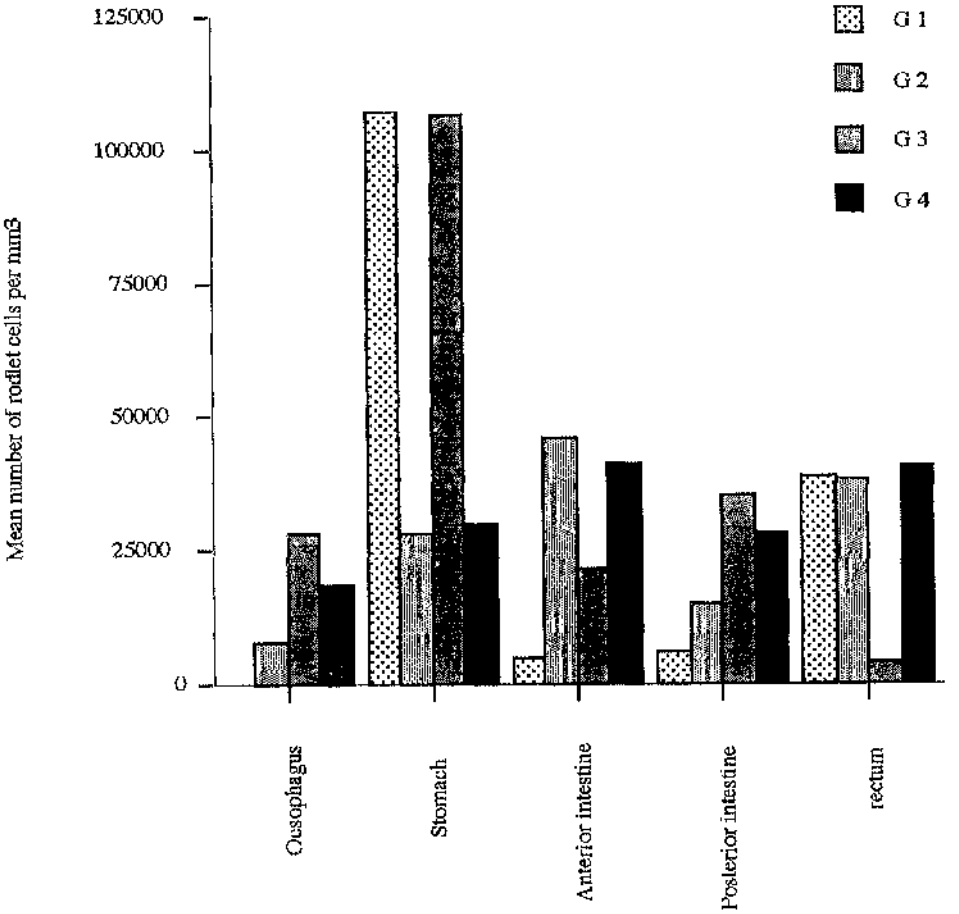


Figure 7.2 Light micrograph of transverse section of anterior intestine showing mucosal epithelium containing many rodlet cells (r). Scale bar: 25 μm.



G 1 Fish held at 10 C and fed twice daily to satiation
G 2 Fish held at 10 C and fed to satiation every 4/5 d
G 3 Fish held at 10 C and fed twice daily on reduced ration
G 4 Fish held at 20 Cand fed twice daily to satiation.

Figure 7.3 Abundance of rodlet cells in different regions of turbot alimentary tract. (n=3 for each group).

many electron lucent vesicles, and were bound by a thickened, fibrillar 'capsule' wall which was absent from the apical region (Figures 7.4; 7.5; 7.6). When cut in longitudinal section, the cells measured an average 5.0 ± 1.1 μm in width and 14.3 ± 3.7 μm in length. The largest rodlet cell measured 5.8 x 21.6 μm and the most narrow 2.6 x 18.7 μm. Both appeared to be in the process of secreting their contents into the lumen. The cells were orientated parallel to the epithelial cells with the basal nucleus furthest from the epithelial surface.

Capsule wall and plasma membrane

Rodlet cells were found to be bound by a single trilamminar membrane (10 nm in thickness) with an underlying fibrillar border of approximately 0.5 μm in diameter which is composed of numerous microfilaments measuring 10 nm in diameter and approximately 0.5 μm in length (Figures 7.7, 7.8). The microfilments were closely spaced and arranged in parallel around the circumference of the cell except in the apical region where a stoma of (1- 2.5 μm) was found (Figures 7.9, 7.10). The stoma was bound only by the plasma membrane and was commonly placed between two adjacent epithelial cells and often seen projecting into the lumen giving the rodlet cell a distinct head and shoulder region (Figures 7.11, 7.12). In longitudinal sections, regularly spaced electron-dense bands were often observed lying underneath the limiting membrane (Figure 7.13). Rodlet cells near the surface epithelium frequently appeared to have communication junctions with neighbouring epithelial cells. These junctions were only observed in the apical region of the rodlet cell (Figures 7.11, 7.13). In some rodlet cells, parts of the limiting membrane appeared to extend into and encompass some of the surrounding cytoplasm (Figure 7.14) Occasionally, the capsule wall had a serrated appearance, although this may have been a fixation artefact (Figure 7.15). Often an intercellular space was present around the circumference of the rodlet cell, especially in the basal region. Vesicles were often found in this intercellular space (Figure 7.16).

Cytoplasmic organization

The basal nucleus of the rodlet cell was commonly roughly spherical in shape and measured approximately 2.8 x 3.3 μm. The nucleus had a pale nucleoplasm with scattered darker areas of heterochromatin, particularly around the periphery. Occasionally the nucleus was irregular in shape, especially when the rodlet cell was very close to the epithelial surface and a distinct apical region with shoulders was present. It is thought that at this stage the cell is usually either about to 'secrete' or is in the process of 'secreting' into the lumen and the basal region of the nucleus becomes flattened or indented along with the basal area of the rodlet cell (Figure 7.15).

The most characteristic part of rodlet cells are the electron dense inclusions referred to as rodlets or rodlet-sacs. These inclusions take up the largest area of cytoplasm above the nucleus

Figure 7.4 TEM depicting general features of rodlet cell in anterior intestine. Elongate, oval cell with large spherical basal nucleus (n), electron dense rodlets (r), many electron lucent vesicles (v) and thick 'fibrillar' capsule wall (c). Scale bar: 1.0 μm.

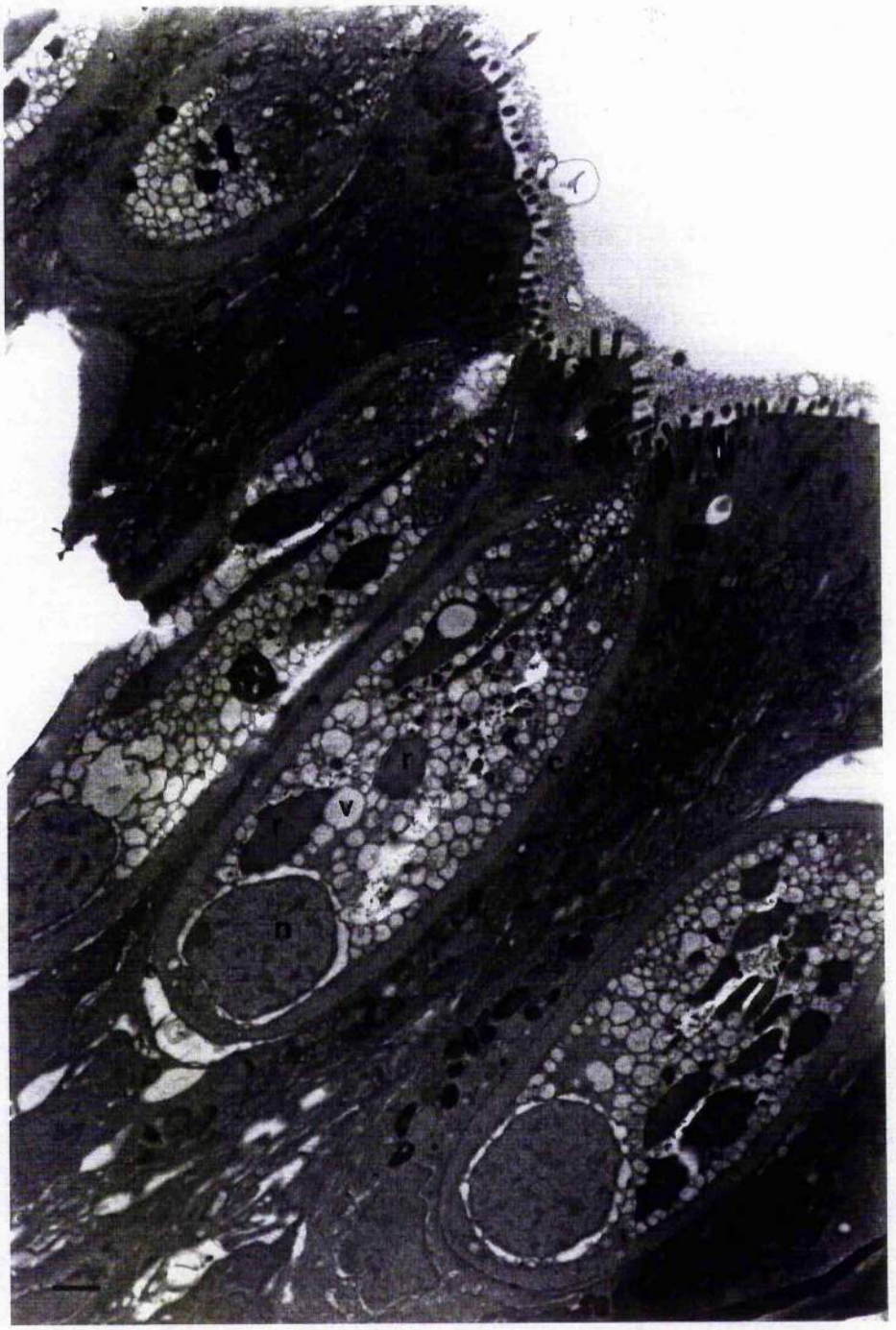


Figure 7.5 TEM showing general features of rodlet cells in the surface epithelium of the stomach. Thick capsule wall (c), large basal nucleus (n), numerous rodlets (r), and vesicles (v). Scale bar: 1.0 μm.

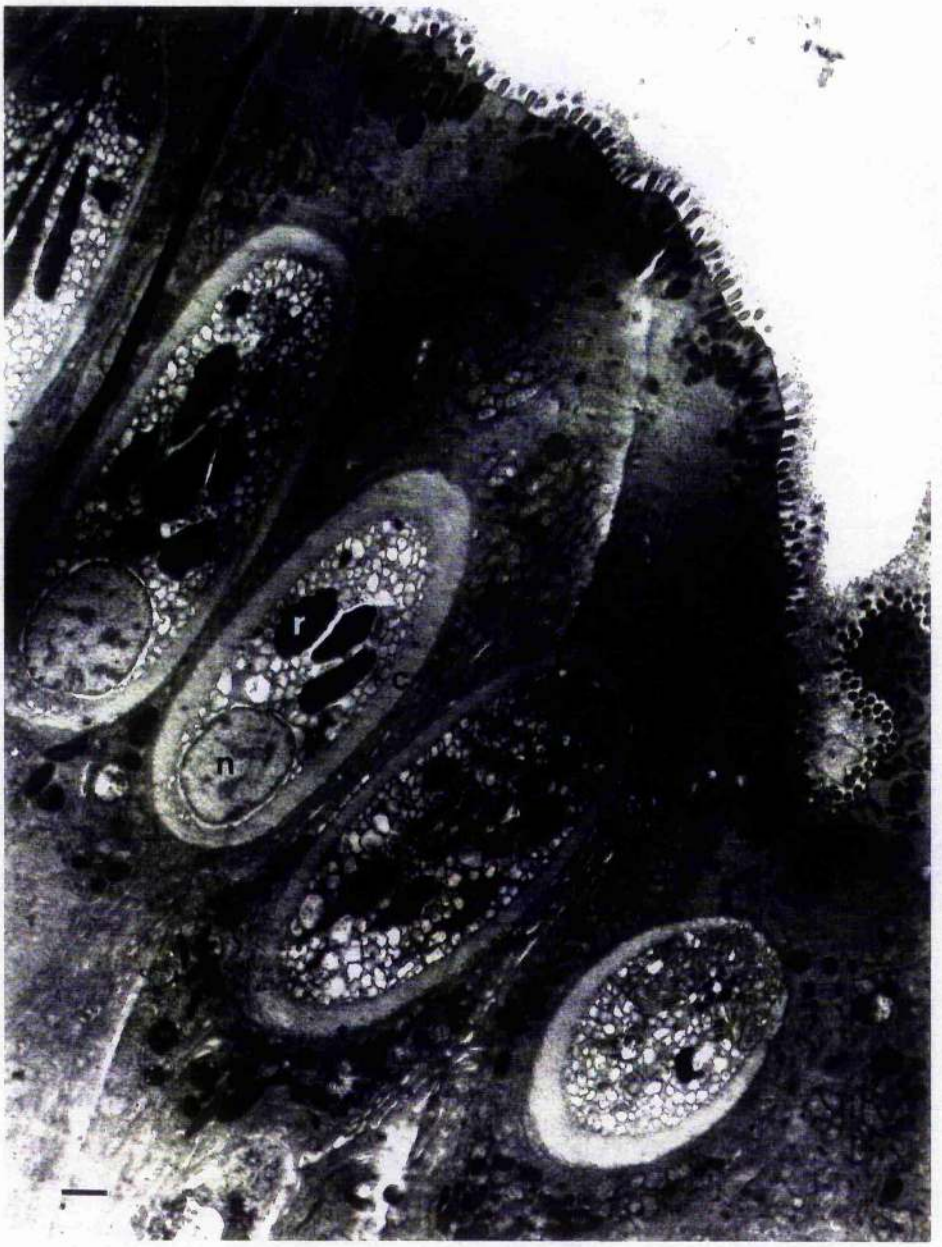


Figure 7.6 TEM depicting rodlet cells in stomach epithelium showing 'fibrillar' capsule wall (c), rodlet inclusions (r), basal nucleus (n) and many translucent vesicles (v). Scale bar: 1.0 μm.

along with a number of large, electron lucent vesicles. In longitudinal section the rodlet sacs are club-shaped, membrane-bound bodies with a rounded basal region and pointed tip and are arranged with their axes converging to form a cone shape, the apex of which lies in the apical region of the cell. Each sac contains a long, narrow, electron dense core which is surrounded in the basal region by an expanded area of fine granular material and a periphery of a course flocculent substance. Occasionally the flocculent material is absent and the peripheral region of the sac appears electron lucent. The rodlet sacs observed ranged from 1.0 - 2.2 μm in diameter and were surrounded by a single, trilaminar membrane. The rodlet cores were approximately 0.1-0.18 μm in width and reached lengths of up to 9.6 μm (Figures 7.17, 7.18). They appeared circular in cross section and occasionally two cores were observed in one rodlet sac (Figure 7.7). The rodlets were positive for Erlich's haematoxylin and eosin and lead citrate. The number of rodlet sacs varied from 4-50 in each cell. They were usually absent from the apical region of the cell where a large number of closely packed, tubular mitochondria were found. When rodlet cells reached the epithelial surface a number of small, electron dense inclusions were present near the apex of the cell. These inclusions varied in size and could have been small rodlets or parts of rodlets which have been broken off. Many ribosomes were observed studded throughout the cytoplasm and often adjacent to the rodlet sacs. The large electron lucent vesicles sometimes contained granular material and occasionally ribosomes. Granular endoplasmic reticulum was found scattered throughout the cell but particularly in the region of the rodlet sacs. Occasionally, a large Golgi apparatus with flattened saccules was observed above the basal nucleus and close to the rodlet sacs (Figure 7.19).

7. 3. 2. 2 'Secretion' of rodlet cell contents into lumen

Rodlet cells near the epithelial surface were longer and narrower than others and had a distinct apical region. Communication junctions were often observed between the apical region of the rodlet cell and adjacent epithelial cells. The fibrillar border was absent from the apical region and a stoma of approximately 0.5-2.5 μm was present, bound only by the plasma membrane. The stoma was often observed protruding into the lumen, often between two epithelial cells. A central, electron lucent shaft bound by narrow lines of electron dense particles which in turn were bordered by a fine granular area was often present in the apical region prior to secretion (Figure 7.11). A gap was sometimes observed at the epithelial surface and rodlets, mitochondria, vesicles and other cytoplasmic constituents appeared to be discharged into the lumen (Figures 7.5, 7.13, 7.20, 7.21, 7.22). 'Secreting' cells were sometimes seen to have a thicker fibrillar border at the base and lateral parts of the cell leaving an intercellular gap (Figure

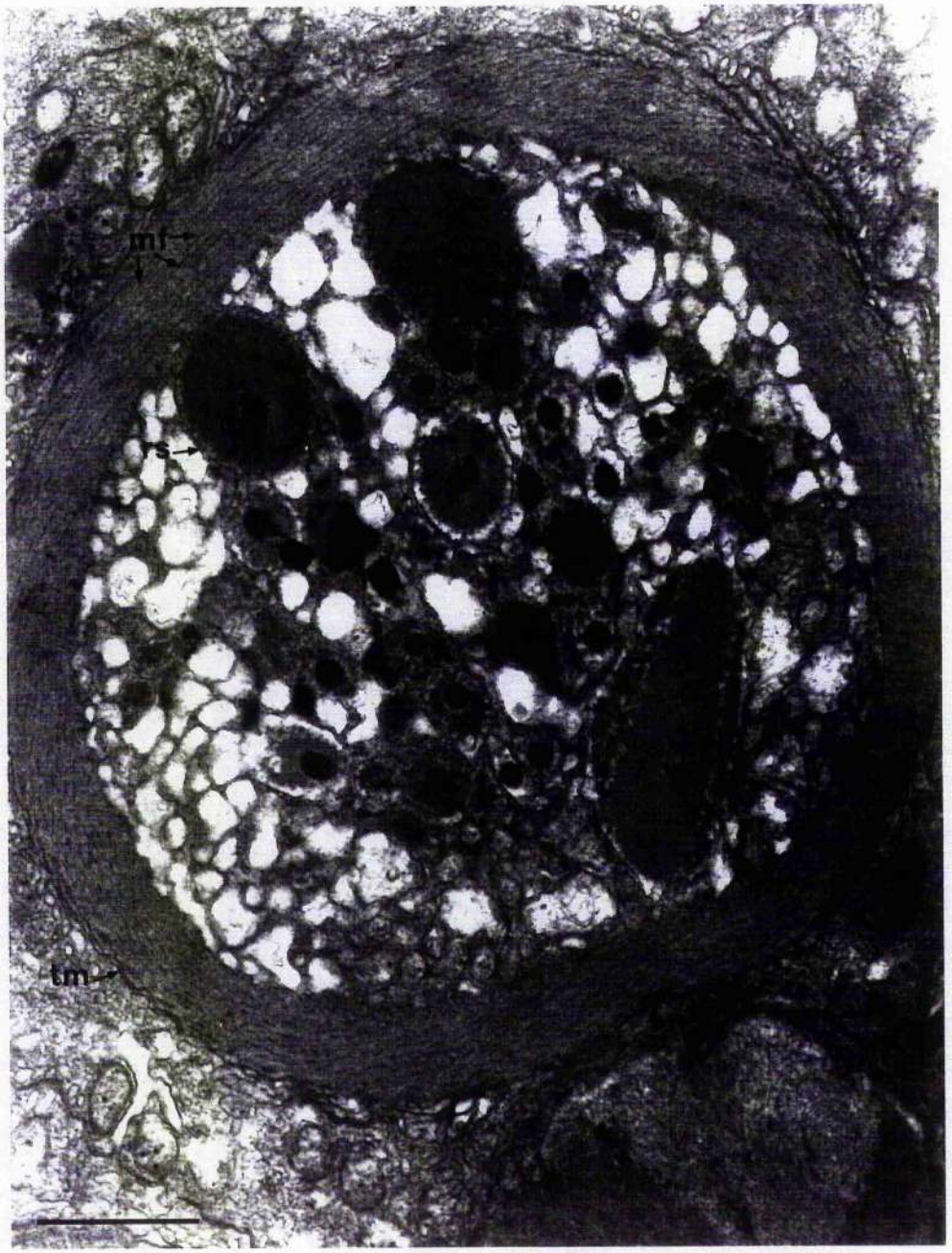


Figure 7.7 TEM of rodlet cell in transverse section showing thick capsule wall composed of numerous microfilaments (mf) and bound by a trilaminar membrane (tm). Many rodlet sacs (rs) with densely stained rodlet cores (rc) are visible and in one of these, two rodlet cores are discernible. Scale bar: 1.0 μm.

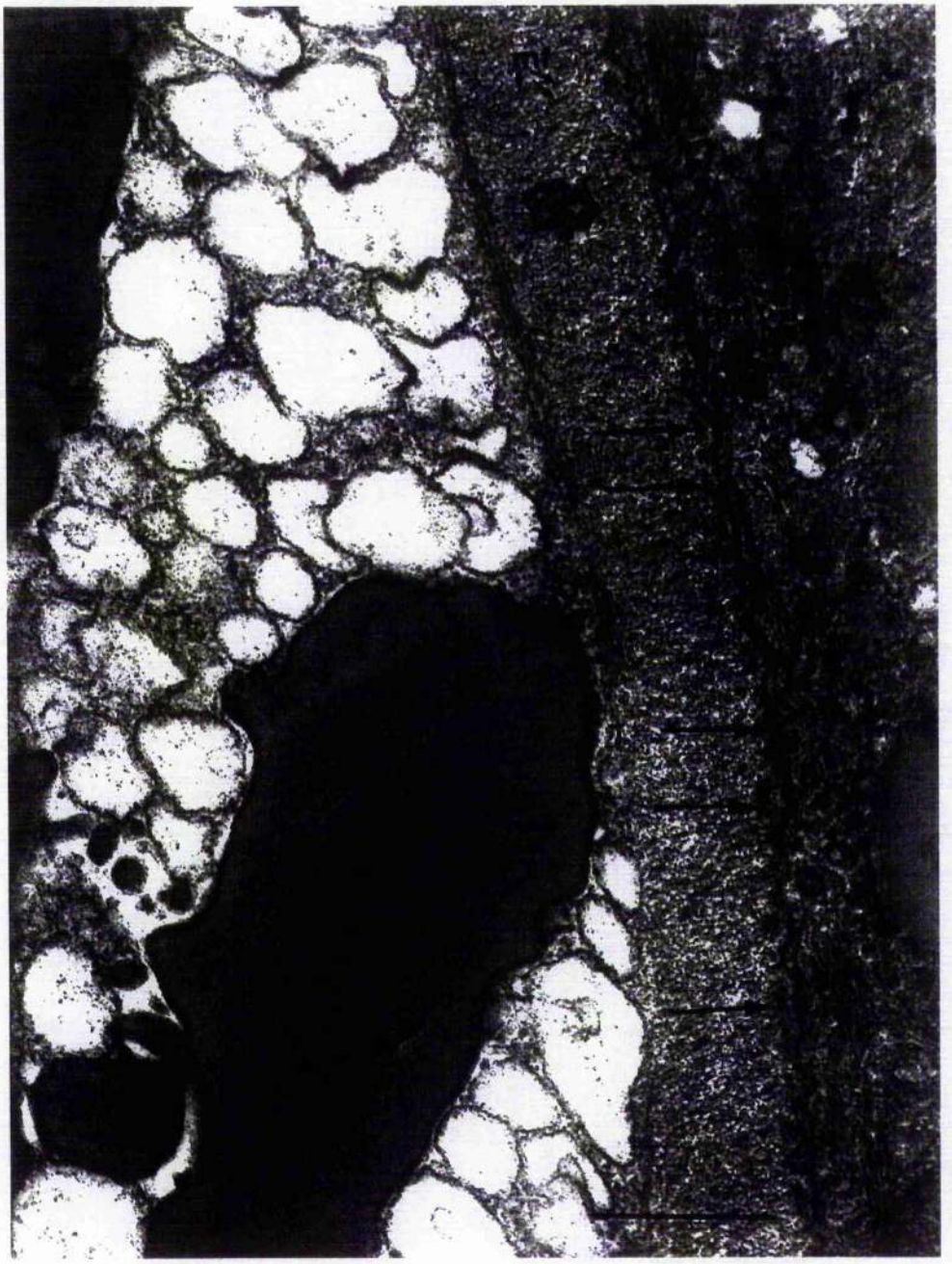


Figure 7.8 TEM showing longitudinal section through capsule wall of rodlet cell showing trilaminar membrane (tm) composed of many transversely sectioned microfilaments (mf) and intermittent dense bands (arrows) which lie next to the membrane. Scale bar: 1.0 μm.

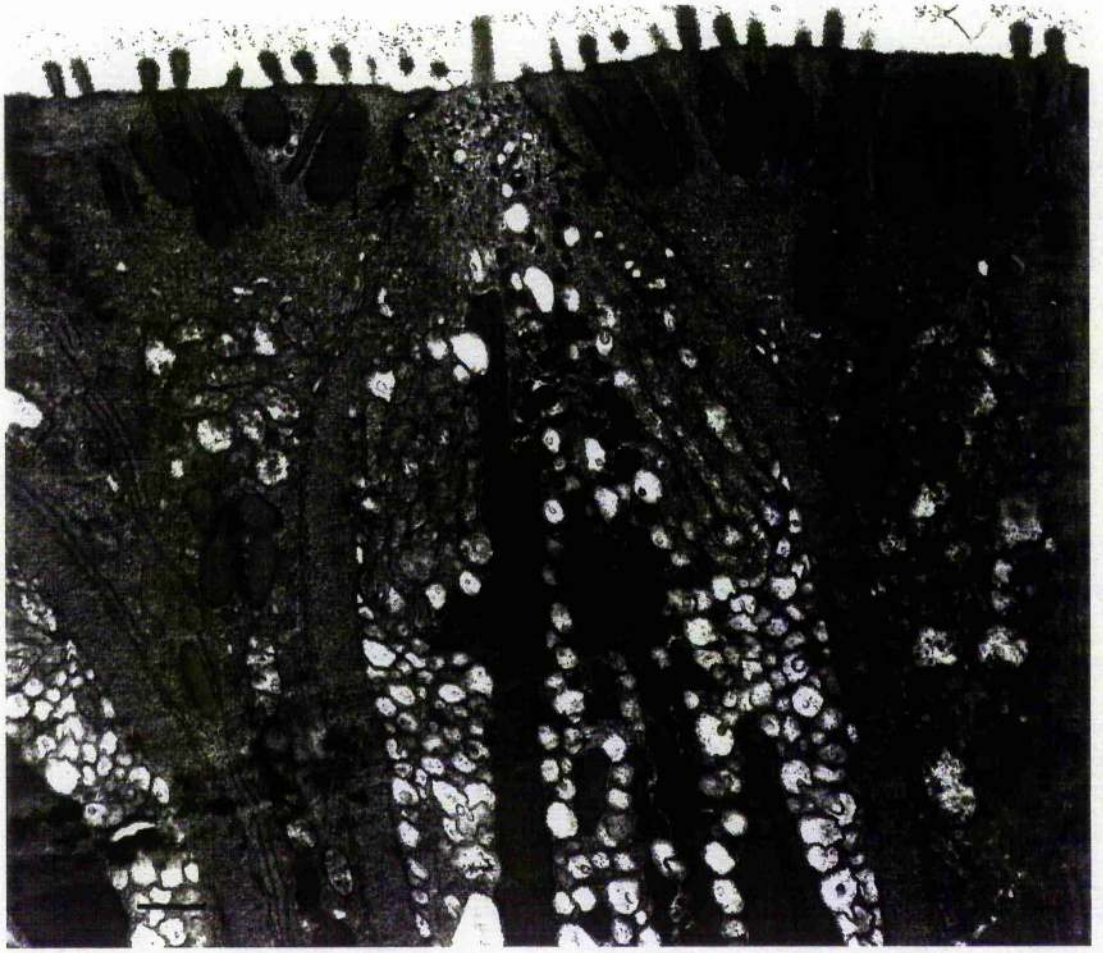


Figure 7. 9 TEM of a longitudinal section through the apical region of a rodlet cell depicting a stoma between two adjacent epithelial cells. Communication junctions (j) between the rodlet cell and neighbouring epithelial cells are visible. An extension from the apical region of the rodlet cell gives has the appearance of a microvillus. Scale bar: 1.0 μm.

Figure 7.10 TEM of longitudinal section through the apical region of a rodlet cell showing a stoma between two adjacent epithelial cells. The apical region of the rodlet cell extends into the lumen of the stomach (l) and has a superficial resemblance to the epithelial microvilli. The characteristic dense concentration of mitochondria (m) can also be seen. Scale bar: 1.0 μm.

Figure 7.11 TEM of a longitudinal section through a rodlet cell near the lumen (l) of the anterior intestine. A distinct head and shoulder region is visible at the apex of the rodlet cell with a central electron-lucent shaft (s) bound by narrow lines of electron-dense particles, bordered by a fine granular area. Communication junctions (j) between apical region of rodlet cell and neighbouring epithelial cells are visible. Scale bar: 1.0 μm.

Figure 7.12 TEM of longitudinal section of rodlet cell. In the apical region a distinct head and shoulder area with electron-lucent shaft (s) is visible. Communication junctions (j) can be seen between the rodlet cell and neighbouring epithelial cells. Scale bar: 1.0 μm.

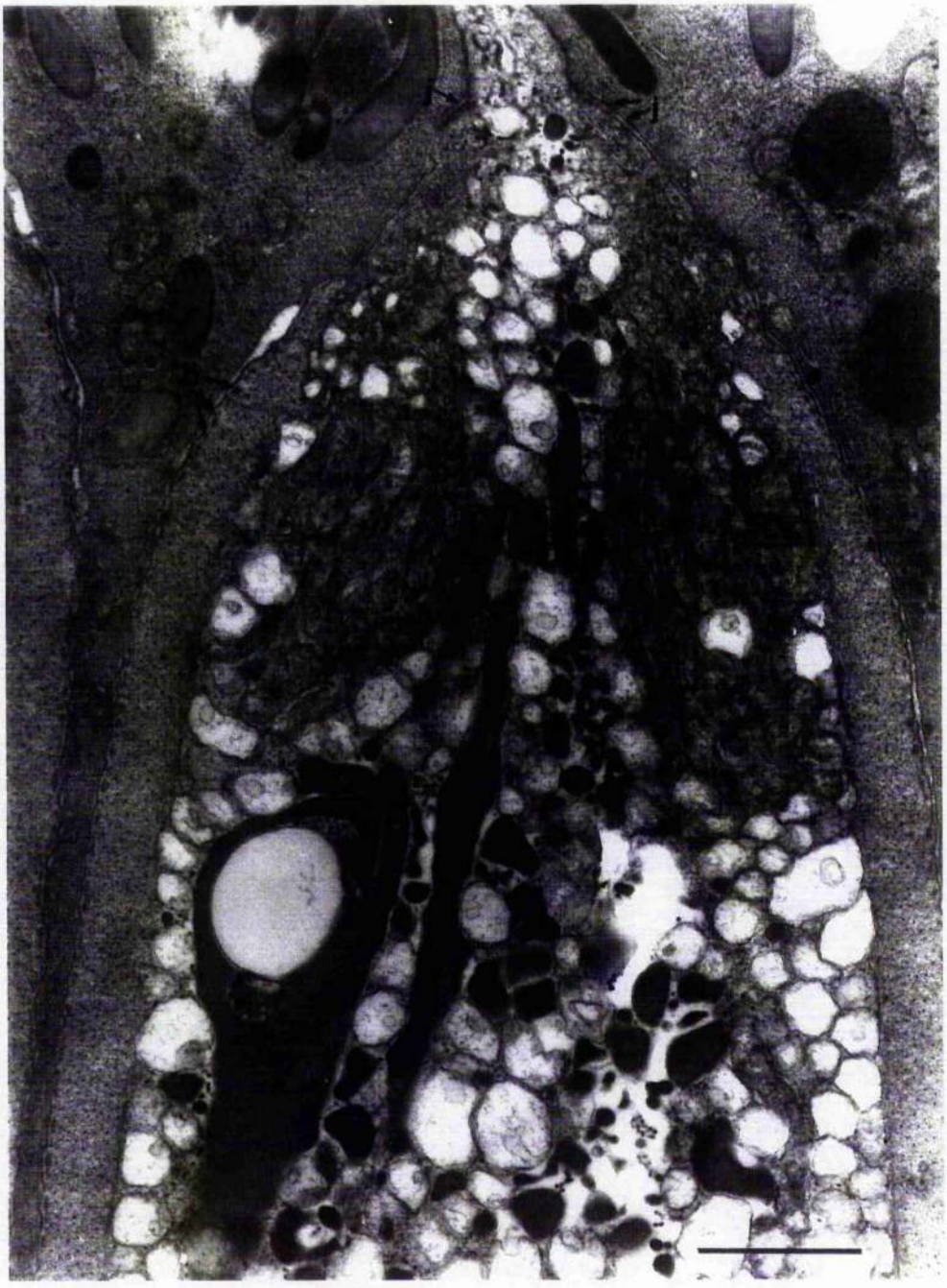


Figure 7.13 TEM of longitudinal section through apical region of rodlet cell showing regularly spaced electron-dense bands (b) in the capsule wall adjacent to the limiting membrane of the rodlet cell. Communication junctions (j) are visible between apical region of rodlet cell and neighbouring epithelial cells. Scale bar: 1.0 μm.

Figure 7.14 TEM of transverse section of rodlet cell showing cytoplasmic extension (ce). Part of the limiting membrane of the rodlet cell can be seen extending into and encompassing some of the surrounding cytoplasm. Scale bar: 1.0 μm.

Figure 7.15 TEM of longitudinal section of rodlet cell depicting capsule wall (c) with cerrated appearance. Scale bar: 1.0 μm.

Figure 7.16 TEM showing rodlet cells in stomach epithelium with intercellular spaces around the perimeter of the cells. A large electron-lucent vesicle (v) is visible in the intercellular space in the basal region of the rodlet cell. Scale bar: 1.0 μm.

Figure 7.17 TEM of anterior region of rodlet cell showing electron-dense rodlet core (rc) and surrounding rodlet sac (rs). Scale bar: 1.0 μm.

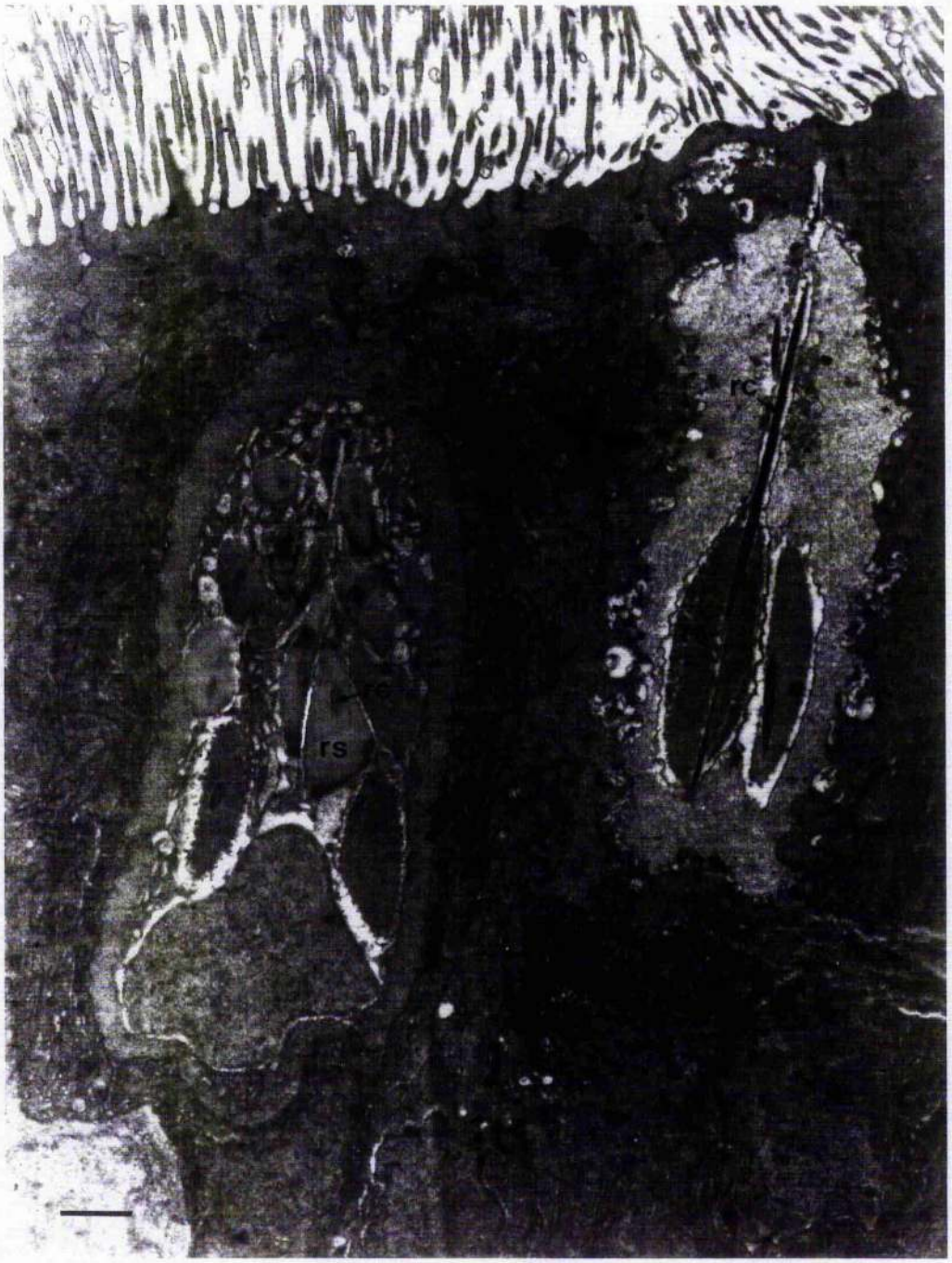


Figure 7.18 TEM of oblique section through rodlet cell showing rodlet sacs (rs) with rodlet cores (rc) and also longitudinal section of disintegrating rodlet cell showing long rodlet shaft and rodlet sac. Scale bar: 1.0 μm.

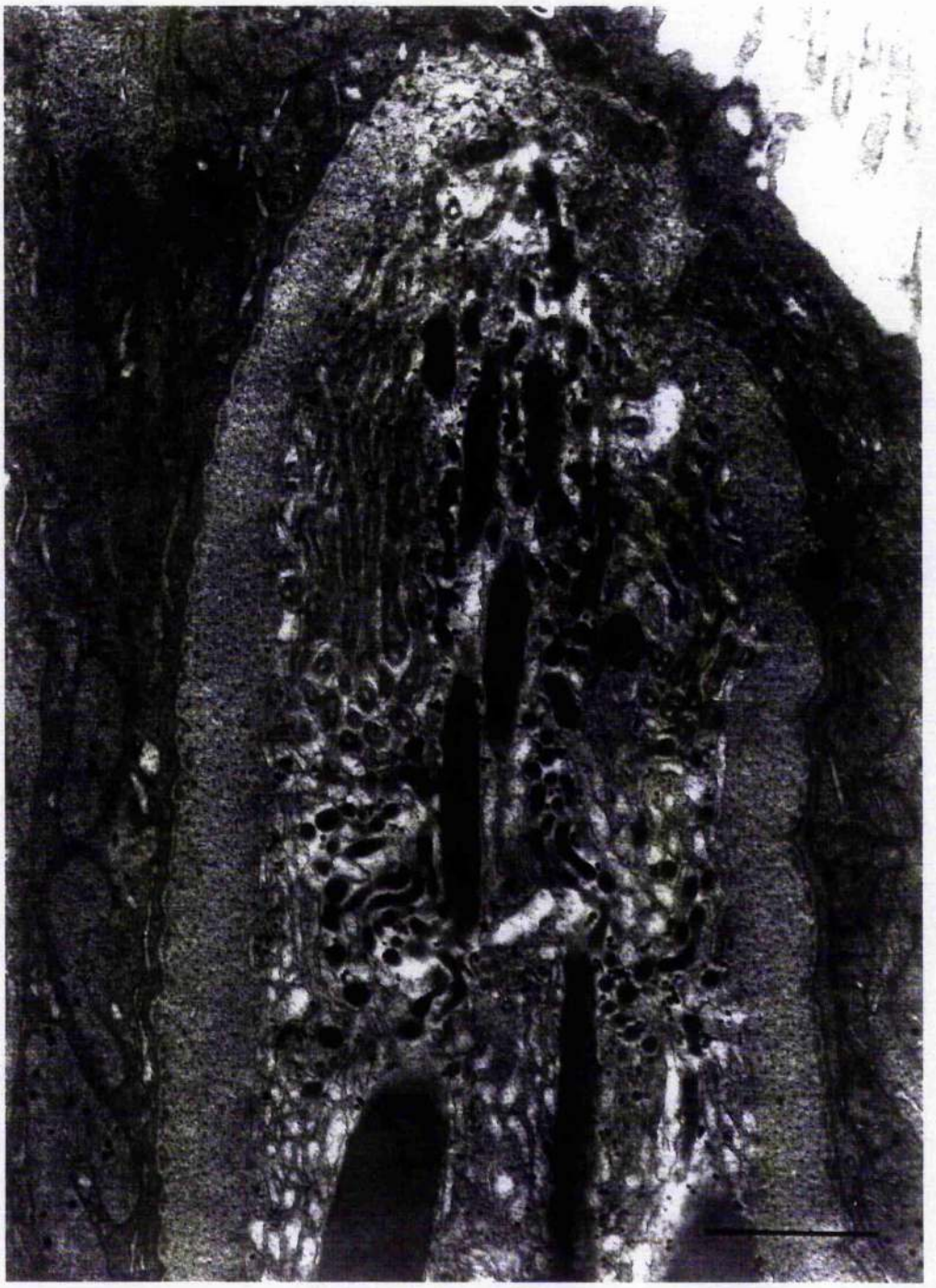


Figure 7.19 TEM of longitudinal section through anterior region of rodlet cell showing large Golgi apparatus (g) with flattened saccules, adjacent to rodlet sacs. Scale bar: 1.0 μm.

7.20). Occasionally, rodlet cells with only the basal nucleus and the fibrillar outer layer remaining were observed at the epithelial surface (Figure 7.20).

7. 3. 2. 3 Morphology of putative early stages of rodlet cell

Putative early stages, as described by Leino (1974), Desser and Lester (1975), Flood *et al.* (1975), Morrison and Odense (1978), Barber *et al.* (1979), and Mattey *et al.* (1979) with no 'capsule' wall, or with a less well developed 'capsule' wall, and containing rodlet sacs in various stages of development and in association with typical secretory organelles such as granular endoplasmic reticulum and Golgi apparatus were not found in this study. However, a cell closely resembling the putative rodlet cell precursor of Smith *et al.* (1995) was observed in the mucosal epithelium of the stomach (Figures 7.23, 7.24). This cell was found in close proximity to other rodlet cells and had many pseudopod-like cytoplasmic extensions which occasionally appeared to be enveloping the rodlet cells. The possible precursor cell was irregular in shape with a length of approximately 9.5 μm and contained a large euchromatic nucleus and fine granular cytoplasm studded with ribosomes, a number of mitochondria and a few darkely stained granules. Occasionally, more than one of these putative precursor cells was found in association with a rodlet cell.

7. 3. 2. 4 Pathological tissue reactions associated with rodlet cells

In all areas of the gut examined, disruption of the histological integrity of the epithelial tissue surrounding rodlet cells was evident to some degree. In some cases the rodlet cells appeared to be flush with the neighbouring cells but mostly an intercellular gap between rodlet cells and neighbouring epithelial cells was observed, even in cells that were not directly next to the epithelial surface and therefore not in the process of secreting (Figure 7.22). Membrane bound vesicles were often observed in this intercellular gap. In some rodlet cells, a cytoplasmic extension was observed projecting into the surrounding epithelial cytoplasm and the plasma membrane appeared to be continuous with the cytoplasmic extension and the fibrillar wall. Vesicles and fine granular material were often found within the extension. This was observed only in 'mature' cells with a thick fibrillar wall (Figures 7.14, 7.25).

Occasionally, a gap was observed in the surface epithelium (Figures 7.20, 7.22). This may represent a rodlet cell that has 'secreted' its contents, possibly the entire cell may be expelled in some cases. More commonly, large intercellular spaces were seen between the base and lateral edges of the rodlet cell and the adjacent epithelial cells, giving the appearance that contraction of the capsule wall has resulted in secretion of the cell contents and is responsible for the intercellular gap (Figure 7.20, 7.22). In some specimens where fixation was incomplete and

Figure 7.20 TEM of longitudinal section through rodlet cell in stomach epithelium. 'Secretions' from rodlet cell appear to be discharged into lumen (l). Scale bar: 1.0 μm.

Figure 7.21 TEM of the stomach epithelium depicting two rodlet sacs (rs) in the lumen (l). Scale bar: 1.0 μm.

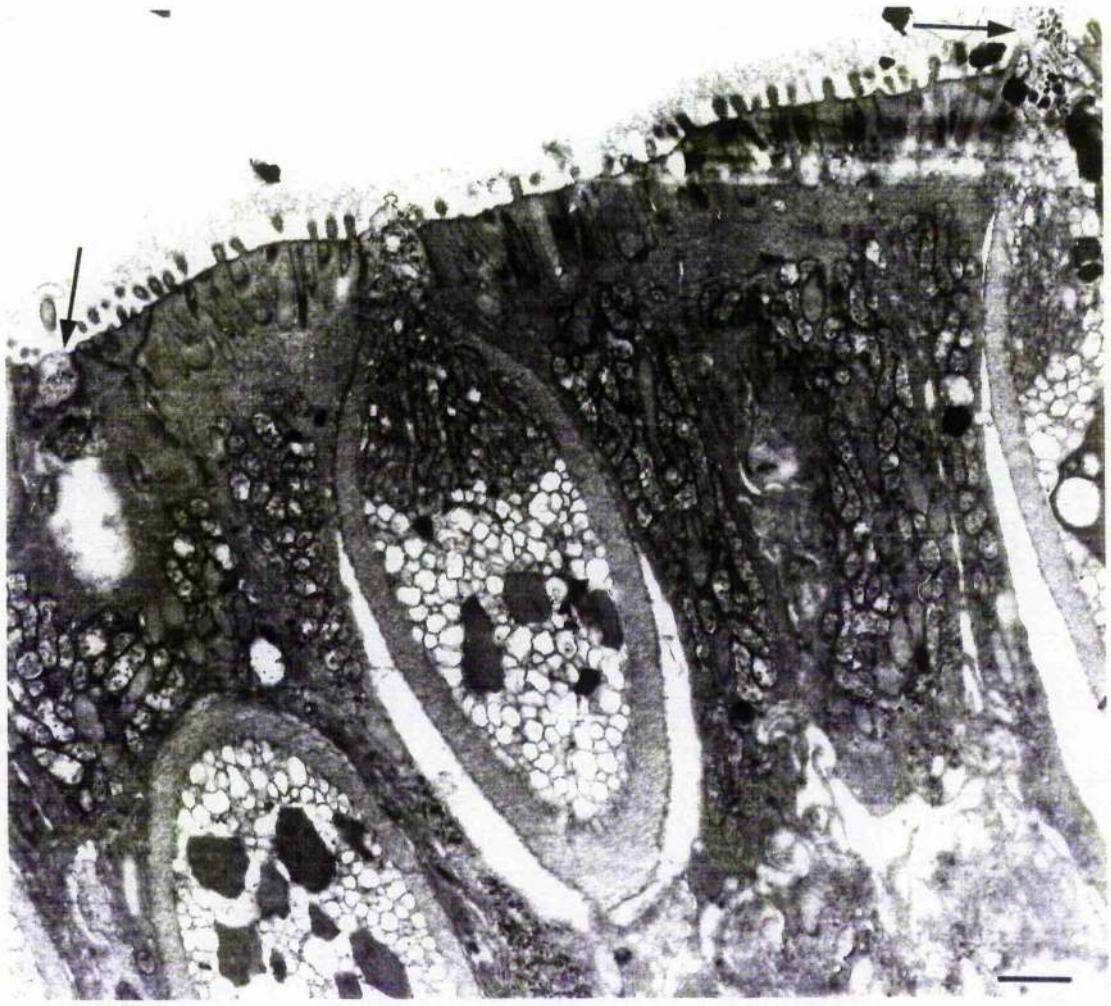


Figure 7.22 TEM of stomach epithelium depicting longitudinally sectioned rodlet cells. The contents of the rodlet cell on the right of the micrograph give the appearance of being discharged into the lumen. The gap on the left of the micrograph may be the site of a 'Discharged' rodlet cell. Scale bar: 1.0 μm.

Figure 7.23 TEM showing putative rodlet cell precursor (pc) in close contact with rodlet cell. Scale bar: 1.0 μm.

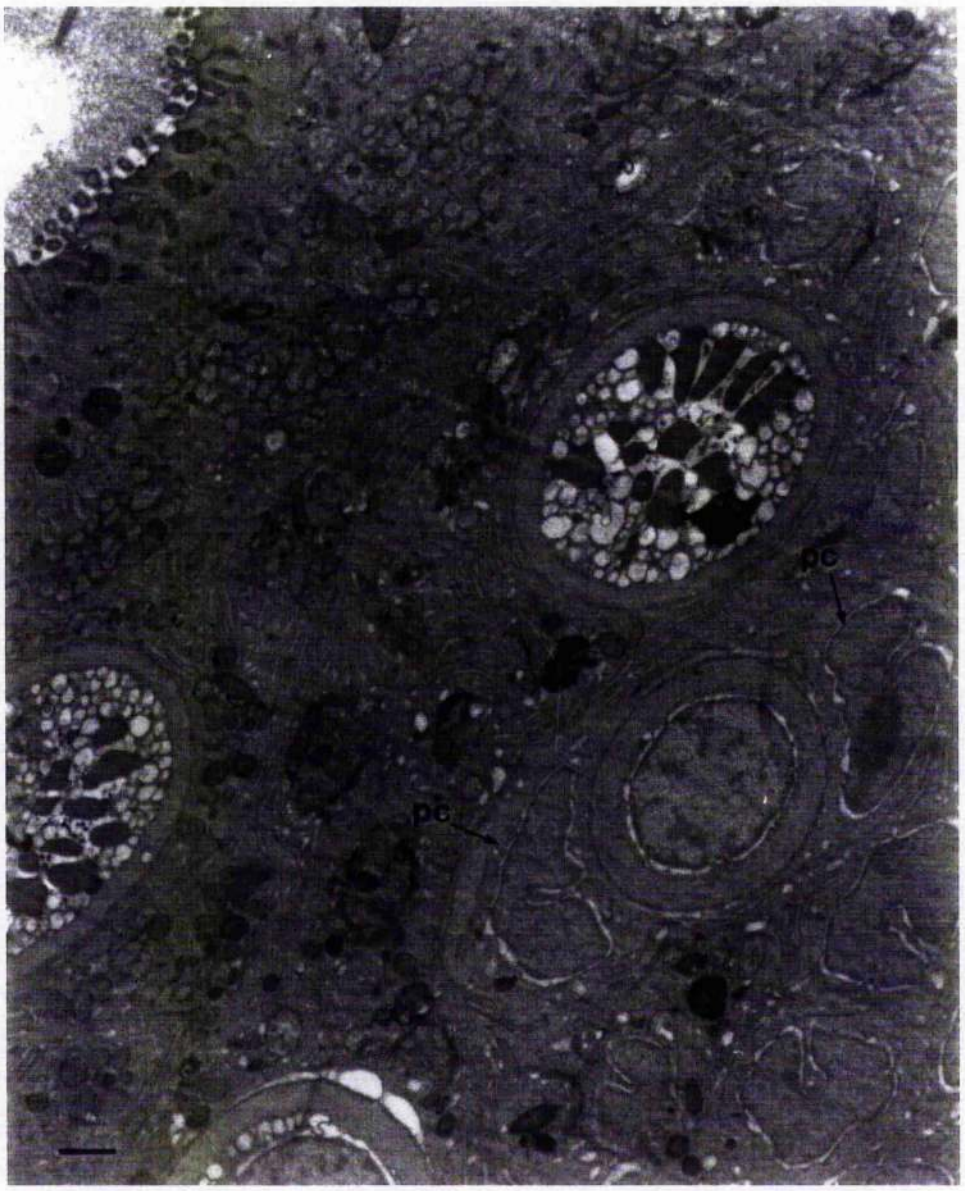


Figure 7.24 TEM showing putative rodlet cell precursor cells (pc) in close contact with rodlet cell. Scale bar: 1.0 μm.

Figure 7.25 TEM of transverse section of rodlet cell showing cytoplasmic extension (ce). Scale bar: 1.0 μm.

Figure 7.26 TEM showing intact rodlet cell surrounded by disintegrating epithelial tissue. Scale bar: 1.0 μm.

Table 7.1. Species of fish in which rodlet cells have been investigated. **at** alimentary tract, **b** brain, **ba** bulbous arteriosus, **bd** bile duct, **bv** blood vessel, **ct** connective tissue, **e** eye, **en** endothelium, **ep** epimysium, **g** gonad, **gb** gall bladder, **gi** gill, **h** heart, **i** intestine, **k** kidney, **kt** kidney tubules, **l** liver, **lp** lamina propria, **m** mesenteries, **mn** meninges, **oe** olfactory epithelium, **p** pancreas, **pc** pyloric caecum, **pe** pharyngeal epithelium, **psb** pseudobranch, **sb** swim bladder, **sk** skin, **sp** spleen, **sv** spiral valve, **wa** whole animal.

Superclass/class	Order	Family	Species	Common name	Rodlet cells observed (+) tentative (?) unobserved (-)	Location
Agnatha	Pteromyzontiformes	Pteromyzonidae	*Lampetra fluviatilis*[48]	river lamprey	?	i
Elasmobranchii	Hypotremata	Rajidae	*Raja ocellata* [26]	skate	-	sv
			Thélohan[1,2]		?	?
Actinopterygii	Acipenseriformes	Acipenseridae	*Acipenser oxyrhynchus* [26]	Atlantic sturgeon	-	gi, h, i
Elopomorpha	Anguilliformes	Anguillidae	*Anguilla fluviatilis* [29]	eel	+	i
			Anguilla vulgaris [26]	eel	+	oc
			Anguilla rostrata [26]	American eel	+	bd, i, k, pd
		Congridae	*Conger oceanicus* [26]	conger eel	+	ct, i, k, m, oe, pc,
Clupeomorpha (herrings)	Clupeiformes	Clupeidae	*Sardina*[1,2]	sardine	+	i
			Clupea harengus harengus [1,2,26,	herring	+	ct, i,
			Alosa pseudoharengus[26]	alewife	-	i
Protacanthopterygii (salmon & pike)	Salmoniformes	Salmonidae	*Salmo gairdneri*[35,14,24,30,26,10]	rainbow trout	+	i, m
			Salmo salar[14,41]	Atlantic trout	+	i
			Salmo trutta[40,14,9,26,44]	brown trout	+	bd, gi, i, l, m
			Salvelinus fontinalis[14,26]	brook charr	+	i
			Salvelinus namaycush[22]	lake trout	+	ba, g, i k, m
			Oncorhynchus tshawytscha[30]	chinook salmon	+	i, gi, sk
			Oncorhynchus kisutch[30]	coho salmon	+	i, gi, sk

Table 7.1 (continued):

Superclass/class	Order	Family	Species	Common name	Rodlet cells Observed (+) Tentative (?) Unobserved (-)	Location
Protacanthopterygii	Salmoniformes	Esocidae	*Esox lucius*[22]	pike	+	ba, g, i, k, m
Ostariophysi (carp & allies)	Cypriniformes	Cyprinidae	*Rutilus rutilus*[9]	roach	+	gi, i, m
			Rutilus rubilio[29]		+	k
			Alburnus alburnus[1,2]	bleak	+	i
			Gobio gobio	gudgeon	+	i
			Catostomus commersoni[34,27,36,37,11,23,32,45]	white sucker	+	bv, e, gi, i, lp, l, m, oe, sp
			Catostomus catostomus[13]	sucker	+	ba, bv, gi, i, l, lp, m
			Cyprinus carpio [40,34,36,35,41,39,43,33]	common carp	+	i, bv
			Semotilus atromaculatus[34,36,37]	northern creek chub	+	gi
			Carassius auratus[24,22]	goldfish	+	i, pd, bd, oe, gi, ba
			Carassius carassius[45]	crucian carp	+	oe
			Tinca tinca [1,2,35]	tench	+	k, gl, ba
			Leuciscus cephalus albus[29]	chubb	+	gb, i, k, l, sb, sp
			Phoxinus phoxinus[15,30]	minnow	+	b, ct, ep, e, g, k, l, mn, oe, pep
			Pachychlon pictum[29]	leucisinae	+	i, l, sb
			Brachydanio rerio[30]	zebra danio	+	ct, i, k, m, oe, pc
			Notropis blennius[22]	river shiner	+	i, k, gi, ba, m
			Notropis umbratilis[22]	redfish shiner	+	i, k, gi, ba, m,
			Notropis cornutus[31]	common shiner	+	at
			Richardsonius balteatus[29]	redside shiner	+	gi, ms, opct, ct
			Xiphophorus helleri[22]	swordtail	+	ba, gi, i, k, m
			Ptychocheilus oregonense[13]	predaceous minnow	+	bv, i, l, lp, m
			Alburnoides bipunctatus ohridanus[29]	leucisinae	+	at, gb, k, sb

Table 7.1 (continued):

Superclass/class	Order	Family	Species	Common name	Rodlet cell Observed (+) Tentative (?) Unobserved (-)	Location
Ostariophysi	Cypriniformes	Cyprinidae	*Chondrostoma nasus ohridanus*[29]	nase	+	l, sb, sp
	Siluriformes	Ictaluridae	*Ictalurus nebulosus* [11]	brown bullhead	-	l,
Paracanthopterygii	Gadiformes	Gadidae	*Lota lota*[22]	burbot	+	i, gi
			Gadus morhua[26]	Atlantic cod	+	i
			Melanogrammus aeglefinus[26]	haddock	+	ct, i, k, m, oe, pc
			Pollacchius virens[26]	pollock	+	ct, i, k, m, oe, pc
			Urophycis tenuis[26]	white hake	-	i, k, oe, pc
	Lophiformes	Lophiidae	*Lophius americanus*[26]	goose fish	?	i
			Cetonurus globiceps[29]	grenadier	+	ub
			Chalinura leptolepis[29]	grenadier	+	ub
			Nezumia stegiolepis[29]	grenadier	+	gb
			Ventrifossa atherodon[29]	grenadier	+	gb
Acanthopterygii (spiny-finned fish)	Atheriniformes	Poeciliidae	*Poecilia reticulata*[26]	guppy	+	at, l, p,
			Gambusia affinis[17]	mosquito fish	+	bd, i, kt, pd
			Fundulus heteroclitus[29]	killifish	+	i
	Gasterosteiformes	Gasterosteidae	*Eucalia inconstans cayuga*[26]	5-spined stickleback	+	k, l, p
			Gasterosteus aculeatus[20]	3-spined stickleback	+	at, k
			Pungitius pungitius[1,2]	10-spined stickleback	+	i
	Perciformes	Labridae	*Crenilabrus (Symphodus) melops*[6]	corckwing	+	en, i, k, l, m, p
			Crenilabrus pavo[6]	wrasse	+	gi, i, k, l, m,
			Labrus bergylta[6]	ballan wrasse	+	gi, i, k, l, m,
			Labrus mixtus[6]	cuckoo wrasse	+	gi, i, k, l, m,
			Julis (Coris) aygula[29]	wrasse	+	at
			Tautogolabrus adspersus[26]	cunner	+	i, m
			Ctenolabrus rupestris[9]	gold-sinny	+	gi, i, k,

Table 7.1 (continued):

Superclass/class	Order	Family	Species	Common name	Rodlet cell Observed (+) Tentative (?) Unobserved (-)	Location
Acanthopterygii	Perciformes	Labridae	*Pseudoscarus harid*[29]	parrotfish	+	at
			Pseudoscarus ghobbam[29]	parrotfish	+	at
		Anarhichidadidae	*Anarrhichas lupus*[26]	Atlantic wolfish	-	i
		Percichthydae	*Dicentrarchus labrax*[28]	bass	+	gi, psb
		Centrarchidae	*Ambloplites rupestris*[22]	rock bass	+	ba, gi, i, k, m
			Micropterus dolomieui[22]	smallmouth bass	+	gi, i, k
		Percidae	*Perca flavescens*[22,32]	yellow perch	+	gi, i, k
			Perca fluviatilis[9]	perch	+	gi, i, m, pc
			Stizostedion vitreum[22]	walleyed pike	+	gi, i, k
		Ammodytidae	*Ammodytes*[29]	sandeel	+	i
		Monodactylidae	*Monodactylus argentus*[26]	fingerfish	+	gi
		Carangidae	*Trachurus trachurus*[1,2]	horse mackeral	+	i
		Stromateidae	*Stromateus fiatola*[29]	harvestfish	+	st
		Nomeidae	*Nomeus gronovii*[29]	man of war fish	+	i
		Cichlidae	*Pterophyllum scalare scalare*[46]	angel fish	+	bv
			Pterophyllum eimiki[38]	angel fish	+	ba
			Oreochromis mossambicus[42]	tilapia	+	gi
			Apistogramma ramirezi[38]	ram cichlid	+	ba
			Hemichromis bimaculatus[38]	jewel fish	+	ba
			Cichlasoma festivum[38]	acara bandeira	+	ba
			Cichlastoma severum[38]	banded cichlid	+	ba
			Pelmatrochromis kribensis[38]	blue acara	+	ba
			Etoplus maculatus[38]	orange chromide	+	ba
		Channidae	*Chaenocephalus aceratus*[29]	snakefish	+	ub
	Scorpaeniformes	Scorpaenidae	*Sebastes inermis*[19,26]	black rockfish	+	pc
		Triglidae	*Trigla corax*[6]	yellow tub gunard	+	gi, i, k, l, m,
			Trigla hirundo[6]	gunard	+	gi, i, k, l, m,

Table 7.1 (continued):

Superclass/class	Order	Family	Species	Common name	Rodlet cell Observed (+) Tentative (?) Unobserved (-)	Location
Acanthopterygii	Scorpaeniformes	Triglidae	*Trigla cuculus*[9,6]	gurnard	+	gi, i, k, l, m,
		Cottidae	*Cottus gobio*[26]	bullhead	÷	en, i, k
			Myoxocephalus octodecemspinosus[26]	bullrout	÷	ct, i, k, m, oe, pc
			Hemitripterus americanus[26]	sea raven	÷	i
		Anaplopomatidae	*Anaplopoma fimbria*[29]	sablefish	+	ub
	Pleuronectiformes	Scophthalmidae	*Scophthalmus maximus*[25,48]	turbot	+	at, k, l,
		Pleuronectidae	*Glyptocephalus cynoglossus*[26]	witch flounder	+	i
			Pleuronectes ferruginea[26]	yellowtail flounder	?	i, pc
			Pseudopleuronectes americanus[26]	winter flounder	+	pc
			Hippoglossus hipoglossus[47]	Atlantic halibut	+	at
			Hippoglossoides platessoides[26]	long rough dab	+	ct, i, k, m, oe
			Pleuronectes platessa[48]	plaice	+	at
Amphibia			*Hyla crucifer*[26]	spring peeper	-	wa
			Triturus vulgaris[26]	red spotted newt	-	wa
			Thélohan[1,2]	hyalid frog	?	?

[1,2]Thélohan (1892a, b); [3]Laguessé (1895); [4,5]Plehn (1906a, b); [6]Duthie (1939); [7,8]Al-Hussaini (1949a, b); [9]Catton (1951); [10]Weinreb & Bilstad (1955); [11]Dawe *et al.* (1964); [12]Vickers (1962); [13]Weisel (1962); [14]Bullock (1963); [15]Hale (1965); [16]Bannister1966; [17]Bullock (1967); [18]Wilson & Westerman (1967); [19]Iwai (1968); [20]Mourier (1970); [21]Fernhead & Fabian (1971); [22]Leino (1974); [23]Desser & Lester (1975); [24]Flood *et al.*(1975); [25]Anderson *et al.* (1976); [26]Morrison & Odense (1978); [27]Barber *et al.* (1979); [28]Mattey *et al.* (1979); [29]Mayberry *et al.* (1979), [30]Modin (1981); [31]Paterson & Desser (1981); [32]Leino (1982); [33]Viehberger & Bielek (1982); [34]Barber &Westermann (1983); [35]Bielek & Viehberger (1983); [36,37]Barber &Westermann (1985); 1986); [38]Leknes (1986); [39]Mayberry *et al.*(1986); [40]Balabanova & Matey (1987); [41]Imagawa *et al.*(1990); [42]Pratap and Bonga (1993); [43]Richards *et al.* (1994); [44]Rocha *et al.* (1994); [45]Wayne & Mayberry (1994); [46]Smith *et al.* (1995); [47]Murray *et al.* (1996); [48]Present study (1997);

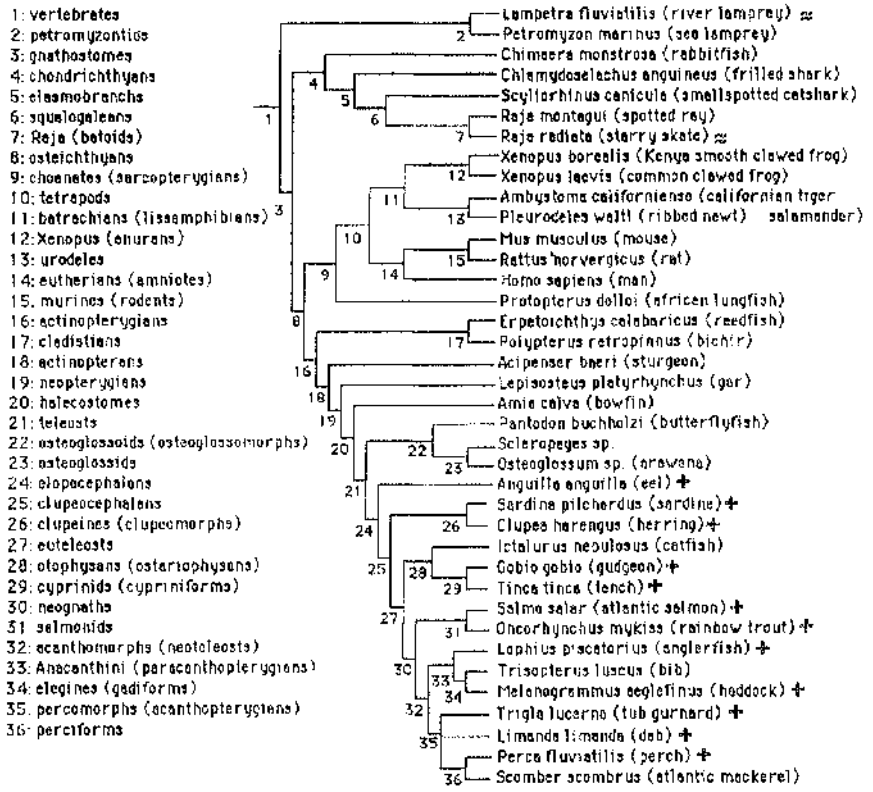


Figure 7.27 Cladogram (adapted from Le *et al.*, 1993) showing the intrarelationships of the gnathostomes and agnatha. + refers to species in which rodlet cells have been observed and ≈ refers to the possible occurrence of rodlet cells in this species but has yet to be confirmed.

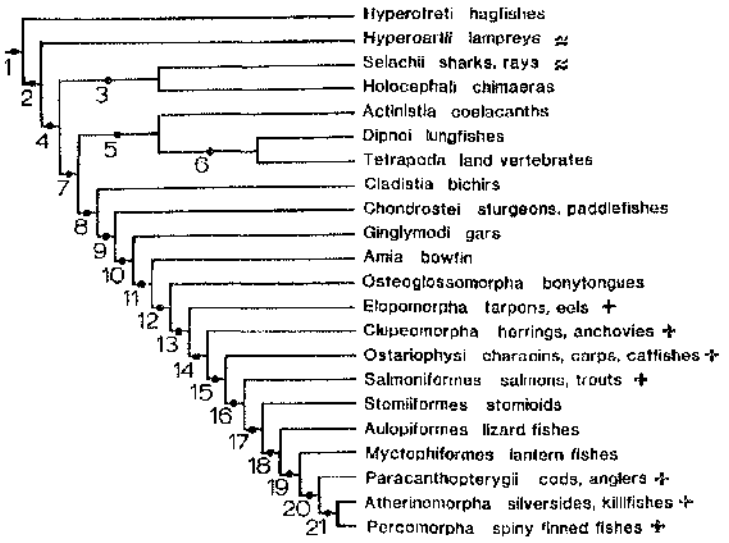


Figure 7.28 Shows the interrelationships of the major fish groups (adapted from Nelson, 1989). + refers to the groups where rodlet cells have been observed and ≈ refers to the possible occurence of rodlet cells in species but has yet to be confirmed.

the epithelial tissues were badly damaged and disintegrating, rodlet cells were observed to be still intact (Figure 7.26).

Defence reactions against rodlet cells or rodlet sacs or their contents as described by Bielek and Vichberger (1983) were not observed in this study but occasionally degenerating rodlet cells which had not secreted their rodlets into the lumen were observed near the surface epithelium (Figure 7.18).

7. 3. 3 Occurrence of rodlet cells in fish species

Fish species and other lower vertebrates which have been investigated for the presence of rodlet cells are listed in taxonomic groups based on the system of classification by Nelson (1994) in Table 7.1. Rodlet cells have been found in many different fish groups including marine, fresh-water and brackish-water fish and are widespread throughout fish tissues. Only in a few of the species studied were no rodlet cells observed. When the phylogenetic relationships of the major fish lineages was examined with respect to those groups in which rodlet cells have been observed, the evolutionary antiquity of the rodlet cell was evident. Figure 7.27 is a cladogram showing the intrarelationships of the gnathostomes and agnatha (from Lauder and Liem, 1983; Maisey, 1986; Le *et al.*, 1993), whilst table 7.28 (from Nelson, 1989) demonstrates the phylogenetic relationships between various teleost groups. The presence of rodlet cells in cyclostomes, elasmobranchs and even hyalid frogs is mentioned by Bannister (1966) citing the work of Thélohan (1892b). I was unable to find evidence of this in Thélohan's paper. Investigation into the possible presence of rodlet cells in the river lamprey, *Lampetra fluviatilis*, in the present work was inconclusive. Using light microscopy, some cells were found in the alimentary tract which resembled rodlet cells but further work using electron microscopical procedures is necessary to confirm this. The presence of rodlet cells in cyclostomes and sharks has therefore yet to be confirmed.

7. 4. DISCUSSION

The distribution and morphology of rodlet cells has been reviewed by Leino (1974), Morrison and Odense (1978), Mattey *et al.* (1979), and Mayberry *et al.* (1979). In general, rodlet cells from different species have general similarities but have been found to vary in size and in morphological detail among species (Barber and Westermann, 1983). The presence of rodlet cells in the turbot, *Scophthalmus maximus* (L.) as reported by Anderson *et al.* (1976) have been confirmed in the present study. In this investigation the rodlet cells of the turbot were found to be similar in distribution and in morphological appearance to those of other species of

fish, with only a few variations. However, despite this apparent consensus on the general features of rodlet cells, most of their characteristics have been used as arguments for both the exogenous, parasitic nature of the cell and for an endogenous, secretory or type of granulocyte role for the cell and so the nature and/or function of the cell remains controversial. These features will be discussed by comparing findings in the present study in the context of information reported in other work.

Rodlet cells have been noted from many different species of marine and freshwater fish and from every type of fish tissue examined. There is some evidence, although this has to be substantiated, that rodlet cells are also found in elasmobranchs and cyclostomes. This would indicate an organism or cell of relatively ancient lineage. This feature and the wide variation in the distribution and abundance of rodlet cells in individuals of the same species has led to conflicting views. Rodlet cells may be absent from some individuals of a species or present in different organs of individuals of a species. In some cases they are sparsely distributed throughout a tissue and in others they form dense aggregations with few or no intermediate epithelial or endothelial cells. Leino (1974) noted an inter- and intra-specific variation in distribution and abundance of rodlet cells correlated with seasonal variations, crowding and ionic concentration and suggested this might be related to a site-specific physiological function. Desser and Lester (1975), Morrison and Odense (1978) and Mattey *et al.* (1979) claimed the wide distribution of rodlet cells in tissues and among different species was not typical of a parasite which normally displays a more rigid host specificity. Bannister (1966), Barber *et al.* (1979) and Mayberry *et al.* (1979) all took the opposing view that the absence of rodlet cells from certain individuals in a species and the variation in distribution and abundance of the cells in different individuals of the same species was not consistent with patterns of distribution of normal tissue cells but rather was typical of an organism foreign to the tissues where it is found or typical of the epidemiology of many parasitic infections.

Leino (1974) noted the presence of rodlet cells in very young, laboratory-reared fish and concluded that the cells were of endogenous origin. Mayberry *et al.* (1986) examined artificially fertilized fish reared in the laboratory under controlled conditions and found 10 day old fish had no rodlet cells whilst 60 day old fry displayed what appeared to be a migration of rodlet cells from connective tissue to the bulbus arteriosus. Although their work discounted the possibility of horizontal transmission between fish, they could not discount the possibility of vertical transmission or the transfer of an infectious stage of rodlet cells from broodstock to offspring. This could be a possible explanation for the presence of rodlet cells in Leino's young fish and may also explain why Anderson *et al.* (1976) found heavy numbers of rodlet cells in 17 day old hatchery-reared turbot. In a later study by Wayne and Mayberry (1994), investigation of

laboratory-reared carp fry under controlled conditions revealed an absence of rodlet cells from all tissues examined from 5-day old and 10-day old fry. The authors concluded the rodlet cells could not be of fish origin and that the absence of rodlet cells was due to the isolation and maintenance of the fish host in a controlled environment.

Anderson *et al.* (1976) assumed the rodlet cell was a parasite and found a correlation between both the numbers of rodlet cells (*Rhabdospora thelohani*) and the sporozoan *Myxidium incurvatum* in the kidney and liver of juvenile turbot, and with the severity of lesions in these organs caused by hepato-renal syndrome. They found very few turbot with no rodlet cells and also observed that many apparently normal organs (with no lesions) had medium levels of infections with rodlet cells. However, they found a general trend of increasing numbers of rodlet cells with increasing severity of lesions and took this as a sign of a parasitic infection. The authors suggested it was not possible to determine whether both *Rhabdospora thelohani* and *Myxosporidium incurvatum* were possible causative agents of the disease or if they played a secondary, opportunistic role. They stated however, that if the latter was the case, then the organisms may contribute to the ultimate death of the fish. They also observed large numbers of rodlet cells in the lower intestine and rectum and fewer numbers in the upper intestine, regardless of whether the fish had liver or kidney infection, and so suggested that the lower intestine and rectum may be the major site of infection with rodlet cells.

However, this observation is not consistent with the present study where large numbers of rodlet cells were present in the stomach and anterior (upper) intestine of juvenile turbot, as well as in the posterior (lower) intestine and rectum. Fewer rodlet cells were observed in the oesophagus than in any other region of the alimentary tract. As with other investigations (Leino, 1974; Morrison and Odense, 1978; Mayberry *et al.*, 1979), the distribution and abundance of rodlet cells in juvenile turbot varied between individual fish and in some fish (electron microscopal observations) no rodlet cells were observed, although this does not prove that they were not present. There could be several reasons for this apparent difference. Firstly, if rodlet cells are parasitic it may be that in heavier infections the parasites establish themselves in less preferable sites. Secondly, if rodlet cells are some type of granulocyte with immune properties they may only be activated under certain circumstances, presently unknown. Thirdly, differences in individual fish, both genetic and phenotypic, could result in variation in abundance and distribution of the parasite, this is common in parasitic infections.

This would seem to follow the pattern of parasitic infection where one might expect the prevalence of infection to vary, as well as the distribution and abundance of the parasite, between individuals of a species. Parasites, on the other hand, usually display more host and site specificity, but it is possible there could be a number of different species of rodlet cell involved

which would explain their wide host, tissue and habitat variation. Also, this could be an organism of very ancient lineage (Barber and Westermann, 1983). This would explain the wide distribution in freshwater and marine fish, the minor morphological variations and the reported association with some cyclostomes, elasmobranchs and even hyalid frogs (Thélohan, 1892a,b; Bannister, 1966). The protozoa are a very ancient and even more diverse group than was presumed until recently. For example, molecular data suggest that different species within the genus *Entamoeba* are more diverse than plants, fungi and the metazoa (Margulis, 1993). Such proven diversity lends support to the idea that rodlet cells could comprise a group of organisms of different species or groups having diverse relationships with different hosts.

There are some parasites which display low host specificity and are referred to as wide generalists (Holmes, 1990). For example, the protozoa *Toxoplasma* and *Trypanosoma* as well as the tapeworms *Diphyllobothrium latum* and *Echinococcosus granulosus* display a lack of host specificity. Some parasites migrate through different tissues before the sexually mature phase localizes in a certain area (*Toxoplasma, Eimeria, Ascaris lumbricoides* and *Dracunculus medinensis*). Also, certain host or environmental factors such as diet, life span, mobility (including the variety of habitats it encounters), population density, and also size, may cause the distribution and abundance of a parasite to vary. If indeed the rodlet cell is a parasite, some of these factors could explain the differences observed between the present results and those of Anderson *et al.* (1976).

Rodlet cells are characterized by their unusually thick 'capsule' wall, basal nucleus and large, electron dense inclusion bodies known as rodlets. They are found in many fish tissues such as intestine, liver, kidney, pancreas, bulbous arteriosus, thymus, gonad, gills, connective tissues, meninges, brain, spinal cord and eye (Bannister, 1966; Mayberry *et al.*, 1979; Pulsford, *et al.*, 1991). However, they are most commonly associated with epithelial or endothelial tissues where they are orientated with their apexes bordering on the free surface. Junctional complexes between the apical region of rodlet cells and neighbouring epithelial or endothelial cells when they are positioned at the free surface have been described by many authors. Desser and Lester (1975) observed tight junctions between rodlet cells and gill epithelium, Leino (1974) noted desmasomes between rodlet cells and neighbouring epithelial cells, Mattey *et al.* (1979) also reported desmasomes between rodlet cells and epithelial cells. They all interpreted this as evidence of the endogenous origin of the rodlet cell. Paterson and Desser (1981) claimed the junctional complexes observed by workers proved that rodlet cells lay between epithelial cells and therefore could not be members of the apicomplexa as these organisms were intracellular in epithelia. Although Smith *et al.* (1995) found no desmasomes in their own study, they argued that the observations of such complexes by other authors was inconsistent with the parasite theory of

rodlet cells. Leknes (1990) observed tight junctions and desmasomes connecting rodlet cells to endothelial cells but was aware that some parasites are known to form junctional complexes with host cells. Barber *et al.* (1979) also considered that the presence of desmasomes and tight junctions between rodlet cells and epithelial cells did not preclude the parasitic nature of rodlet cells, noting that *Giardia* may induce cytoplasmic filaments in the host cell encircling the sites of attachment of the trophozoites (Erlandsen and Chase, 1974). Mayberry *et al.* (1979) observed desmasomes connecting rodlet cells to epithelial cells and interpreted the cells as a parasite, although they did not discuss the junctional relationship. Flood *et al.* (1975) described a junctional relationship between rodlet cells and adjacent epithelial cells as well as between neighbouring rodlet cells and explained this as an active function of the fibrous layer. Bielek and Viehberger (1983) commented on other authors observations that junctional complexes indicated a type of tissue cell but noted that such complexes are also found between parasites and host cells (Aikawa and Kilejian, 1979).

In this study, desmasomes and tight junctions were observed between the apical region of rodlet cells and intestinal epithelial cells of turbot. Anderson *et al.* (1976) did not carry out electron microscopal studies and therefore did not observe desmasome contact between rodlet cells and epithelial cells in their study on turbot. However, they commented on Mourier's (1970) observation of desmasome-like connections between pairs of rodlet cells but not between rodlet cells and epithelial cells and claimed that this, combined with their observation of a separation between rodlet cells and epithelial cells, indicated a lack of intimate contact between rodlet cells and the neighbouring host cells. Many studies since then have shown junctional complexes may occur between parasites and host cells (Aikawa & Kilejian, 1979).

Many protozoa and other parasites invade host tissues but the physiology of cell recognition, cell adhesion and penetration is both complex and inadequately understood (Chappell, 1993). For example with malarial parasites, after recognition of the red blood cell by the merozoite, junctions are formed between the apical complex of the merozoite and the red blood cell prior to invasion. Perhaps the junctions observed connecting rodlet cells to epithelial or endothelial cells are similar to those of certain stages of Apicomplexa but instead of being involved in cell invasion they are used when the rodlet cell is secreting material into the lumen. The mitochondrial ultrastructure suggests that the rodlet cell may be in a metabolically inactive state when it is situated next to a free surface. It is possible therefore that communication junctions are formed with epithelial cells in order to obtain the energy required for secretion.

Mayberry *et al.* (1979) considered rodlet cells to be members of the Apicomplexa and argued that the apical region of the rodlet cell bore many similarities to the apical complex in motile stages of the coccidia. They compared the organelles of the rodlet cell to the conoid, polar

ring, subpellicular microtubules, micronemes and rhoptries of members of the Apicomplexa, although they also stated neither a conoid or polar ring were present in rodlet cells. Paterson and Desser (1981) argued that the tubular organelles at the cell apex, described as micronemes by Mayberry *et al.* (1979), were, in fact, the dense mitochondria usually found in the apical region of mature rodlet cells which had probably been preserved inadequately and claimed that the rodlet sacs bore only a superficial resemblance to the rhoptries of the Apicomplexa.

Paterson and Desser (1981) also noted the similarity of the structure of the rodlet cells of the common shiner, *Notropis cornutus,* to that of those of the white sucker, *Catostomus commersoni,* as reported by Desser and Lester (1975), in respect to the posession of apical microvilli. Apical microvilli have not been reported in the rodlet cells of other fish or by other workers investigating the ultrastructure of rodlet cells from *Catastomus commersoni.* Rodlet cells in the present study did not appear to possess apical microvilli. However, in cells positioned at the free surface of the epithelium, a well defined apical cap was observed projecting into the extracellular space. Occasionally, these cells possessed a projection which resembled the neighbouring microvilli but which might also be explained as the commencement of the secretory process (Figures 6.8 and 6.9). This cone-shaped area had features similar to those of the penetration organs of some parasites and did have some resemblance to the apical complex of the Apicomplexa. However, the apical complex is used to assist the parasite in invasion of the host cell and rodlet cells are not intracellular. It has been suggested that the apical region may assist in the secretory process of the rodlet cells but is unlike the usual secretory patterns such as merocrine, holocrine or apocrine secretion of normal secretory cells (Smith *et al.* 1995).

Barber *et al.* (1979) described a cytoplasmic extension projecting from the fibrillar 'capsule' into the neighbouring epithelial cell cytoplasm. Desser and Lester (1975) also illustrated a cytoplasmic extension, but did not discuss it in their paper. Cytoplasmic extensions were observed projecting from the fibrillar wall of rodlet cells into adjacent epithelial cells in the present study (Figures 7.14 and 7.28). Barber *et al.* (1979) suggested this may be similar to the extracapsular cytoplasm seen in *Lankesterella* species for which Stehbens (1966) postulated a feeding function. Occasionally, vesicles are observed in the protoplasmic extension of the rodlet cell and these possibly indicate some form of nutrient uptake. Flood *et al.* (1975) described an amorphous substance surrounding their semi-encased and fully-encased rodlet cells and suggested that this may be a secretion of the 'encasing' layer or of the surrounding vascular tissue. They noted the resemblance to a series of observations made by Bowers and Korn (1967, 1968, 1969) on the ultrastuctural changes which take place in encysting amoeba. The authors also compared the similarities of the cellular activity which accompanies the construction of the encasing layer in amoebae and its structure to that accompanying the production of the fibrillar wall of the rodlet

cell. However, they pointed out that the capsule-like wall of the rodlet cell is not a 'resistant envelope' but a specialized fibrillar cytoplasm.

The thick, 'capsule' wall of the rodlet cell is yet another characteristic which has been interpreted as either a feature of an endogenous cell or as an indication of an exogenous or parasitic origin. Some have taken it to represent a cyst-like wall characteristic of an isolated parasite (Bannister, 1966; Iwai, 1968c) whilst others state the 'capsule' wall is clearly not a cyst wall and is part of the cytoplasm with no membrane separation (Flood *et al.*, 1975; Mattey *et al.*, 1979; Smith *et al.*, 1995). Many have attributed contractile properties to the microfilaments within the capsule wall (Leino, 1974; Desser and Lester, 1975; Mattey *et al.*, 1979; Mayberry *et al.*,1979 and Barber and Westermann, 1983). Mattey *et al.* (1979), described the general appearance of the 'capsule' wall as similar to that of smooth muscle cell cytoplasm and Desser and Lester (1975) compared the appearance and size of the microfilaments to the actin filaments of skeletal muscle suggesting the microfilamentous 'girdle' could squeeze the cell contents through the thin apical region onto the apical surface. Morrison and Odense (1978) noted the capsule wall appeared darker when the rodlet cells were secreting and suggested the filaments became disorientated and overlapped as the capsule contracts. This could possibly explain the irregular shape often observed in secreting cells.

In the present study, rodlet cells apparently in the process of secreting were occasionally observed with irregular outlines, but often they appeared relatively smooth in shape. Sometimes electron dense bands lying immediately underneath the plasma membrane were observed. These are superficially reminiscent of the hemidesmasomes of the coccidea (Smith *et al.*, 1995). There appeared to be no changes in the staining properties of the capsule wall of rodlet cells during secretion, although these cells appeared narrower and longer than cells further down in the epithelium. This is in accordance with Leino (1974) who reported that rodlet cell width decreased from 6 μm to 2 μm during contraction and that this supplied the force necessary to propel the rodlet sacs and other cytoplasmic contents out into the lumen. It was observed in the present study with juvenile turbot that the width of the 'capsule' wall remained relatively constant at approximately 0.5 μm, although occasionally the fibrillar border at the basal portion of the secreting cell appeared to be thicker. Frequently, an intercellular gap between the base and lateral edges of the rodlet cell and adjacent epithelial cells was observed. Such intercellular gaps were observed between both secreting and non-secreting rodlet cells and adjacent epithelial cells. These gaps were also reported in turbot by Anderson *et al.* (1976), who suggested they indicated a lack of intimate contact between the rodlet cell and adjacent host cells.

It is possible that the 'capsule' wall has contractile properties but it seems unlikely that this is the method of secretion employed. Secretory cells usually employ methods such as

merocrine, holocrine and apocrine secretion (Rhodin, 1974). Smith *et al.* (1995) likened the secretion of the contents of the rodlet cell to a modified form of holocrine secretion, where only part of the cytoplasm is released. They suggested that the rodlets are lysed intracellularly, then released in amorphous form. This is in some agreement with this study where, occasionally, disintegration of the nucleus and other organelles appeared to occur prior to secretion of the rodlets. There has also been some suggestion that the form of secretion may vary between different species of fish. Discharged, intact rodlets have been reported by Leino (1974), Desser and Lester (1975) and Barber *et al.* (1979) whilst Morrison and Odense (1978) noted discharged rodlets near 'ruptured' cells and suggested the rodlets had caused the rupture of the cell. In other cases, large vesicles, small electron dense bodies and intact rodlets were observed in the lumen.

Many studies have reported rodlet cells secreting their products, especially the rodlets, into the extracellular space. This has led to a great deal of speculation as to the properties of this secretion and/or its function.

The interpretations of the rodlet inclusions have included: sporozoites (Thélohan 1892 a,b, Laguessé, 1895,1906, Mayberry *et al.*, 1979); trichocysts (Mourier, 1970); merozoites (Richards *et al.*, 1994); secretory products of a gland cell (Plehn, 1906a,b; Leino, 1974; Morrison and Odense, 1978; Mattey *et al.*, 1979; Leknes, 1986); a type of blood cell (Duthie, 1939; Catton, 1951; Weinreb and Bilstad, 1955; Smith *et al.*, 1995). Proponents of the endogenous theory for rodlet cells have attributed a variety of functions to these secretions. These include ion transport and osmoregulation (Vickers, 1962; Fernhead and Fabian, 1971; Leino, 1974; Morrison and Odense 1978; Mattey *et al.*, 1979), reduction of friction (Desser and Lester, 1975), antibiotic effect or cells associated with defence (Leino, 1974, 1982; Mattey *et al.*, 1979; Leknes, 1986; Balabanova and Matey, 1987; and Imagawa *et al.*, 1990). A number of earlier workers believed the secretion may be similar to that of mucous glands (*e.g.* Al-Hussaini, 1949a,b) but Morrison and Odense (1978) pointed out that the polysaccharide of the rodlet gives different histochemical reactions to those of goblet cell mucous. Also, it would seem unlikely for a cell with an exocrine secretion like mucous or even a cell with an endocrine or sensory function would be found in so many different tissue sites. Morrison and Odense (1978) argued that the absence of rodlet cells in elasmobranchs supported the theory that they are involved in ion transport as elasmobranchs use urea for osmoregulation. However, Bannister (1966) claimed that Thélohan (1892b) reported the cells from elasmobranchs and this would seem to contradict their theory. Neither would this explain the absence of rodlet cells from certain individuals of the same species. Mayberry *et al.* (1979) noted the presence of intact rodlet cells as well as rodlets in the lumen and considered this was not consistent with a secretory cell hypothesis and suggested this may be a method of escape for the parasite from the host's body.

A secretory cell would be expected to develop in the germinal epithelium from an undifferentiated cell type and therefore early stages of the cell should be evident. Leino (1974), Desser and Lester (1975), Flood *et al.* (1975), Morrison and Odense (1978), Barber *et al.* (1979) and Mattey *et al.* (1979) all described what they believed were developmental stages of rodlet cells. They described cells with organelles in progressive stages of development. Generally, the immature or 'active' cells displayed a gradual appearance of a 'capsule' wall and rodlet sacs, combined with a temporary increase in the number of ribosomes and granular endoplasmic reticulum, which is indicative of increased protein synthesis. This was followed by mature or 'inactive' stages showing a decrease in granular endoplasmic reticulum and a rearrangement of the cell organelles with the mitochondria forming a dense aggregation at the apex of the cell in the region where junctional complexes are made with epithelial or endothelial cells. The rest of the mature cell was taken up by the basal nucleus with a number of rodlet sacs and large, electron luscent, membrane bound vesicles lying between the nucleus and the mitochondria. Leino, (1974); Desser and Lester, (1975); Morrison and Odense (1978) and Mattey *et al.* (1979) all believed the formation of rodlet cells in the basal and intermediate zones of the epithelium or endothelium and their migration to the surface showed they were normal host cells. Desser and Lester (1975) commented on the similarity between the formation of rodlet cells and mucous cells. Mattey *et al.* (1979) believed the granular endoplasmic reticulum and Golgi complex of the immature cell explained the formation of the rodlet sacs. Morrison and Odense suggested that the mitochondria, ribosomes and Golgi apparatus moved to the apex of the cell so that material could be added to the apical ends of the rodlets as they became elongated. However, Flood *et al.* (1975) believed these could be different stages of a parasite and compared the progressional development to the stages undergone by pre-encysting amoebae, although they did emphasize the different natures of the capsule of the amoebae and the cytoplasmic fibrillar wall of the rodlet cell. They noted the mitochondria of the 'inactive' or 'fully encased' stage were irregular in size and shape, were more opaque than the mitochondria of earlier stages and appeared to lack internal cristae. They suggested the lack of internal structure of the mitochondria may be a sign of a depressed metabolic state with a correspondingly reduced respiratory activity (Vickerman, 1960, 1962; Band and Mohrlok, 1969).

Cells with organelles in progressive stages of development were not observed in this study, although cells similar to the 'rodlet cell precursor' of Smith *et al.* (1995) were observed. According to Smith *et al.* (1995) these cells had euchromatic nuclei, contained numerous small cytoplasmic vesicles and were often found with pseudopod-like cytoplasmic extensions enveloping nearby mature or nearly mature rodlet cells. Similar cells were observed in close association with mature rodlet cells in this study.

If rodlet cells are some form of protozoan parasite it is possible that the developmental stages occur in another species. Mayberry *et al.* (1986) pointed out that the developmental stages of *Myxosoma cerebralis* and *Triactinomyxon* were described from their respective fish and tubificid hosts but until recently were not recognized as alternating phases of a single parasite life cycle (Wolf and Markiw, 1984).

The proposition that rodlet cells are some type of blood cell (Duthie, 1939; Catton, 1951; Weinreb and Bilstad, 1955; Smith *et al.*, 1995) would certainly agree with the widespread distribution of some defence cells. However, it does not explain one of its main morphological characteristic, the 'capsule' wall, and does not explain the nature of the rodlets. It would seem that explaining the chemical nature of the rodlets may hold the answer to the enigmatic rodlet cell.

Morphologically, the rodlet sac consists of a club-shaped, membrane bound sac with a long, narrow electron-dense core surrounded in the basal region by a fine granular inner area and a coarse, flocculent outer zone. There is a reasonable consensus on the composition of the cortical layer of the rodlet but not on the core material. The rodlet cortices appear to consist of a neutral glycoprotein (Morrison and Odense, 1978; Mattey *et al.*, 1979; Leino, 1982; Bielek and Viehberger, 1983). Studies have demonstrated the rodlet core to be negative for polysaccharides (Bannister, 1966; Morison and Odense, 1978; Leino, 1982). A negative Feulghen reaction for DNA (Bannister, 1966; Barber and Westerman, 1975; Bielek and Viehberger, 1983) was interpreted by some as evidence against the parasitic nature of the rodlets but Feulghen-negative DNA is known to exist in the cytoplasm of amoebae, insects and plants (Lima-de-Faria, 1962). More recently, the rodlet cores have been shown to be positive for DNA of an unusual configuration (Viehberger and Bielek, 1982; Bielek and Viehberger, 1983; Barber and Westerman, 1986b), whilst there is some evidence that the nucleus of the rodlet cell contains a similar quantity of DNA to other cell nuclei from the same fish species (Barber and Westermann, 1986a). This would appear to indicate that the rodlet cell itself is of teleost origin whilst the rodlets may be the transport units of genetic material. The possibility that only the rodlets are parasitic stages was argued against by Bannister (1966) who pointed out that the nucleus of the rodlet cell remained healthy and showed no signs of degeneration as one might expect in a healthy cell. However, as Mayberry *et al.*(1986) suggested the rodlets may have been parasitic at some stage and have evolved in a similar fashion to mitochondria. A symbiotic relationship between the rodlets and host cell could explain many of the properties of rodlet cells. However, again the overall diversity and the unknown nature of many individual protozoa should be remembered. For example, Bielek and Viehberger (1983) noted the electron dense rods of the Haplosporidians which develop into internal bipartite structures of yet unknown nature, and of which DNA is indicated as a possible constituent. Until the rodlets are isolated from rodlet cells and tested for

DNA the nature of the rodlets will remain uncertain. Sequencing of any DNA present should provide answers to many of the currently unanswered questions.

Finally, the lack of a distinct pathological tissue reaction has often been used as evidence against the parasitic nature of rodlet cells. Schaperclaus (1979), however, pointed out that sporozoa often cause no significant defence mechanisms in their host and also many authors have observed some degree of tissue reaction against rodlet cells. For example, Bielek and Viehberger (1983) noted a phagocytic reaction against rodlets by macrophages and by heterophilic granulocytes. Richards *et al.* (1994) observed phagocytosis of both rodlet cells and released rodlets by macrophages and neutrophils. Dawe *et al.* (1964) suggested there was a relationship between rodlet cells and hepatic neoplasia in *Catostomus commersoni* where he observed rodlet cells replaced 50 % of the neoplastic tissue. Also, the isolation of rodlet cells from the surrounding host tissue would seem to suggest a parasitic origin for the cells (Anderson *et al.* 1976; Morrison and Odense, 1978 and Bielek and Viehberger, 1983). Mayberry *et al.* (1986) used the term 'rhabdosporosis' to describe the heavy invasion of host tissue by large numbers of rodlet cells which they believed to be disrupting the histological integrity of the host tissue. Evidence in the present study also indicates some degree of tissue disruption does take place. For example, intercellular gaps between rodlet cells and surrounding host tissue were often observed. The observation that in poorly preserved sections rodlet cells remained intact amidst autolysing host tissue suggests that they differ significantly form most of the hosts cells.

7. 5. CONCLUSIONS

When the evidence and views accumulated over the last century on rodlet cells are examined it seems possible, but not conclusive, that rodlet cells, or at the least the rodlet inclusions, are of a parasitic (or exogenous) origin. The strongest evidence for a parasitic origin lies in the fact that the rodlets appear to contain DNA different from the host DNA and of an unusual configuration. Also, the evidence that some form of tissue reaction to the rodlet cells, or to the rodlets, appears to take place and that there is isolation of the cells from adjacent tissue, point to a parasitic origin. The protozoa are an enormously diverse group of very ancient origin about which much remains unknown. Rodlet cells too, would appear to be of relatively ancient origin. That the rodlet cell is a protozoan of unknown origin would seem the most likely conclusion from the present evidence. Clearly, however, the taxonomic category is unknown at this stage. Isolation of the rodlets and identification of the type of DNA they contain would appear to be a crucial experimental approach to undertake. The use of a chitinase enzyme to test if the 'capsule' wall contains chitin might also be a useful approach in determining whether the

rodlet cell is of exogenous origin. If rodlet cells represent a parasitic infection then the mode of transmission between hosts must be ascertained and the course of infection within individual hosts established. Although no severe pathology has been recorded, there is some evidence indicating disruption of the integrity of epithelial and endothelial tissue and this may cause a decrease in metabolic efficiency. This would be of particular significance since rodlet cells are so widespread amongst fish species and are known to occur in species of fish of great economic importance such as turbot, salmon and trout. If the rodlets alone are of parasitic origin it is possible that some form of parasitic relationship is in the process of evolution.

7.6 SUMMARY

1 The presence of rodlet cells was recorded throughout the mucosal epithelium of the alimentary tract of juvenile turbot from the oesophagus to the rectum. The basic morphology of the cells agreed with the description of 'mature' rodlet cells of other workers. They are large ovoid cells (5-10 μm x 10-20 μm) with a large spherical basal nucleus, large electron dense rod-like inclusions and a thickened capsule wall. The rodlet cells were intercellular.

2 A slight variation in shape was observed between rodlet cells in the stomach and those in the intestine. Rodlet cells in the stomach were more ovoid than those in the intestine which appeared longer and narrower.

3 Rodlet cells were not observed in every fish examined.

4 The distribution of rodlet cells throughout the alimentary tract varied between individuals but generally the greatest numbers were found in the stomach.

5 Rodlet cells near the surface epithelium were frequently observed to form communication junctions between the apical region of rodlet cells and neighbouring epithelial cells.

6 A distinct apical region with a stoma was observed in rodlet cells adjacent to the surface epithelium. Often the rodlets and other contents of the cell appeared to be 'secreted' into the lumen.

7 Occasionally in some rodlet cells part of the limiting membrane appeared to extend into and encompass part of the surrounding cytoplasm forming a cytoplasmic extension.

8 Intercellular gaps were often observed between rodlet cells and neighbouring epithelial cells especially in those nearest the lumen. A certain amount of disruption of the histological integrity of the mucosal epithelial tissue was evident.

9 The distribution of rodlet cells among fish species was examined phylogenetically. Rodlet cells have been described from many fish groups including the Clupeomorpha, Ostariophysi, Salmoniformes, Paracanthopterygii, Atherinomorpha and the Percomorpha showing the wide distribution among fish groups and demonstrating they are probably of ancient phylogenetical origin. Cells were found in the river lamprey, *Lampetra fluviatilis*, which resembled rodlet cells. If this observation along with Thélohan's tenuous report of rodlet cells in elasmobranchs is substantiated this would confirm an even more ancient phylogenetic origin for these cells.

10 Based on all the evidence available, it is suggested that rodlet cells are probably parasitic in nature. However further research is necessary to substantiate this hypothesis; the rodlets must be isolated from rodlet cells and any DNA present must be identified and Koch's postulates must also be proven.

CHAPTER 8

GENERAL DISCUSSION

This research investigation dealt with factors influencing feeding, digestion and absorption in juvenile turbot and explored the partitioning of energy from ingested food. Energy use under varying intrinsic and extrinsic conditions was examined, thus giving a deeper insight into the interrelationships of factors affecting the metabolic scope and hence growth in turbot. The application of science to fish farming was very much in its infancy when this study was undertaken, little was known of the basic biology of turbot and observations were also made on less obvious factors which may influence the energy partitioning in turbot, such as parasitic infection.

A multi-disciplinary approach was adopted. The research strategy involved a study of the gross morphology, histology and ultrastructure of the alimentary tract of turbot; experimental investigations into the effect of various environmental and intrinsic factors on the relationship between ingestion rate, growth rate and conversion efficiency; the rate of transit of food through the gut, assimilation efficiency; maintenance and feeding oxygen metabolism; body composition; condition factor and hepato-somatic index. A study of the host-parasite relationship between turbot and the tapeworm, *Bothriocephalus scorpii* was undertaken. Data were collated from the above and used to construct energy partitioning tables for turbot. A review of infectious diseases of turbot and an investigation into the nature of the rodlet cell were also carried out.

Research has shown that temperature, salinity, quantity and composition of food, feeding frequency, size of fish, structure of alimentary tract, health of fish and parasitic infection may influence food intake, digestion and absorption in fish and that these are interrelated (Kapoor *et al.*, 1975; Fänge and Grove, 1979; Talbot, 1985; Hoole, 1994). Braber and de Groot (1973b) showed there was an ontogenic shift in the relative lengths of different parts of the alimentary tract of turbot reflecting the changes in their feeding patterns throughout their development. It was thought that this plasticity in gut morphology due to changes in diet might also be reflected in differences in the length of the alimentary tract between wild turbot fed on a natural diet and cultured turbot fed on a highly nutritious, easily digested, protein rich diet. Results from the present study indicate that indeed there may be a shortening of the overall length of the alimentary tract in turbot fed on an artificial, high protein, easily digested diet in comparison with that of wild fish eating natural prey items. This was more pronounced in fish held at 20°C compared with those held at 10°C. The length of the alimentary tract in turbot is approximately equal to the body length of the fish and is typical for carnivorous fish. The histological study of the alimentary tract undertaken in the present study revealed the organization of the turbot alimentary tract to be very similar to that of other teleost fish and to carnivorous fish in particular (discussed in detail in Chapter 3). No differences were observed in the histological structure of turbot acclimated to different temperatures and feeding regimes, although more refined, quantitative

techniques are probably necessary before major conclusions can be drawn (McFadzen *et al.*, 1994). However, notably long microvilli were observed in the intestine and rectum as well as rodlet cells in the epithelial layer throughout the length of the alimentary tract, meriting further investigation using electron microscopal techniques.

The ultrastructure of the epithelial layer of the intestine of turbot was examined to see if there was any apparent link between the feeding habits and digestion of the fish and the structure of the epithelial brush border. Strikingly long microvilli of 2.0-5.6 μm were found in the intestine and rectum of juvenile turbot, compared with an average of 0.6-2.0 μm in other fish (Iwai, 1968a, b; Krementz and Chapman, 1975; Stroband and Debets, 1978; Kuperman and Kuz'mina, 1994). The length of the microvilli appeared to decrease from the anterior intestine to the rectum (Chapter 3). Kuperman and Kuz'mina (1994) recently examined the ultrastructure of the intestinal epithelium of three species of fish with different feeding habits and found substantial variations in the height of the microvilli in different regions of the gut. Since microvilli greatly increase the digestive and absorptive area of the intestine, it is likely that the total area for these purposes is greatly increased in turbot and this may possibly contribute to a highly efficient digestive system in young turbot which in turn may play an important role in the very fast growth rate of young turbot.

Comparisons were also made of the ultrastructure of the epithelial layer of the intestine between fed and fasted turbot. There appeared to be no difference between the number and height of the microvilli in these two groups although this may be because a period of two weeks fasting was not long enough for any identifiable changes to occur. Also, more refined techniques, such as quantified digital image analysis of size and number of microvilli, are probably necessary to examine this in greater detail (McFadzen *et al.*, 1994). However, there were distinct signs of cellular degeneration in the fasted fish and the appearance of large, supranuclear vacuoles which may possibly indicate the occurrence of autophagy. The use of endogenous energy frequently occurs in fish as a survival mechanism as many fish species are faced with fluctuating food supplies or periods of winter fasting.

Investigations into the digestive enzymes of turbot were carried out to elucidate the process of food digestion. The enzymes indicated were found to be consistent with those of carnivorous fish fed on a high protein diet, where pepsin is the main proteolytic enzyme in the stomach and trypsin and chymotrypsin are the main enzymes in the intestine. These findings were later confirmed by Munilla-Moran and Stark (1990). Cousin *et al.* (1987) commented on the intensity of alkaline phosphatase activity in the brush border of the intestine of turbot and linked this with the importance of alkaline phosphatase in the absorptive process. Research has shown that the regulation of enzyme production and activity is under neuro-hormonal control and

that distension of the stomach muscles probably acts as an important stimulus to the vagal-cholinergic nerves (De Silva and Anderson, 1995). The amount and composition of food consumed, frequency of feeding, rate of passage of food through the gut and temperature, all influence the rate of digestive secretions and are all interrelated.

The food intake of fish is controlled by appetite, metabolic debt and by foregut fullness (Colgan, 1973; Elliott, 1975a, b; Grove *et al.*, 1978; Grove and Crawford, 1980; Godin, 1981; De Silva and Anderson, 1995). The rate of transit of food through the gut depends on quantity of food, feeding frequency, method of feeding, fish size, temperature, gastric motility and capacity of the intestine. Since appetite is believed to return as the foregut empties, some workers suggest that in fish fed on a regular basis regulation of meal size follows so that digestion is complete before the next meal arrives (De Silva and Anderson, 1995).

In the present study the rate of transit of food through the alimentary tract of turbot was found to be faster in fish fed to satiation twice daily than in fish fed on a reduced ration daily or for those fed to satiation once every 4-5 d. An increase in the time since the last meal slowed the rate of gastric evacuation. There was only a slight increase in transit time in fish acclimated to 20 °C compared with those acclimated to 10°C, possibly due to compensatory changes occurring with acclimation to temperature. Alternatively, this may partly reflect the only slightly greater ingestion rate of the 20°C fish, possibly due to restriction in the size of the holding tanks. Force-feeding greatly increased the transit time of food through the gut compared with fish fed voluntarily and similarly fish fed on a high protein, easily digested pelleted diet had a faster digestion rate than that of fish fed on natural prey items (discussed in detail in Chapter 3.4). It seems possible that turbot may have a high degree of control over the process of digestion and evacuation. Work by Grove *et al.* (1985) and Bromley (1987) has shown that turbot may regulate the flow of chyme into the small intestine at an optimum rate to ensure completion of digestion and absorption of nutrients.

Ingested food is only useful to the fish if it is digested and absorbed (assimilated) and therefore assimilation efficiency and the factors affecting this are very important in determining how much of the ingested energy is available for maintenance and growth of the fish. Assimilation efficiency is found to be 40-80 % for herbivorous fish where a high proportion of indigestible material in the food means significant losses of energy and nutrients in the faeces, whilst values of 70-95 % are common for carnivorous fish where a large amount of protein is found in the food (Brett and Groves, 1979; Cui and Liu, 1990). The assimilation efficiency, in terms of energy and protein content, was found to be very high (96 %) for juvenile turbot aged 3 months. When expressed in terms of energy content, the assimilation efficiency decreased with increase in age (to 82 % for 6-9 month old turbot), and with increasing temperature (to 75 %),

and also with less frequent feeding (to 73 %). When expressed in terms of protein content, the assimilation efficiency remained high (94 %) with increase in age and decreased only slightly with increasing temperature and decrease in frequency of feeding (89.0-90.0 %). This may be due to the very high protein content of the diet. Incremental replacement of the protein content of the diet with starch produced no significant difference in either the protein or energy assimilation efficiency in 3 month old turbot. The protein and energy assimilation efficiencies of juvenile turbot held at 10°C were generally found to be very high and were similar to data subsequently obtained by Grove *et al.* (1985) for turbot held at 16°C.

It is clear that a number of intrinsic and extrinsic factors may influence the ingestion rate and processes of digestion and absorption in turbot. That these factors may be manipulated to achieve optimum food intake to give highest assimilation efficiencies is of great importance so that the maximum energy and nutrients are available for maintenance and growth. However, it should be noted that these conditions are most likely to be species-specific, they may show ontogenic shifts within species, and the results may be highly dependent on the length of time the fish are subjected to the conditions. For instance, the fish may reduce their activity in response to a decrease in rations in order to preserve their food supplies. Ultimately the fish may adapt metabolically. Also, the conditions for highest food intake and for highest assimilation efficiency may not produce maximum growth rates as the metabolic costs to other physiological processes may be too high.

One method of investigating the relationship between food intake and growth is to produce growth-ration curves for a variety of experimentally controlled variables. In the present study, growth-ration curves were constructed from data obtained in feeding experiments to give some insight into the relationship between mean specific growth rate and ingestion rate and between ingestion rate, mean specific growth rate, conversion efficiency and temperature for juvenile turbot. The results are discussed in detail in Chapter 4 but generally it was found that although fish held at 20°C had a higher ingestion rate than those acclimated to 10°C, on the same feeding regime, the 10°C acclimated fish had a higher conversion efficiency whether expressed in terms of grams of food eaten or as energy intake. Jones *et al.* (1981) found the temperature for maximum growth for young turbot was 18.9°C but the temperature for maximum food conversion efficiency was 16.2°C. The growth rate of young turbot (< 100 g) has since been shown to be greatest between 16-20°C (Brown *et al.*, 1984; Scherrer, 1984; Imsland *et al.*, 1996). Fish in general tend to have a temperature range which is optimal for growth and survival (Brett and Groves, 1979; Weatherley and Gill, 1987; Gadomski and Caddell, 1991; Imsland *et al.*, 1996). This often changes with age and size of fish (*i.e.* there is an ontogenic shift) as juveniles of a great many species prefer warmer waters than adults (McAuley and

Huggins, 1979; Pedersen and Jobling, 1989; Fonds *et al.*, 1992; Hallaråker *et al.*, 1995; Björnsson and Tryggvadóttir, 1996; Imsland *et al.*, 1996). This is of great significance to both the natural distribution of different life stages and to the optimal rearing of the species under culture conditions. Imsland *et al.* (1996) observed that the temperature optimum producing the maximum growth rate in turbot decreased rapidly with increasing size, and for 100 g turbot was 13-16°C. The optimum environmental requirements for maximum growth in young turbot are still poorly understood due to the variation in techniques used and the conditions fish were held under, thus making comparisons of data difficult (Person-Le-Ruyet, 1990).

The energetic cost of maintenance, feeding and activity metabolism are also dependent on various intrinsic and extrinsic factors and a knowledge of how the assimilated energy is partitioned between these three components of metabolism and growth, under different conditions, is therefore essential. It is important to have empirical knowledge of the effects of, for instance, salinity, temperature, stocking density, water quality, variations in diet and feeding regimes and body size on the cost of metabolism in order that predictions may be made on the amount of assimilated energy available for growth.

The relationship between body size and standard metabolism was investigated in the laboratory for juvenile turbot acclimated to 10°C and 20°C. These results are discussed and compared with those obtained for routine metabolism of juvenile turbot by Brown *et al.* (1984) under field conditions and Waller (1992) under laboratory conditions (Chapter 4). The effects of short and long-term acclimation to temperature and how the fish adapt to these changes were also examined and the results are discussed in Chapter 4. Generally, the exponent for the relationship between body weight and standard metabolism at 10°C was slightly higher at 0.77 than the 0.75 obtained for routine metabolism of turbot by Brown *et al.*, (1984) for fish acclimated to 9-10°C and the 0.699 obtained by Waller (1992) (exact temperature not given). This finding emphasizes the fact that caution should be taken about the transference of physiological data from laboratory to field set-up and how important it is to communicate the exact conditions under which the experiments are performed. The length of time the fish have been held under the experimental conditions is very important as partial or complete compensation in standard metabolic rate may occur to accommodate for changes in temperature. The rate and amount of compensation may depend on whether there is an increase or decrease in temperature, whether the temperature is in the mid-range or near the upper or lower limits of tolerance of the fish, on the nutritional and thermal history of the fish, on salinity and oxygen levels and also on hormonal condition. In the present study partial adaptation to an increase of 10°C in juvenile turbot fed twice daily to satiation occurred after 3 weeks with further acclimation to temperature taking place after 10 weeks; complete compensation was not observed. Rapid temperature changes gave a

corresponding rise or fall in respiration rate and therefore, where possible, temperature changes should be gradual to avoid respiratory stress to the fish.

The thermic affect of feeding (SDA) was also examined and revealed a definite rise in respiration rate from 1-3 h after feeding and lasting for a period of approximately 24 h. The size and length of time of this increase was positively correlated with the size of the meal administered and is in agreement with similar work on other species of fish (Brett and Groves, 1979; Jobling and Davies, 1980; Jobling, 1981b, 1983a; Knights, 1985; Cho and Bureau, 1995). However, it should be noted that thermic effect of feeding will also be influenced by the nutritional quality of the food, the energy value of the food, the method of feeding (force feeding causes an increase in transit of food through the gut) and temperature of acclimation.

A bioenergetics model was constructed for turbot held under different conditions using data obtained for the energy value of the food consumed, ingestion rates and assimilation efficiencies (Chapter 3); the energy used for respiratory metabolism (Chapter 4); data obtained from growth experiments (Chapters, 3, 4); and information on the energy consumption of the tapeworm, *Bothriocephalus scorpii* (Chapter 6).

Energy partitioning data revealed fluctuations in the assimilation efficiency between the experimental groups. Generally, less of the consumed energy was lost in the faeces of fish fed regularly and held at 10°C, whether fed to satiation or on a reduced ration, than in those fish fed once every few days. Assimilation efficiency also decreased with an increase in temperature.

Further variation was observed in the amount of assimilated energy apportioned between standard metabolism, feeding metabolism, parasitism and growth depending on the experimental conditions. According to Priede (1985) there is a continual conflict between SDA, active metabolism and growth. If a fish is swimming at maximum speed it can't cope with digestion but this is also dependent on other influences including temperature, stress, salinity, and parasite status. In the present study fish fed on a reduced ration used a greater proportion of assimilated energy for standard metabolism (maintenance) than fish fed to satiation. Fish held at 20°C partially acclimatized to the rise in temperature, and used only a slightly greater amount of the assimilated energy for standard metabolism than the 10°C fish. Fish fed on a reduced ration used even less energy on feeding metabolism. The amount of assimilated energy likely to be consumed by tapeworms of infected fish varied with the size of fish. Smaller fish had a higher parasite index and in this case the worms would be likely to use a higher proportion of the assimilated energy than in larger fish. This effect was assumed to be exacerbated by reduced rations and an increase in temperature as tapeworms derive all their energy and nutrient requirements for growth, development and the maintenance of physiological functions from the host's intestine (Ash *et al.*, 1984). Data from growth experiments revealed a similar proportion of

the assimilated energy was used for growth under all conditions apart from the 10°C fish fed to satiation where only slightly more energy was used than in the other fish groups. A large proportion of the assimilated energy was used for active metabolism although this appeared to be very labile between the experimental groups. This supports the observation by Boisclair and Sirois (1993) that activity rates can be a large and variable component of a fish's energy budget.

The proportion of energy used for growth (anabolism) in turbot was quite small in comparison with studies on rainbow trout, *Salmo gairdneri* (Cho and Bureau, 1995), on white sturgeon, *Acipenser transmontanus* (Cui *et al.*, 1996), and on the southern catfish, *Silurus meridionalis* (Cui and Wooten, 1988a,b,c,d; Xie and Sun, 1990, 1992a,b, 1993; Cui *et al.*, 1996). A number of factors may have influenced this result. For instance, fish held at 20°C experienced a growth check at the start of the experiment, probably whilst adjusting to the large rise in temperature. This undoubtedly caused stress to the fish and resulted in energy being wasted. The small size of the tanks used may have influenced the results, although fish fed to satiation at both 10 and 20°C did have a higher mean specific growth rate than those on a reduced ration, however, they did use a similar proportion of the assimilated energy for growth. It is also possible that the fish were poor growers having been selected from a small size scale at Ardtoe (Pedersen and Jobling, 1989). Also, flatfish are more efficient when swimming at speeds for long periods as they have no swimbladder and have negative buoyancy. It is energetically costly for flatfish to actively lift off from the bottom and therefore swimming for short periods is inefficient (Priede, 1985). This may have adverse implications in fish farms where space may be limited and may have even more extreme effects for fish held in small, laboratory tanks.

Errors may occur in energy budgets when some of the parameters are unknown and estimates are made, especially from unknown activity data and from unjustified borrowing of physiological values from other species (Ney, 1993), or from the same species, but under different conditions. Different growth rates at different stages in the life history (ontogenic shift) can also result in errors when extrapolation of weight-dependent power functions are made (Imsland *et al.*, 1996). Variation in experimental techniques used by researchers may make comparisons of physiological data difficult. For instance, differences exist between the measurement of routine metabolism under farmed conditions and the measurement of the respiration rate in the laboratory of isolated fish in a small respiratory chamber which undoubtedly induces stress and may also induce a growth check. Weighing the fish during the course of growth experiments can also cause stress and induce growth checks (Brafield, 1985).

Despite these inherent problems, the development of energy budgets over the past 15 years has grown from single-species studies of growth and food consumption to studies of food-web dynamics, nutrient cycling within food-webs, food requirements of single animals, whole

populations and communities of fish, predator-prey interactions at various spatial and temporal scales to assessment of production potential at eco-system level (Brandt and Hartman,1993; Hansen *et al.*, 1993). Much of the advance has come from the integration of bioenergetics approaches with new technologies (hydroacoustics, physiological telemetry) and ecological theory (*e.g.* energetics approach to diel vertical migration of fishes where combined effects of several selective forces such as predator avoidance, foraging efficiency and bioenergetic efficiency are at play (Bevelhimer and Adams, 1993)). However, improved estimates are still required for model parameters such as the metabolic costs of activity, and more complete studies of the bioenergetics of different ontogenic stages of fish species, especially larval and juvenile stages. According to Hansen *et al.*, (1993) future research on bioenergetics should include both laboratory and field measurements of important model parameters such as weight-dependent optimum consumption, respiration and activity and the thermal habitats actually occupied by the fish, thus improving predictions of fish growth. Recent work by Imsland *et al.*, (1996) clearly shows the need for such information where the optimal temperature for juvenile turbot decreased substantially for fish of 100 g compared with that for fish between 25-75 g, reflecting their natural spatial distribution differences. Data by Gaumet *et al.*, (1995) also supports this where full strength sea-water was found not to be the optimum salinity for growth in juvenile turbot over periods > 3 months.

Bioenergetics models can predict the amount of energy available for growth but it is also necessary to know what type of growth has occurred. Turbot are a luxury fish and any changes in the relative proportions in chemical composition of the fish tissue may result in spoilage of the product. Accordingly, chemical analyses were carried out on the body composition of juvenile turbot held under different experimental conditions. The largest variations were found in the percentage of lipid in both the carcass and in the liver. Carcass lipid levels (% dry weight) were highest in fish fed to satiation at both 10°C and 20°C whilst liver lipid levels (% dry weight) were far greater in the 20°C acclimated fish. The percentage carcass protein was fairly stable in all experimental groups with a slightly higher level occurring in the 20°C fish. Healthy fish tend to have a relatively stable percentage of carcass protein for a given species with little seasonal variation except during periods of starvation and gonad development where non-fatty fish may draw on their carcass protein (Love, 1970; Chellappa *et al.*, 1995). The percentage of carcass and liver lipid may, however, vary considerably in fish (Love, 1970). In the Atlantic cod, *Clupea harengus*, a non-fatty fish where the muscle lipid concentration is 0.5 %, the liver is used to store large quantities of lipid of up to 65 % liver weight (Jangaard *et al.*, 1967). The liver lipid was very high in turbot held at 20°C at 52.9 % dry weight suggesting that the liver may be a major store of lipid.

Hepato-somatic index (HSI) is a measure of the relative weight of the liver and is often used as an indication of the energy stores in fish, especially in non-fatty fish (Chellappa *et al.*, 1995). In turbot the HSI was found to be lowest in fish acclimated to 20°C. However, as this group of fish were also found to have the greatest level of liver lipids and the highest carcass energy levels, this suggests that HSI may not be a suitable index of energy stores in turbot, although this may be a reflection of the small sample size and could be worth further investigation.

In fish farming it is often useful to employ rapid, non-invasive methods of indicating growth-rates. Commonly, the condition factor, a traditional method of measuring the body condition or, 'fatness' or well-being of a fish, is employed. This is calculated as a ratio between weight and length of a fish and is often expressed in the form W/L^3 (Chapter 4). In turbot the condition factor increased over the experimental period in fish held at 10°C and fed to satiation from 1.6 to 1.8, whilst in the other experimental groups the condition factor remained relatively stable. Again, since the 20°C acclimated fish had the highest liver lipid and the highest carcass energy levels it would suggest that condition factor may not be an ideal index of energy reserves. However, large samples with a large size range are important for accuracy of condition factor (Chellappa *et al.*, 1995) as CF is known to vary considerably at any given fish length (Weatherley and Gill, 1987). This partly arises due to variations occurring in the growth rates of different parts of the body of an organism and highlights the necessity to measure growth in more detail such as the relative growth of tissues in terms of lipid, protein and energy content. It should also be remembered that the measurement of weight and length at different time intervals are necessary to obtain data on the condition factor. Since large numbers are required for this and it is known that handling induces stress resulting in growth checks, it may be more accurate to take a sample of fish and run more detailed morphometric measurements and chemical analyses.

Most studies of the energetics of fish either fail to note diseases of fish, or they may give them a very cursory mention. Instead, this research area tends to be dealt with in isolation. In the present study, the effect of disease on the morbidity and mortality of turbot was very apparent from the outset. The prevalence of the tapeworm, *Bothriocephalus scorpii* was quite high at 40-60 %. Mortalities occurred in the early stages of research due to the bacterium, *Vibrio anguillarium*. Light and electron microscopal studies revealed the presence of the rodlet cell, reported as possibly being a protozoan parasite, *Rhabdospora thelohani* (Thélohan, 1892a, b; Laguessé, 1895; Dawe *et al.*, 1964; Hale, 1965; Bannister, 1965, 1966; Iwai, 1968c; Anderson *et al.*, 1976; Mayberry *et al.*, 1979; Vichberger and Bielek, 1982; Barber and Westermann, 1983; Bielek and Viehberger, 1983; Richards *et al.*, 1994; Wayne and Mayberry, 1994), in the

alimentary tract of turbot. That parasites and disease adversely affect the host's nutrition and may therefore ultimately affect their growth seemed very probable and worthy of investigation in a study on the energetics of turbot.

A review of infectious diseases of turbot was therefore carried out and the importance of disease in intensive fish culture was discussed in Chapter 5. Generally, an increase in the intensive culture of turbot in the last 15 years has led to a concomitant rise in the pathological problems of turbot. Whilst most research has been carried out on bacterial and viral infections, the pathogenicity of some parasitic protozoa and their threat to turbot stocks has become evident and has led to recent research in this area. For instance, infection of turbot with the microsporidian *Tetramicra brevifilum* resulted in a reduction in growth rate of 50 % and mortalities of 11.5 % (Figueras *et al.*, 1992). It would seem prudent to incorporate a more vigilant approach to the possible effects of infectious diseases in studies of the energetics of fish.

Intestinal helminths of fish, including cestodes, have generally been considered to be a less serious problem than other diseases and consequently less research has been carried out on this topic. There remains something of a controversy over the pathogenicity of tapeworms. However, intestinal parasites are known to adversely affect their hosts in a number of ways. Firstly, the parasite receives its energy and nutrition from the host's assimilated energy. This may have little effect in a well nourished and healthy host with unlimited amounts of food but if infection is combined with other environmental stressors (physical, chemical, dietary or other disease) which have a limiting effect on the host's food intake then the proportion of the hosts food intake used by the parasite will increase (Ash *et al.*, 1984). There have been numerous reports of fish infected with fresh water bothriocephalid worms (*B. acheilognathi*) becoming sluggish and emaciated, ceasing to feed and swimming closer to the surface (Hoole, 1994). The presence of the parasite in the intestinal tract may alter the gastric evacuation rate by impeding the passage of food through the gut and therefore may decrease the host's food intake by impeding digestion and absorption of nutrients by the intestinal lining (Ash *et al.*, 1984). This would have the effect of increasing the proportion of the host's food intake consumed by the worm as the worm's nutrient and energy intake remain the same. Current evidence suggests that species of fresh water bothriocephalids affect host intestinal enzymes including trypsin, chymotrypsin, phosphatases and amylase (Matskási, 1984; Kurovskaya and Kititsyna, 1986) and therefore probably effect host nutrition. Parasitism of sockeye salmon, *Oncorhynchus nerka* by *Eubothrium salvelini* had a deleterious effect on the growth, survival and swimming performance of the fish (Boyce, 1979). A 10 % loss of growth was recorded in farmed Atlantic salmon with low level infections of *Eubothrium* species and this was more pronounced in males than in female fish Bristow and Berland (1991). They estimated a direct loss of millions of dollars per

year in Norway due to the infection as well as noting the loss of food and the possible increased susceptibility to disease in infected fish. Generally, the greater the degree of pathogenicity invoked by a parasite the greater the decrease in food intake of the host. This could have a profound effect when the fish is suffering from a second infection caused by a more highly pathogenic organism such as *Vibrio anguillarium*.

Investigations were therefore carried out into the host-parasite relationship between turbot and the tapeworm, *Bothriocephalus scorpii* and these results are discussed in detail in Chapter 6. Data were gathered on the prevalence and intensity of infection of turbot of different sizes and used to estimate the amount of energy used by the tapeworm in fish held under different conditions. Local tissue pathology was also investigated. It was estimated that the tapeworm used between of 1.5-8.0 % of the turbot's assimilated energy depending on various intrinsic and environmental factors (Chapters 4, 6). With this kind of information, estimates of energetic losses due to intestinal helminth infection can be made for fish at different ontogenic stages and informed decisions on when and whether to treat the infection with anthelminthics can be made.

Light and electron microscopal investigations were carried out into the nature of the rodlet cell in turbot. These findings and their implications are discussed in detail and in the context of other research in Chapter 7. It was considered that if this were a protozoan parasite as suggested (Thélohan,1892a, b; Laguessé, 1895; Dawe *et al.*, 1964; Hale, 1965; Bannister, 1965, 1966; Iwai, 1968c; Anderson *et al.*, 1976; Mayberry *et al.*, 1979; Viehberger and Bielek, 1982; Barber and Westermann, 1983; Bielek and Viehberger, 1983; Richards *et al.*, 1994; Wayne and Mayberry, 1994), then this might have important implications on the health and energetics of turbot.

When the evidence and views accumulated over the last century on rodlet cells are examined together with results from the present study it seems possible, but not conclusive, that rodlet cells, or at the least the rodlet inclusions, are of parasitic (or exogenous) origin. The most convincing evidence for a parasitic origin lies in the fact that the rodlets appear to contain DNA different from the host DNA and of an unusual configuration (Viehberger and Bielek, 1982; Bielek and Viehberger, 1983; Barber and Westerman, 1986b). That some form of tissue reaction to the rodlet cells, or to the rodlets, appears to take place and that there is isolation of the cells from adjacent tissue, also points to a parasitic origin (Anderson *et al.*, 1976; Morrison and Odense, 1978; Bielek and Viehberger, 1983; Mayberry *et al.*, 1986; Richards *et al.*, 1994; present study).

Protozoa are an enormously diverse group of very ancient origin about which much remains unknown. The distribution of rodlet cells among fish species was examined phylogenetically in the present study. Rodlet cells appear to be of relatively ancient origin

having been described from many fish groups including the Clupeomorpha, Ostariophysi, Salmoniformes, Paracanthopterygii, Atherinomorpha and the Percomorpha. If observations of rodlet cells from elasmobranchs and cyclostomes are substantiated (Thélohan, 1892a, b; Bannister, 1966; present study) then an even more ancient phylogenetic origin will be realised. That the rodlet cell is a protozoan of unknown origin would seem the most likely conclusion from the present evidence. Clearly, however, the taxonomic category is unknown at this stage. Isolation of the rodlets and identification of the type of DNA they contain would appear to be a crucial experimental approach to undertake. If rodlet cells represent a parasitic infection then the mode of transmission between hosts must be ascertained and the course of infection within individual hosts established *i.e.* Koch's postulates must be realised.

Although no severe pathology has been recorded, there is some evidence indicating disruption of the integrity of epithelial and endothelial tissue and this may cause a decrease in metabolic efficiency. This would be of particular significance since rodlet cells are so widespread amongst fish species and are known to occur in species of fish of great economic importance such as turbot, salmon and trout. If the rodlets alone are of parasitic origin it is possible that some form of parasitic/symbiotic relationship is in the process of evolution.

An understanding of the life history pattern and ecology of parasitic infections of turbot is necessary if effective control measures are to be employed in fish farming. Quantitative information on the energy cost of infectious diseases is clearly lacking and would also be of great benefit for use in bioenergetics models.

Elaborations of the mass-balance energy equation (energy budget) that partition the energy of consumed food into its various physiological fates have flourished over the last 15 years into sophisticated bioenergetics models (Ney, 1993). They have grown from simple predictions of the growth of fish as a function of quality and quantity of food and temperature to more powerful simulation tools applied to a variety of research and management questions relating to fish stocks, populations, food-webs and ecosystems (Hansen, *et al.*, 1993). Applications include, estimates of the intensity and dynamics of predator-prey interaction, nutrient cycling in food webs at different trophic structure, food requirements of single fish, populations of fish and of communities groups of fish species. Despite criticism by some that bioenergetics models lack sufficient detail to produce reliable results in field applications, many studies have achieved notable predictive success. For instance, bioenergetics models were used to predict the effect of increased lake trout *Salvelinus namaycush* predation of rainbow smelt *Osmerus mordax*, following chemical control of the sea lamprey, *Pteromyzon marinus* (Labar, 1993). Predictive models were also used to assess the environmental impact of a salmon farm off

the west coast of Ireland and to ensure any environmental impacts were minimized and kept within acceptable limits (Hartnett, 1993).

It is essential to know how aquaculture interacts with the environment. Maintenance of the water column avoiding deoxygenation is necessary. It is important to alleviate the effect of sediments on the benthos avoiding hypernutrification, eutrophication and sediment accumulation. Prevention of the detrimental effects of chemicals and drugs used in aquaculture thus protecting natural fish populations as well as bird and mammal populations is very important. The alteration of the natural environment due to food, chemical pollution, the introduction of new diseases and parasites from farmed fish to wild populations and *vice-versa* and the displacement of native stocks by escaped fish are all serious potential consequences of the aquaculture industry. The conflict and interrelationships between maximum growth in fish, pollution of the environment and disease in fish is clear. Mawdesley-Thomas (1972) stated that " .... disease *per se*, is not an entity in itself. Disease is the end result of an interaction between a noxious stimulus and a biological system and to understand disease is to understand all aspects of that system" (Snieszko, 1974). Bioenegetics models can be used to monitor the effects of aquaculture such as the flow of nitrogenous waste, the effects of stress, the increase in risk of infection, the energetic cost of infection, and ensure that whilst the industry grows and develops the impacts on the environment are minimized.

In conclusion, the present study has shown how factors affecting growth in fish are complicated and interrelated and the more factors examined the more accurate the bioenergetics model. Undoubtedly improvements can be made on the accuracy of some parameters of the model, such as improved estimates of the metabolic cost of activity. Further examination of the host-parasite relationship between turbot and the tapeworm, *Bothriocephalus scorpii*, including more detailed studies on the energetic cost of stress and disease would be advantageous. Detailed epidemiological studies of the infectious diseases of wild and captive turbot, including methods of control and the implications for public health are essential. The determination of the status of the rodlet cell remains as elusive and as intriguing as it appeared over one century ago and the isolation and accurate identification of the DNA remains a challenge for future research. Despite the time gap, these laboratory studies give a deeper insight into the interrelationships of factors affecting metabolic scope and hence growth in turbot. Although a few of these findings have since been repeated, others add to the knowledge of this area and are still relevant today.

REFERENCES

Abbott, J. C. and Dill, M. (1989). The relative growth of dominant and subordinate juvenile steelhead trout (*Salmo gairdneri*) fed equal rations. *Behaviour*, **108,** 1104-113.

Aikawa, M. and Kilejian, A. (1979). Invasion procedures and intracellular localization in parasitic protozoa. In: *Lysosomes in Applied Biology and Therapeutics* edited by J. T. Dingle., P. J. Jaques and I. H. Shaw. North Holland Publishing Company: Amsterdam, New York, Oxford, pp. 31-48.

Al-Hussaini, A. H. (1949a). On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: anatomy and histology. *Quarterly Journal of Microscopical Science*, **90,** 109-139.

Al-Hussaini, A. H. (1949b). On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: cytology and physiology. *Quarterly Journal of Microscopical Science*, **90,** 323-354.

Alderdice, D. F. (1988). Osmotic and ionic regulation in teleost eggs and larvae. In: *Fish Physiology Vol. XIA, Eggs and larvae* edited by W. S. Hoar and D. J. Randall. New York: Academic Press, pp. 163-252.

Alderson, R. (1979). The effects of ammonia on the growth of juvenile Dover sole, *Solea solea* (L) and turbot, *Scophthalmus maximus* (L). *Aquaculture*, **17,** 291-309.

Allen, J. R. M. and Wooten, R. J. (1982). The effect of ration and temperature on the growth of the three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, **20,** 409-422.

Amigo, J. M., Garcia, M. P., Rius, M., Salvado, H., Maillo, P. A., Vivares, C. P. (1996). Longevity and effects of temperature on the viability and polar-tube extrusion of spores of *Glugea stephani*, a microsporidian parasite of commercial flatfish. *Parasitology Research*, **82,** 211-214.

Anderson, C. D., Roberts, R. J., MacKenzie, K. and McVicar, A. H. (1976). The hepato-renal syndrome in cultured turbot (*Scophthalmus maximus* L.). *Journal of Fish Biology*, **8,** 331-334.

Anderson, R. M. (1978). The regulation of host population growth by parasitic species. *Parasitology*, **76,** 119-157.

Anderson, R. M. (1979). The influence of parasitic infection on the dynamics of host population growth. In: *Population Dynamics* edited by R.M Anderson, B. M. Turner and L. R. Taylor. Blackwell: Oxford, England, pp.245-281.

Anderson, R. M. (1993). Epidemiology. In: *Modern Parasitology: a Textbook of Parasitology* edited by F. E. G. Cox. Blackwell Scientific Publishers: London, Edinburgh, Boston, pp. 75-116.

Anderson, R. M. and Gordon, D. M. (1982). Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities. *Parasitology,* **85,** 373-378.

Anderson, T. A. (1986). Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata* (Pisces, Kuphosidae), in relation diet. *Journal of Morphology,* **190,** 109-119.

Angelescu, V. and Gneri, F. S. (1949). Adaptaciones del aparto diestivo al regimen alimenticio en algunos peseos del Rio Uruguay y del Rio de la Plata. *Revista del Instituto Nacional de Investigacion de la Ciencias Natureles anexo al Museo Argentino de Ciencias Natura les "Bernardo Rivadavia", Buenos Aires, Ciencias Zoológicas,* **1,** 161-272.

Angulo, L., Lopez, J. E., Vilente, J. A. and Saborido, A. M. (1994). Hemorrhagic areas in the mouth of farmed turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases,* **17,** 163-169.

Arme, C. and Owen, R. W. (1967). Infections of the three-spined stickleback, *Gasterosteus aculeatus* L., with the plerocercoid larva of *Schistocephalus solidus* (Müller, 1776), with special reference to pathological effects. *Parasitology,* **57,** 301-314.

Arme, C. and Walkey, M. (1970). The physiology of fish parasites. In: *VIII Symposium of the British Society for Parasitology* edited by A. E. R. Taylor. Blackwell Scientific Publications: Oxford. pp. 79-101.

Arme, C., Bridges, J. F. and Hoole, D. (1983). Pathology of cestode infections in the vertebrate host. In: *Biology of the Eucestoda,* Volume 2 edited by C. Arme and P. W. Pappas. pp. 499-538.

Ash, C., Crompton, D. W. T. and Keymer, A. (1984). Nature's unfair food tax. *New Scientist,* **101,** 17-20.

Austin, B., Stobie, M., Robertson, P. A. W., Glass, H. G., Stack, J. R and Mudarris, M. (1993). *Vibrio alginolyticus* - the cause of gill disease leading to progressive low level mortalities among juvenile turbot, *Scophthalmus maximus* L in a Scottish aquarium. *Journal of Fish Diseases,* **16,** 277-280.

Balabanova, L. V. and Matey, V. E. (1987). The ultrastructure of rodlet cells from different organs in the carp and brook trout. *Tsitologiya,* **29,** 776-770.

Band, R. N. and Mohrlok, S. (1969). The respiratory metabolism of *Acanthamoeba rhysodes* during encystation. *Journal of General Microbiology,* **59,** 351-358.

Bannister, L. H. (1965). The structure of *Rhabdospora thelohani,* a parasitic protozoan of uncertain affinities. *Intern. Congr. Ser.* **91,** 130. Excerpta Medica: Amsterdam.

Bannister, L. H. (1966). Is *Rhabdospora thelohani* (Laguesse) a sporozoan parasite or a tissue cell of lower vertebrates? *Parasitology,* **56,** 633-638.

Barber, D. L. and Westermann, J. E. M. (1975). 'Rodlet cells' in *Catastomus commersoni* (Teleostei: Pisces): Secretory cell or parasite? *Experientia,* **31,** 924-925.

Barber, D. L. and Westermann, J. E. M. (1983). Comparison of nuclear DNA content of rodlet cells and erythrocytes in some freshwater teleosts. *Journal of Fish Biology,* **22,** 477-484.

Barber, D. L. and Westermann, J. E. M. (1985). Reappraisal of nuclear DNA content compared to several cell-types from fresh-water teleosts, using 2 methods of microdensitrometry. *Journal of Fish Biology,* **27,** 817-826.

Barber, D. L. and Westermann, J. E. M. (1986a). Comparison of the DNA of nuclei of rodlet cells and other cells in the chub *Semotilus atromaculatus*: hybridization in situ. *Canadian Journal of Zoology,* **64,** 801-804.

Barber, D. L. and Westermann, J. E. M., (1986b). The rodlet cell of *Semotilus atromaculatus* and *Catastomus commersoni* (Teleostei): studies on its identity using histochemistry and DNase 1 - gold, RNase 1 - gold, and S1 nuclease - gold labelling techniques. *Canadian Journal of Zoology,* **64,** 805-813.

Barber, D. L., Westermann, J. E. M. and Jensen, D. N. (1979). New observations on the rodlet cell (*Rhabdospora thelohani*) in the white sucker *Catostomus commersoni* (Lacapede): LM and EM studies. *Journal of Fish Biology,* **14,** 277-284.

Barrington, E. J. W. (1957). The alimentary canal and digestion. In: *The Physiology of Fishes* edited by M. E. Brown, Volume 1. Metabolism. Academic Press: New York, pp. 109-161.

Beamish, F. W. H. (1974). Apparent specific dynamic action of largemouth bass, *Micropterus salmoides. Journal of the Fisheries Research Board of Canada,* **31,** 1763-1769.

Beamish, F. W. H., Sitjabobadilla, A., Jebbink, J. A. and Woo, P. T. K. (1996). Bioenergetic cost of cryptobiosis in fish, rainbow trout *Oncorhyhncus mykiss* infected with *Cryptobia salmositica* and with an attenuated live vaccine. *Diseases of Aquatic Organisms,* **1-2,** 1-8.

Beamish, F. W. H., Niimi, A. J. and Lett, P. F. K. P. (1975). Bioenergetics of teleost fishes: Environmental influences. In: *Comparative Physiology - Functional Aspects of Structural Materials* edited by L. Bolis, H. P. Maddrell and K. Schmidt-Nielsen. North Holland Publishing Company: Amsterdam, pp. 187-209.

Bevelhimer, M. S. and Adams, S. M. (1993). A bioenergetics analysis of diel vertical migration by Kotanee salmon, *Oncorhynchus nerka. Canadian Journal of Fisheries and Aquatic Sciences,* **50,** 2236-2349.

Bielek, E and Vielhberger, G. (1983). New aspects on the 'rodlet cell' in teleosts. *Journal of Sumicroscopal Cytology,* **15,** 681-694.

Bishop, C. and Odense, P. H. (1966). Morphology of the digestive tract of the cod *Gadus morhua. Journal of the Fisheries Research Board of Canada,* **23,** 1607-1615.

Björnsson, B. and Tryggvadóttir, S. V. (1996). Effect of size on optimal temperature for growth efficiency of immature Atlantic halibut (*Hippoglossus hipoglossus* L.). *Aquaculture,* **142,** 33-42.

Bloch, B. and Larsen, J. L. (1993). An iridovirus-like agent associated with systemic infection in cultured turbot *Scophthalmus maximus* fry in Denmark. *Diseases of Aqatic Organisms,* **15,** 235-240.

Bloch, B., Cravningen, K. and Larsen, J. L. (1991). Encephalomyelitus among turbot associated with a picornavirus-like agent. *Diseases of Aquatic organisms*, **10,** 65-70.

Boggs, C. H. (1991). Bioenergetics and growth of nothern anchovy *Engraulis mordax. Fishery Bulletin*, **89,** 555-566.

Boisclair, D. and Sirois, P. (1993). Testing assumptions of fish bioenergetics models by direct estimation of growth, consumption, and activity rates. *Transactions of the American Fisheries Society,* **122,** 784-796.

Bower, C. E. and Bidwell, J.P. (1978). Ionization of ammonia in seawater: effects of temperature, pH and salinity. *Journal of the Fisheries Research Board Canada,* **35,** 1012-1016.

Bowers, B. and Korn, D. E. (1967). Fine structural changes during encystation of amoeba. *Journal of Cell Biology,* **35,** 15A.

Bowers, B. and Korn, D. E. (1968). The fine structure of *Acanthamoeba castellani.* I. The trophozoite. *Journal of Cell Biology,* **39,** 95-111.

Bowers, B. and Korn, D. E. (1969). The fine structure of *Acanthamoeba castellani.* II. Encystment. *Journal of Cell Biology,* **41,** 786-805.

Boyce, N. P. (1979). Effects of *Eubothrium salvelini* (Cestoda: Pseudophyllidea) on the growth and vitality of sockeye salmon, *Oncorhynchus nerka. Canadian Journal of Zoology,* **57,** 597-602.

Braber, L. and de Groot, S. J. (1973a). The food of five flatfish species (pleuronectiformes) in the southern North Sea. *Netherlands Journal of Sea Research,* **6,** 163-172.

Braber, L. and de Groot, S. J. (1973b). On the morphology of the alimentary tract of flatfishes (Pleuronectiformes). *Journal of Fish Biology,* **5,** 147-153.

Brafield, A. E. (1985). Laboratory studies of energy budgets. In: *Fish Energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London and Sydney, pp. 257-281.

Brandt, S. B. and Hartman, K. J. (1993). Innovative approaches with bioenergetics models - future applications to fish ecology and management. *Transactions of the American Fisheries Society,* **122,** 731-735.

Bråten, T. and Hopkins, C. A. (1969). The migration of *Hymenolepis diminuta* in the rat's intestine during normal development and following surgical transplantation. *Parasitology,* **59,** 891-905.

Brett, J. R. (1958). Implications and assessments of environmental stress in the investigation of fish power problems. *H.R. MacMillan Lectures on Fisheries.* University of Colombia. (cited in Roberts, 1989).

Brett, J. R. (1962). Some considerations in the study of respiratory metabolism in fish, particularly salmon. *Journal of the Fisheries Research Board of Canada,* **19,** 1025-1030.

Brett, J. R. (1965). The relation of size to rate of oxygen consumption and swimming speed of sockeye salmon, *Oncorhynchus nerka. Journal of the Fisheries Research Board of Canada,* **22,** 1491-1501.

Brett, J. R. (1970). The energy cost of living. In: *Marine aquaculture* edited by W. J. McNeil. Oregon State University Press: Corvallis, pp. 37-52.

Brett, J. R. (1971). Starvation time, apetite and maximum food intake of sockye salmon, *Oncorhynchus nerka, Journal of the Fisheries Research Board of Canada,* **28,** 409-415.

Brett, J. R. (1972). The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. *Respiratory Physiology,* **14,** 151-170.

Brett, J. R. (1979). Environmental factors and growth. In: *Fish Physiology. Volume VIII, Bioenergetics and Growth* edited by W. S. Hoar, D. J. Randall and J. R. Brett, pp. 599-675.

Brett, J. R. (1983). Life energetics of sockeye salmon, *Oncorhynchus nerka.* In: *Behavioural Energetics: The Cost of Survival in Vertebrates* edited by W. P. Aspey and S. I. Lustick. Ohio State University Press: Columbus, pp. 29-63.

Brett, J. R. and Glass, N. R. (1973). Metabolic rate and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. *Journal of the Fisheries Research Board of Canada,* **30,** 379-387.

Brett, J. R. and Groves, T. D. D. (1979). Physiological energetics. In: *Fish Physiology. Vol. VIII, Bioenergetics and Growth,* edited by W. S. Hoar, D. J. Randall and J. R. Brett. Academic Press, New York, pp. 280-344.

Brett, J. R., Shelbourn, J. E. and Shoop, C. T. (1969). Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka,* in relation to temperature and ration size. *Journal of the Fisheries Research Board of Canada,* **26,** 2363-2394.

Bristow, G. A. and Berland, B. (1991). The effect of long term, low level *Eubothrium* sp. (Cestoda: Pseudophyllidea) infection on growth in farmed salmon (*Salmo salar*). *Aquaculture,* **9,** 325-330.

Bromley, P J. (1987). The effects of food type, meal size and body weight on digestion and gastric evacuation in turbot, *Scophthalmus maximus* L. *Journal of Fish Biology,* **30,** 501-512.

Bromley, P. J. (1994). The role of gastric evacuation in quantifying the feeding rates of predatory fish. *Reviews in Fish Biology and Fisheries,* **4,** 36-66.

Brown, J. A. G., Jones, A. and Mattey, A. J. (1984). Oxygen metabolism of farmed turbot (*Scophthalmus maximus*) I. The influence of fish size and water temperature on metabolic rate. *Aquaculture,* **31,** 31-40.

Buchanan, J. S. and Madeley, C.R. (1978). Studies on *Herpesvirus scopthalmi* infection of turbot *Scophthalmus maximus* (L) ultrastructural observations. *Journal of Fish Diseases,* **1,** 283-295.

Buchanan, J. S., Richards, R. H., Sommerville, C. and Madeley, C. R. (1978). A herpes-type virus from *Scophthalmus maximus* (L.). *Veterinary Record,* **2,** 527-528.

Bucke, D. (1971). The anatomy and histology of the alimentary tract of the carnivorous fish the pike *Esox lucius* L. *Journal of Fish Biology*, **3**, 421- 431.

Buddington, R. K. and Diamond, J. M. (1986). Aristotle revisited: the function of pyloric ceca in fish. *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 8012-8014.

Bullock, W. L. (1963). Intestinal histology of some salmonid fishes with particular reference to the histopathology of acanthocephalan infections. *Journal of Morphology*, **112**, 23-25.

Bullock, W. L. (1967). The intestinal histology of the mosquito fish *Gambusia affinis* (Baird and Girard). *Acta Zoologica*, **48**, 1-17.

Calow, P. (1985). Adaptive aspects of energy allocation. In: *Fish Energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London, Sydney, pp. 3-31.

Campbell, S. and Love, R. M. (1978). Energy reserves of male and female haddock (*Melanogrammus aeglefinus* L.) from the Moray Firth. *Journal du Conseil International pour l'Exploration de la Mer*, **38**, 120-121.

Cannon, C. E. and Mettrick, D. E. (1970). Changes in the distribution of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea) within the rat intestine during prepatent development. *Canadian Journal of Zoology*, **48**, 761-769.

Castric, J. and Dekinkelin, P. (1984). Experimental study of the susceptibility of 2 marine fish species, sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) to viral hemorrhagic septicaemia. *Aquaculture*, **41**, 203-212.

Castric, J., Baudinlaurencin, F., Coustans, M. F. and Aufret, M. (1987). Isolation of pancreatic necrosis virus, AB serotype, from an epizootic in farmed turbot, *Scophthalmus maximus*. *Aquaculture*, **67**, 117-126.

Catton, W. T. (1951). Blood cell formation in certain teleost fishes. *Blood*, **6**, 39-60.

Chandler, A. C. (1939). The effects of number and age of worms on development of primary and secondary infections with *Hymenolepis diminuta* in rats, and on investigation into the true nature of "premunition" in tapeworm infections. *American Journal of Hygiene*, **29**, 105-114.

Chappell, L. H. (1993). Physiology and Nutrition. In: *Modern Parasitology: a Textbook of Parasitology* edited by F. E. G. Cox. Blackwell Scientific Publications: Oxford, pp. 157-192.

Chellappa, S., Huntingford, F. A., Strang, R. H. C. and Thomson, R. Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology*, **47**, 775-787.

Cho, C. Y. and Bureau, D. P. (1995). Determination of the energy requirements of fish with particular reference to salmonids. *Journal of Applied Icthyology*, **11**, 141-163.

Cho, C. Y., Slinger, S. J. and Bayley, H. S. (1982). Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comparative Biochemistry Physiology*, **73B**, 25-41.

Christiansen, J. S., Ringo, E. and Jobling, M. (1989). Effects of sustained exercise on growth and body composition of first-feeding Arctic charr, *Salvelinus alpinus*. *Aquaculture*, **79**, 329-335.

Coles, J. M. (1971). The early settlement of Scotland: excavations at Morton Fife. *Proceedings of the Prehistoric Society*, **31**, 284-366.

Colgan, P. (1973). Motivational analysis of fish feeding. *Behaviour*, **45**, 38.

Cook, R. M., Sinclair, A. and Stefánsson, G. (1997). Potential collapse of North Sea cod stocks. *Nature*, **385**, 521-522.

Cooper, A. R. (1918). North American pseudophyllidean cestodes from fishes. *Illinois Biological Monographs*, **4**, 288-541.

Cousin, J. C. B., Baudin-Laurencin, F. and Gabaudan, J. (1987). Ontogeny of enzymatic activities in fed and fasting turbot, *Scophthalmus maximus* L. *Journal of Fish Biology*, **30**, 15-33.

Crompton, D. W. T. (1973). The sites occupied by some parasitic helminths in the alimentary tract of vertebrates. *Biological Reviews*, **48**, 27-83.

Crompton, D. W. T. (1991). Nutritional interactions between hosts and parasites. In: *Parasite-Host Associations: Coexistence or Conflict?* edited by C. A. Toft, A. Aeschlimann and L. Bolis. Oxford University Press: Oxford, New York, Toronto, pp. 228-257.

Cui, Y. and Liu, J. (1990). Comparison of energy budget among six species of teleosts. III. Growth rate and energy budget. *Comparative Biochemistry and Physiology*, **97A**, 381-384.

Cui, Y. and Wooten, R. J. (1988a). Pattern of energy allocation in the minnow, *Phoxinus phoxinus* (L.) (Pisces: *Cyprinidae*). *Functional Ecology*, **2**, 57-62.

Cui, Y. and Wooten, R. J. (1988b). The metabolic rate of the minnow, *Phoxinus phoxinus* (L.), in relation to ration, body size and temperature. *Functional Ecology*, **2**, 157-161.

Cui, Y. and Wooten, R. J. (1988c). Bioenergetics of the growth of a cyprinid, *Phoxinus phoxinus* (L.): the effect of ration, temperature and body size on food consumption, faecal production and nitrogenous excretion. *Journal of Fish Biology*, **33**, 431-433.

Cui, Y. and Wooten, R. J. (1988d). Bioenergetics of a cyprinid, *Phoxinus phoxinus* (L.): the effect of ration and temperature on growth rate and efficiency. *Journal of Fish Biology*, **33**, 763-773.

Cui, Y., Hung, S.S.O. and Zhu, X. (1996). Effect of ration size on the energy budget of juvenile white sturgeon. *Journal of Fish Biology*, **49**, 863-876.

Davey, J. Y. and Peachey, J. E. (1968). *Bothriocephalus scorpii* (Cestoda: Pseudophyllidea) in turbot and brill from British coastal waters. *Journal of the Marine Biological Association*, **48**, 335-340.

Davis, L. E. and Schreck, C. B. (1997). The energetic response to handling stress in juvenile coho salmon. *Transactions of the American Fisheries Society*, **126**, 248-258.

Davis, S. J. M. (1987). *The Archaeology of Animals.* Yale University Press: New Haven and London, 224 pp.

Dawe, C. J., Stanton, M. F. and Schwartz, F. J. (1964). Hepatic neoplasms in native bottom-feeding fish of Deep Creek Lake, Maryland. *Cancer Research,* **24,** 1194-1201.

de Groot, S. J. (1971). On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). *Netherlands Journal of Sea Research,* **5,** 121-196.

De Silva, S. S. and Anderson, T. A. (1995). *Fish Nutrition in Aquaculture.* Chapman and Hall: Glasgow, London, New York, 319 pp.

Desser, S. S. and Lester, R. (1975). An Ultrastructural study of the enigmatic "rodlet cell" in the white sucker, *Catastomus commersoni* (Lacapede) (Pisces: Catastomidae). *Canadian Journal of Zoology,* **53,** 1483-1494.

Devesa, S., Barja, J. L. and Toranzo, A. E. (1989). Ulcerative and fin lesions in reared turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases,* **12,** 123-333.

Diamant, A. and Paperna, I. (1995). Protozoan diseases of fish in Mediterranean marine aquaculture. In: Second European Cogress on Protistology and Eighth European Conference on Ciliate Biology. Clermont, France. Abstract 56, p. 40.

Dick, T. A. and Choudhury, A. (1995). Cestoidea (Phylum Platyhelminthes). In: *Fish diseases and Disorders Vol. 1. Protozoan and Metazoan infections* edited by P. K. Woo. CAB international: Cambridge University Press, pp. 391-414.

Dick, G. C., Machin, D., Roberts, R. J. and Anderson, C. D. (1976). The hepato-renal syndrome of cutured turbot. Its effect on a 2-year trial of farm reared turbot (*Scophthalmus maximus*). *Aquaculture,* **8,** 241-249.

Dorucu, M. (1996). *Ecology of helminth infections in salmonid fish.* PhD thesis, University of Glasgow, 200pp.

Durán, M. L. S., Caamaño-García, F., Casal, J. F., Leiro, J. and Ubeira, F. M. (1989). Anthelminthic activity of Praziquantel, Niclosamide, Netobimin and Mebendazole against *Bothriocephalus scorpii* naturally infecting turbot (*Scophthalmus maximus*). *Aquaculture,* **76,** 199-201.

Duthie, E. S. (1939). The origin, development and function of the blood cells in certain marine teleosts. Part 1. Morphology. *Journal of Anatomy,* **73,** 396-412.

Dyková, I. (1995). Pathogenicity of some protozoan parasites of cultivated fishes. In: *Second European Congress of Protistology and Eighth European Conference on Ciliate Biology.* Clermont, France. Abstract 70, p. 44.

Dyková, I. and Figueras, A. (1994). Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate. *Diseases of Aquatic Organisms,* **18,** 5-9.

Dyková, I., Figueras, A. and Novoa, B. (1995). Amebic gill infection of turbot *Scophthalmus maximus*. *Folia Parasitologica*, **42**, 91-96.

Eckert, J. (1991). Interactions between cestodes and their vertebrate hosts. In: *Parasite-Host Associations: Coexistence or Conflict?* edited by C. A. Toft, A. Aeschlimann and L. Bolis. Oxford University Press: Oxford, New York, Toronto, pp. 201-227.

Economou, A. N., Daoulas, C. and Psaras, T. (1991). Growth and morphological development of chub, *Leuciscus cephalus* (L.), during the first year of life. *Journal of Fish Biology*, **39**, 393-408.

Eddy, F.B. (1981). Effects of stress on osmotic and ionic regulation in fish. In *Stress and Fish* edited by A.D. Pickering, Academic Press: London, New York, pp. 77-95.

Edwards, A. (1995). Stress in fish. *Fish Farmer*, **18**, 15.

Edwards, D. J. (1971). Effect of temperature on rate of passage of food through the alimentary canal of the plaice *Pleuronectes platessa* L. *Journal of Fish Biology*, **3**, 433-439.

Edwards, R. R. C., Finlayson, D. M. and Steele, J. H. (1969). The ecology of o-group plaice and common dabs in Loch Ewe. II. Experimental studies of metabolism. *Journal of Experimental Marine Biology and Ecology*, **3**, 1-17.

Edwards, R. R. C., Blaxter, J. H. S., Gopolan, U. K. and Mathew, C. V. (1970). A comparison of standard oxygen consumption of temperate and tropical bottom-living marine fish. *Comparative Biochemistry and Physiology*, **24**, 491-495.

Elliot, J. M. (1972). Rates of gastric evacuation in brown trout, *Salmo trutta* L. *Freshwater Biology*, **2**, 1-18.

Elliot, J. M. (1975a). Weight of food and time required to satiate brown trout, *Salmo trutta* L. *Freshwater Biology*, **5**, 51-64.

Elliot, J. M. (1975b). Number of meals in a day, maximum weight of food consumed in a day and maximum rate of feeding for brown trout. *Freshwater Biology*, **5**, 287-303.

Elliot, J. M. (1976a). The growth rate of brown trout, *Salmo trutta* L., fed on maximum rations. *Journal of Animal Ecology*, **44**, 805-821.

Elliot, J. M. (1976b). The growth rate of brown trout, *Salmo trutta* L., fed on reduced rations. *Journal of Animal Ecology*, **44**, 823-842.

Elliot, J. M. (1976c). The energetics of feeding metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. *Journal of Animal Ecology*, **45**, 923-948.

Elliot, J. M. and Persson, L. (1978). The estimation of daily rates of food consumption for fish. *Journal of Animal Ecology*, **47**, 977-991.

Ellis, A. E. (1981). Stress and the Modulation of Defence Mechanisms in Fish. In: *Stress and Fish* edited by A.D. Pickering, Academic Press: London, New York, pp. 147-165.

Emmans, G. C. (1994). Effective energy: a concept of energy utilization applied across species. *British Journal of Nutrition,* **71,** 801-821.

Erlandsen, S. L. and Chase, D. G. (1974). Morphological alterations in the microvillus border of villous epithelial cells produced by intestinal microorganisms. *American Journal of Clinical Nutrition.* **27,** 1277-1286.

Esch, G. W. and Fernandez, J. L. (1993). *A Functional Biology of Parasitism: Ecological and Evolutionary Implications.* Chapman and Hall: London, Glasgow, New York, 337 pp.

Esch, G.W. and Hazen, T.C. (1978). Thermal ecology and stress: a case history for red-sore disease in largemouth bass. In: *Energy and Environmental Stress in Aquatic ecosystems* edited by J.H. Thorpe and J. W. Gibbons. Technical Information Centre, U. S. Department of Energy. CONF-771114, pp. 331-363.

Estevez, J., Iglesias, R., Leiro, J., Ubeira, F. M. and Sanmartin, M. L. (1992). An unusual site of infection by a microsporean in the turbot *Scophthalmus maximus. Diseases of Aquatic Organisms*, **13,** 139-142.

Estevez, J., Leiro, J., Toranzo, A. E., Barja, J. L. and Ubeira, F. M. (1994). Role of serum antibodies in protection of vaccinated turbot (*Scophthalmus maximus*) against vibriosis. *Aquaculture,* **123,** 197-204.

Euzet, L. and Combes, C. (1980). Les problèmes de l'éspèce chez les animaux parasites. In: *Les problèmes de l'éspèce dans le règne animal.* Tome III, Paris, France: Société Zoologique de France, No. 40. (*Mems. Soc. Zool. Fr.*) pp. 239-283, cited in Williams and Jones, 1994.

Euzet, L. and Raibaut, A. (1985). Les maladies parasitaires en pisculture marine. *Symbioses,* 17, 51-68.

Evans, M. H. (1937). A comparative study of the brains in Pleuronectidae. *Proceedings of the Royal Society* (B) **122,** 308-343.

Fagerlund, U. H. M., McBride, J. R. and Stone, E. T. (1981). Stress-related effects of hatchery rearing density on coho salmon. *Transactions of the American Fisheries Society,* **110,** 644-649.

Fänge, R. and Grove, D. (1979). Digestion. In: *Fish Physiology Volume VIII, Bioenergetics and Growth* edited by W. S. Hoar, D. J. Randall, and J. R. Brett. Academic Press: London, pp. 162-260.

Farbridge, K. J. and Leatherland, J. F. (1987). Lunar cycles of coho salmon, *Oncorhynchus kisutch. Journal of Experimental Biology,* **129,** 165-178.

Farmer, G. J and Beamish, F. W. H. (1969). Oxygen consumption of *Tilapia nilotica* in relation to swimming speed and salinity. *Journal of the Fisheries Research Board of Canada,* **26,** 2907-2921.

Ferguson, H. W. and Roberts, R. J. (1975). Myeloid leucosis associated with sporozoan infection in cultured turbot (*Scophthalmus maximus*). *Journal of Comparative Pathology,* **85,** 317-326.

Fernhead, E. A. and Fabian, B. L. (1971). The ultrastructure of the gill of *Monodactylus argenteus* (an euryhaline teleost fish) with particular reference to morphological changes associated with changes in salinity. *South African Association of Marine Biology Research, Oceanographic Research Institute, Investigation Report No.* 26, 39 pp.

Figueras, A., Novoa, B., Santarem, M., Martinez, E., Alvarez, J. M., Toranzo, A. E. and Dykova, I. (1992). *Tetramicra brevifilum*, a potential threat to farmed turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms*, **14,** 127-135.

Fish, G. R. (1960). The comparative activity of some digestive enzymes in the alimentary canal of *Tilapia* and perch. *Hydrobiologia*, **15,** 161-178.

Fletcher, T. C., White, A. and Baldo, A. (1980). Isolation of a phosphorylcholine-containing component from the turbot tapeworm, *Bothriocephalus scorpii* (Muller) and its reaction with C-reactive protein. *Parasite Immunology*, **2,** 237-248.

Flood, M. T., Nigrelli, R. F. and Gennaro, J. F. (1975). Some aspects of the ultrastructure of the 'Stabchendrusenzellen', a peculiar cell associated with the endothelium of the bulbus arteriosus and with other fish tissues. *Journal of Fish Biology*, 7, 129-138.

Flowerdew, M. W. and Grove, D. J. (1979). Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. *Journal of Fish Biology*, **14,** 229-238.

Fonds, M., Cronie, R., Vethaak, A. D. and van der Puyl, P. (1992). Metabolism, food consumption and growth of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) in relation to fish size and temperature. *Netherlands Journal of Sea Research*, **29,** 127-143.

Forsberg, O. I. (1995). Empirical investigations on growth of post-smolt Atlantic salmon (*Salmo salar*) in land-based farms: evidence of a photoperiodic influence. *Aquaculture*, **133,** 235-248.

Forster, J. R. M. and Gabbot, F. A. (1971). The assimilation of nutrients from compounded diets by the prawns *Palameon serratus* and *Pendalus platyceros*. *Journal of Marine Biological Association. U.K.*, **51,** 943-961.

Forstner, H. (1983). An automated multiple-chamber intermittent-flow respirometer. In: Polarographic Oxygen Sensors edited by A Gnaiger and H. Forstner. Springer Verlag: Heidelberg, pp. 111-126.

Fouz, B., Larsen, J. L., Nielsen, B., Barja, J. L. and Toranzo, A. E. (1992). Characterization of *Vibrio damsela* strains isolated from turbot. *Diseases of Aquatic Organisms*, **12,** 155-166.

Fox, M. (1991). Food consumption and bioenergetics of young of the year walleye (*Stizostedion vitreum vitreum*) model predictions and population density effects. *Canadian Journal of Fisheries and Aquatic Sciences*, **48,** 434-441.

Fry, F. E. J. (1957). The aquatic respiration of fish. In: *The Physiology of Fishes Volume 1. Metabolism* edited by M. E. Brown. Academic Press: New York, pp. 1-63.

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In: *Fish Physiology volume VI, Environmental Relations and Behaviour* edited by W. S. Hoar and D. J. Randall. Academic Press Incorporated: New York, London, pp. 1-98.

Gadomski, D. M. and Caddell, S. M. (1991). Effects of temperature on early-life-history stages of California halibut *Paralichthys calaforniicus*. *Fisheries Bulletin*, **89,** 567-576.

Gaumet, F., Boeuf, G., Severe, A., Le Roux, A. and Mayer-Gostan, N. (1995). Effects of salinity on the ionic balance and growth of juvenile turbot. *Journal of Fish Biology*, **47,** 865-876.

Glass, H. J., MacDonald, R. M. and Stark, J. R. (1989). Digestion of protein in different marine species. *Comparative Biochemistry and Physiology*, **94B,** 607-611.

Godin, J. J. (1981). Effect of hunger on the daily pattern of feeding rates in juvenile pink salmon, *Oncorhyncus gorbuscha* Walbaum. *Journal of Fish Biology*, **19,** 63-71.

Grabda, J. (1991). *Marine Fish Parasitology: An Outline.* Polish Scientific Publishers: Warszawa, 306 pp.

Granath, W. O. and Esch, G. W. (1983). Temperature and other factors that regulate the composition and infrapopulation densities of *Bothriocephalus achellognathi* (Cestoda) in *Gambusia affinis* (Pisces). *Journal of Parasitology*, **69,** 1116-1124.

Grodzinski, W. and Klekowski, R. Z. (1975). IBP Handbook No. 24. *Methods for ecological bioenergetics.* Blackwell Scientific Publishers: Oxford, 367 pp.

Grove, D. J. and Crawford, C. (1980). Correlation between digestion rate and feeding frequency in the stomachless teleost, *Blennius pholis* L., *Journal of Fish Biology*, **16,** 235-247.

Grove, D. J., Lozoides, L. and Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. *Journal of Fish Biology*, **12,** 507-516.

Grove, D. J., Moctezuma, M. A., Flett, H. R. J., Foot, J. S., Watson, T. and Flowerdew, M. W. (1985). Gastric emptying and the return of appetite in juvenile turbot, *Scophthalmus maximus* L., fed on artificial diets. *Journal of Fish Biology*, **26,** 339-354.

Hackenbrock, C. R. and Caplan, A. I. (1969). Ion induced ultrastructural transformations in isolated mitochondria. The energized uptake of calcium. *Journal of Cell Biology*, **42,** 221-234.

Hadjichristophorou, M. and Grove, D. J. (1983). A study of appetite, digestion and growth in juvenile green turtle (*Chelonia mydas* L.) fed on artificial diets. *Aquaculture*, **30,** 191-201.

Hale, P. A. (1965). The morphology and histology of the digestive systems of two freshwater teleosts. *Poeclia Reticulata* and *Gasterosteus aculeatus*. *Journal of Zoology*, **146,** 132-149.

Hallaråker, H., Folkvord, A. and Stefansson, S. O. (1995). Growth of juvenile halibut (*Hippoglossus hippoglossus*) related to temperature, light period and feeding regime. *Netherlands Journal of Sea Research*, **34,** 139-147.

Hansen, M. J., Boisclair, J. F., Lucas, M. C. and Ney, J. J. (1993). Applications of bioenergetics models to fish biology and management - where do we go from here? *Transactions of the American Fisheries Society,* **122,** 1019-1030.

Hartman, K. J. and Brandt, S. B. (1995). Comparative energetics and the development of bioenergetics models for sympatric estuarine piscivores. *Canadian Journal of Fisheries and Aquatic Sciences,* **52,** 1647-1666.

Hartnett, M. (1993). Water quality aspects of offshore fish farming. *Proceedings of the Institution of Civil Engineers - Water Maritime and Energy,* **101,** 223-235.

Hilmy, I. S. (1929). *Bothriocephalus scorpii* (Mueller, 1776) Cooper, 1917. *Annals of Tropical Medicine and Parasitology,* **23,** 385-396.

Hazel, J. R. and Prosser, C. L. (1971). Mechanisms of thermal compensation. *Physiological Reviews,* **54,** 620-677.

Hochachka, P. W. and Somero, G. N. (1971). Biochemicla adaptation to the environment. In: *Fish Physiology,* Volume 6, edited by W. S. Hoar and D. J. Randall. Academic Press, New York, pp. 99-156.

Hoffmann, R. Kennedy, C. R. and Meder, J. (1986). Effects of *Eubothrium salvelini* (Schrank, 1790) on Arctic charr, *Salvelinus alpinus* in an Alpine Lake. *Journal of Fish Diseases,* **9,** 153-157.

Holmes, J. C. (1973). Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. *Canadian Journal of Zoology,* **51,** 333-347.

Holmes, J. C. (1990). Helminth communities in marine fish. In: *Parasite communities: patterns and processes* edited by G. E. Esch, A. Bush and J. Aho. Chapman and Hall: London, New York, pp.101-130.

Hoole, D. (1994). Tapeworm infections in fish: past and future problems. In: *Parasitic Diseases of Fish* edited by A. W. Pike and J. W. Lewis. Samara Publishing Ltd.: London, pp. 119-140.

Horn, M. and Gibson, R. (1988). Les poissons intertidaux. *Pour la Science,* **125,** 72-80.

Horppila, J. and Peltonen, H. (1997). A bioenergetic approach on food consumption of roach (*Rutilus rutilus* (L)) in a eutrophic lake. *Archiv Fur Hydrobiologie,* **139,** 207-222.

Horne, M. T., Richards, R. H., Roberts, R. J. and Smith, P. C. (1977). Peracute vibriosis in juvenile turbot. *Journal of Fish Biology,* **11,** 355-361.

Houston, A. H., Madden, J. A., Wards, R. J. and Miles, H. M. (1971a). Some physiological effects of handling and tricaine methanesulphonate anaesthetization upon brook trout *Salvelinus fontinalis. Journal of the Fisheries Research Board of Canada,* **28,** 625-633.

Houston, A. H., Madden, J. A., Wards, R. J. and Miles, H. M. (1971b). Variations in blood and tissue chemistry of brook trout *Salvelinus fontanalis* subsequent to handling, anaesthesia and surgery. *Journal of the Fisheries Research Board of Canada,* **28,** 600-606.

Hughes, G. M., Albers, C., Muster, D. and Gotz, K. H. (1983). Respiration of the carp, *Cyprinus carpio* L., at 10 and 20°C and the effect of hypoxia. *Journal of Fish Biology,* **22,** 613-628.

Hunn, J. B., Schetlger, R. A. and Willford, W. A. (1968). Turnover and urinary excretion of free and acetylated MS222 by rainbow trout *Salmo gairdneri. Journal of the Fisheries Research Board of Canada,* **25,** 25-31.

Huntingford, F. A., Metcalfe, N. B. and Thorpe, J. E. (1993). Social status and feeding in Atlantic salmon *Salmo salar* parr: the effect of visual exposure to a dominant. *Ethology,* **94,** 201-206.

Hutchinson, S. and Hawkins, L. E. (1990). The influence of salinity on water balance in 0-group flounders, *Platichthys flesus* (L.). *Journal of Fish Biology,* **36,** 751-764.

Huxley, J. S. (1932). *Problems of relative growth.* Methuen: London, 276 pp.

Imagawa, T., Hashimoto, Y., Kon, Y. and Sugimura, M. (1990). Lectin histochemistry as special markers for rodlet cells in carp, *Cyprinus carpio* L. *Journal of Fish Diseases,* **13,** 537-540.

Imsland, A. K., Sunde, L. M., Folkvord, A. and Stefansson, S. O. (1996). The interaction of temperature and fish size on growth of juvenile turbot. *Journal of Fish Biology,* **49,** 926-940.

Iversen, E. S. (1996). *Living Marine Resources: Their Utilization and Management.* Chapman and Hall: New York, London, Singapore, 403 pp.

Ivlev, V. S. (1939). Energy balance of carps. *Zoologicheskit Zhurnal,* **18,** 303-318.

Iwai, T. (1968a). The comparative study of the digestive tract of teleost larvae - V. Fat absorption in the gut epithelium of goldfish larvae. *Bulletin of the Japanese Society of Scientific Fisheries,* **34,** 973-978.

Iwai, T. (1968b). Fine structure and absorption patterns of the intestinal epithelial cells in rainbow trout alevins. *Zeitschrift fue Zellforschung,* **91,** 366-379.

Iwai, T. (1968c). Notes on the pear-shaped cell (rodlet cell) in the epithelium of the digestive tract of fishes. *Bulletin of the Japanese Society of scientific Fisheries.* **34,** 133-137.

Iwai, T. (1969). Fine structure of gut epithelial cells of larval and juvenile carp during absorption of fat and protein. *Archives of Histology* (Japanese), **30,** 183-199.

Jangaard, P. N., Brockerhoff, H., Burgher, R. D. and Hoyl, R. J. (1967) Seasonal changes in general condition and lipid content of cod from inshore waters. *Journal of the Fisheries Research Board of Canada,* **24,** 607-612.

Jobling, M. (1978). Aspects of food consumption and energy metabolism of farmed plaice, *Pleuronectes platessa* L. PhD Thesis. University of Glasgow.

Jobling, M. (1980). Gastric evacuation in plaice, *Pleuronectes platessa*: effects of dietary energy level and food consumption. *Journal of Fish Biology,* **17,** 187-196.

Jobling, M. (1981a). Mathematical models of gastric emptying and the estimation of daily rate of food consumption for fish. *Journal of Fish Biology,* **19,** 245-258.

Jobling, M. (1981b). The influence of feeding on the metabolic rate of fishes: a short review. *Journal of Fish Biology,* **18,** 385-400.

Jobling, M. (1982). A study of some factors affecting rates of oxygen consumption in plaice, *Pleuronectes platessa* L. *Journal of Fish Biology,* **20,** 501-516.

Jobling, M. (1983a). Towards an explanation of specific dynamic action (SDA). *Journal of Fish Biology,* **23,** 549-555.

Jobling, M. (1983b). A short review and critique of methodology used in fish growth and nutrition studies. *Journal of Fish Biology,* **23,** 685-703.

Jobling, M. (1994). *Fish Bioenergetics.* Chapman and Hall: London, Glasgow and New York, 309 pp.

Jobling, M. and Davies, P. S. (1980). Effects of feeding on the metabolic rate and specific dynamic action in plaice, *Pleuronectes platessa* L. *Journal of Fish Biology,* **16,** 629-638.

Jobling, M., Gwyther, D. and Grove, D. J. (1977). Some effects of temperature, meal size and body weight on gastric evacuation time in the dab, *Limanda limanda* (L.). *Journal of Fish Biology,* **10,** 291-298.

Jones, A. (1972). Some aspects of the biology of the turbot (*Scophthalmus maximus* L.) with special reference to feeding and growth in the juvenile stage. PhD Thesis, University of East Anglia, 145 pp.

Jones, A. (1973). The ecology of young turbot, *Scophthalmus maximus* (L.), at Borth, Cardiganshire, Wales. *Journal of Fish Biology,* **4,** 367-383.

Jones, A. (1975). The morphology of *Bothriocephalus scorpii* (Müller) (Pseudophyllidea, Bothriocphalidae) from littoral fishes in Britain. *Journal of Helminthology,* **49,** 251-261.

Jones, A., Brown, J. A. G., Douglas, M. T., Thompson, S. J. and Whitfield, R. J. (1981). Progress towards developing methods for the intensive farming of turbot (*Scophthalmus maximus* L.) in cooling water from a nuclear power station. In: *Proceedings of World Symposium on Aquaculture in Heated Effluents and Recirculation Systems,* edited by K. Tiews. Technical Paper F. A. O.: Paris, pp. 481-496 (cited in Imsland *et al.,* 1996).

Jørgensen, E. H., Christiansen, J. S. and Jobling, M. (1993). Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Salvelinus alpinus*). *Aquaculture,* **110,** 191-204.

Kapoor, B.G., Smit, H. and Verighana, I. A. (1975). The alimentary canal and digestion in teleosts. In: *Advances in Marine Biology* edited by Russell and Yonge, Volume 13. Academic Press: London, New York, San Fransisco, pp. 109-239.

Karas, P. (1990). Seasonal changes in growth and standard metabolic rate of juvenile perch, *perca fluviatilis. Journal of Fish Biology,* **37,** 913-920.

Kaufmann, R., Forstner, H. and Weisner, W. (1989). Respirometry-methods and approaches. In: *Techniques in Comparative Respiratory Physiology. Society for Experimental Biology Seminar Series* 37 edited by C.R. Bridges and P.J. Butler. Cambridge University Press: Cambridge, New York, Sydney, pp. 51-76.

Kennedy, C. R. (1983). General ecology. In: *Biology of the Eucestoda,* Volume 1 edited by C. Arme and P. W. Pappas. Academic Press: London, pp. 27-83.

Kennedy, C. R. (1994). The ecology of introductions. In: *Parasitic Diseases of Fish* edited by A. W. Pike and J. W. Lewis. Samara Publishing Ltd.: London, pp. 189-208.

Kent, M. L. and Fournie, J. W. (1993). Importance of marine fish diseases: an overview. In: *Pathobiology of Marine and Estuarine Organisms, Advances in Fisheries Series,* edited by J. A. Couch and J. W. Fournie. CRC Press: Boca Raton, Ann Arbor, London, Tokyo, pp. 1-24.

Ker, W. C. A. (1968). Loeb Classical Library, *Martial Epigrams* with an English translation by W. C. A. Ker. Volume 1. William Heinemann Ltd.: London, pp. 200-201.

Kerr, S. R. (1971a). Analysis of laboratory experiments on growth efficiency of fishes. *Journal of the Fisheries Research Board of Canada,* **28,** 801-808.

Kerr, S. R. (1971b). Prediction of fish growth efficiency in nature. *Journal of the Fisheries Research Board of Canada,* **28,** 809-814.

Kerr, S. R. (1971c). A simulation model of lake trout growth. *Journal of the Fisheries Research Board of Canada,* **28,** 815-819.

Kerr, S. R. (1971d). Niche theory in fisheries ecology. *Transactions of the American Fisheries Society.* **109,** 254-260.

Kirmse, P. D. (1978). *Haemogregarina sachai* n. sp. from cultured turbot *Scophthalmus* (L.) in Scotland. *Journal of Fish Diseases,* **1,** 337-342.

Kirmse, P. D. (1980). Observations on the pathogenicity of *Haemogregarina sachai,* Kirmse, 1978 in farmed turbot *Scophthalmus maximus* (L). *Journal of Fish Diseases,* **3,** 101-114.

Kitchell, J. F., Stewart, D. J. and Weininger, D. (1977). Applications of bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vvitreum*). *Journal of the Fisheries Research Board of Canada,* **34,** 1922-1935.

Kititsyna, L. A. and Nikitenko, A. G. (1986). Oxygen consumption by yearling grass carp infested with cestodes. *Hydrobiological Journal,* **22,** 58-62.

Kleiber, M. (1975). The fire of life. R. E. Kleiber publishing Co.: New York. 453 pp.

Knights, B. (1985). Energetics and fish farming. In: *Fish energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London and Sydney, pp. 309-341.

Knutsen, J. A. (1992). Feeding behaviour of North-sea turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) larvae elicited by chemical stimuli. *Marine Biology,* **113,** 543-548.

Kociecka, W. (1987). Intestinal cestodiasis. In: *Intestinal Helminthic Infections* edited by Z. S. Pawlowski. Clinical Tropical Medicine and Communicable Diseases, Volume 2. Baillière Tindall: London, pp. 677-694.

Koven, W. M., Henderson, R. J. and Sargent, J. R. (1994a). Lipid digestion in turbot (*Scophthalmus maximus*). 1. Lipid class and fatty-acid composition of digesta from different segments of the digestive tract. *Fish Physiology and Biochemistry*, **13,** 69-79.

Koven, W. M., Henderson, R. J. and Sargent, J. R. (1994b). Lipid digestion in turbot (*Scophthalmus maximus*). 2. Lipolysis in vitro of C-14 labelled triacylglycerol, cholesterol ester and phosphatidylcholine by digesta from different segments of the digestive tract. *Fish Physiology and Biochemistry*, **13,** 275-283.

Krementz, A. B. and Chapman, C.B. (1975). Ultrastructure of the posterior half of the intestine of the channel catfish, *Ictalurus punctatus*. *Journal of Morphology*, **145,** 441-482.

Krogh, A. (1914). The quantitative relation between temperature and standard metabolism in animals. *Int. Z. Phys. Chem. Biol.*, **1,** 491-508.

Kuperman, B. I. and Kuz'mina, V. V. (1994). The ultrastructure of the intestinal epithelium in fishes with different types of feeding. *Journal of Fish Biology*, **44,** 181-193.

Kurovskaya, L. Ya. and Kititsyna, L. A. (1986). Physiological - biochemical features of the white amur infested with helminths. *Soviet Journal of Ecology*, **17,** 168-172.

Labar, G. W. (1993). Use of bioenergetics models to predict the effect of increased lake trout predation on rainbow smelt following sea lamprey control. *Transactions of the American Fisheries Society*, **122,** 942-950.

Laguessé, E. (1895). Sur le pancreas du Crenilabre et particulierement sur le pancreas intra-hepatique. *Rev. Biol. Nord France* **7,** 343-363.

Lange, Ø. N. O. (1962). Development of the intestine in *Rutilus rutilus* (L.). *Voprosy Ikhtiologii*, **2,** 336-349.

Larkin, P. A. (1996). Concepts and issues in marine ecosystem management. *Reviews in Fish Biology and Fisheries*, **6,** 139-164.

Lauder, G. V. and Liem, K. F. (1983). The evolution and the interrelationships of the actinopterygian fishes. *Bulletin of Museums comparative Zoology*, Cambridge (Massachusetts), **150,** 95-197.

Lazarus, R. S. (1971). The concepts of stress and disease. In: *Society Stress and Disease.* Volume 1. *The Psychosocial Environment and Psycosomatic Diseases* edited by L. Levi. Oxford University Press: London, pp.53-58.

Le, H. L. V., Lecointre, G. and Perasso, R. (1993). A 28S rRNA-based phylogeny of the gnathostomes: first steps in the analysis of conflict and congruence with morphologically based cladograms. *Molecular Phylogenetics and Evolution*, **2,** 31-51.

Leino, R. L. (1974). Ultrastructure of immature, developing and secretory rodlet cells in fish. *Cell and Tissue Research*, **155,** 367-381.

Leino, R. L. (1982). Rodlet cells in the gill and intestine of *Catastomus commersoni* and *Perca flavescens* a comparison of their light and electronmicroscopic cytochemistry with that of mucous and glandular cells. *Canadian Journal of Zoology,* **60,** 2768-2782.

Leknes, I. L. (1986). Fine structure and cyto-chemistry of the endothelial cells and rodlet cells in the bulbus arteriosus in species of cichlidae (Teleostei). *Journal of Fish Biology,* **28,** 229-36.

Lester, R. J. G. (1971). The influence of *Schistocephalus* plerocercoids on the respiration of *Gasterosteus* and a possible resulting effect on the behaviour of the fish. *Canadian Journal of Zoology,* **49,** 361-366.

Lima-de-Faria, A. (1962). Progress in Triticum autoradiography. *Progress in Biophysics and Chemistry,* **12,** 281-317 (cited in Bielek and Viehberger, 1983).

Lloyd, R. (1961). Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology, 38,* 447-455.

Lom, J. and Bouix, G. (1995). Parasitic protozoa and Aquaculture. In: *Protistological Actualities - Proceedings of the Second European Congress of Protistology* edited by G. Brugerolle and J. P. Mignot, pp. 204-210.

Love, R. M. (1970). *The Chemical Biology of Fishes.* Academic Press Incorporated: London. 547 pp.

Lupiani, B., Subramanian, K., Hetrick, F. M. and Samal, S. K. (1993). A genetic probe for the identification of the turbot aquareovirus in infected cultures. *Diseases of Aquatic Organisms*, **15,** 187-192.

Mann, K. H. (1965). Energy transformations by a population of fish in the River Thames. *Journal of Animal Ecology*, **34**, 253-257.

Maisey, J. G. (1986). Heads and tails: A chordate phylogeny. *Cladistics,* **2,** 201-256.

Margulis, L. (1993). *Symbiosis in Cell Evolution: Microbial Communitied in the Archean and Proterozoic Eons.* Freeman and Company: New York, 452 pp.

Markowski, S. (1935). O cyklu rozwojowym *Bothriocephalus scorpii* (Müller, 1776), *Bull. Int. Acad. P.* (Acad. Pol. Sci.), **11,** 1-17, (cited in Rees, 1958).

Matskási, I. (1984). The effect of *Bothriocephalus acheilognathi* infection on the protease and alpha-amylase activity in the gut of carp fry. *Symposia Biologica Hungarica,* **33,** 119-125.

Mattey, D. L., Morgan, M. and Wright, D. E. (1979). Distribution and development of rodlet cells in the gills and pseudobranch of the bass, *Dicentrachus labrax* (L). *Journal of Fish Biology* **15,** 363-370.

Mawdesley-Thomas, L. E. (1972). (Ed). *Diseases of fish.* Academic Press: London. p.11.

Mayberry, L. F., Marchiondo, A. A., Ubelaker, J. E. and Kazic, D. (1979). *Rhabdospora thelohani* Laguesse, 1895 (Apicomplexa): New host and geographic records with taxonomic considerations. *Journal of Protozoology,* **26,** 168-178.

Mayberry, L. F., Bristol, J. R., Sulimanovic, D., Fijan, N. and Petrinec, Z. (1986). *Rhabdospora thelohani* - epidemiology of and migration into *Cyprinus carpio* bulbus arteriosus. *Fish Pathology,* **21**, 145-150.

Maynard, L. A. and Loosli, J. K. (1969). *Animal Nutrition* (sixth edition). McGraw-Hill: New York, 613 pp.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society,* **106,** 201-212.

McAuley, R. W. and Huggins, N. W. (1979). Ontogenic and non-thermal seasonal effects on thermal preferenda of fish. *American Zoologist,* **19,** 267-271.

McFadzen, I. R. B., Lowe, D.M. and Coombs, S. H. (1994). Histological changes in starved turbot larvae (*Scophthalmus maximus*) quantified by digital image analysis. *Journal of Fish Biology,* **44,** 255-262.

McGinnis, A. J and Kasting, R. (1964). Comparison of gravimetric and chromic oxide methods for measuring percentage utilization and consumption of food by phytophagous insects. *Journal of Insect Physiology,* **10,** 989-995.

McKinnon, A. D. and Featherston, D. W. (1982). Location and means of attachment of *Bothriocephalus scorpii* (Müller) (Cestoda: Pseudophyllidea) in Red Cod, *Pseudophycis bacchus* (Forster in Bloch & Schneider), from New Zealand waters. *Australian Journal of Marine and Freshwater Research,* **33,** 595-598.

McVicar, A. H. and MacKenzie, K. (1977). Effects of different systems of monoculture on marine fish parasites. In: *Origins of pest, parasite, disease and weed problems* (18th Symposium of the British Ecological Society. Bangor) edited by J. M. Cherrett and G. R. Sagar. Blackwell Scientific Publications: Oxford, pp.163-182.

Meehan, W. R. and Miller, R. A. (1978). Stomach flushing: effectiveness and influence on survival and condition of juvenile salmonids. *Journal of the Fisheries Research Board of Canada,* **35,** 1359-1363.

Mettrick, D. F. (1980). The intestine as an environment for *Hymenolepis diminuta.* In: *Biology of the tapeworm Hymenolepis diminuta* edited by H. P. Arai. Academic Press: London, New York. pp. 281-356.

Mettrick, D. E. and Podesta, R. B. (1974). Helminth-host interactions. In: *Advances in Parasitology Volume 12* edited by B. Dawes, pp.183-278.

Michel, C. (1995). Health control policies in fish viral diseases. *Veterinary Research,* **26,** 369-373.

Mo, T. A. (1994). Status of *Gyrodactylus salaris* problems and research in Norway. In: *Parasitic Diseases of Fish* edited by A. W. Pike and J. W. Lewis. Samara Publishing Ltd.: London, pp. 43-58.

Modin, J.C. (1981). *Microsporidium rhabdophilia* n.sp. from rodlet cells of salmonid fishes. *Journal of Fish Diseases,* **4,** 203-211.

Möller, H. (1986). Pollution and parasitism in the aquatic environment. In: *Parasitology - Quo Vadit?. Proceedings of the sixth International Congress of Parasitology* edited by M. J. Howell, pp. 353-361.

Molnár, G. Y. and Tölg, I. (1962). Relation between water temperature and gastric digestion of largemouth bass, *Micropterus salmoides. Journal of the Fisheries Research Board of Canada,* **19,** 1005-1012.

Molnár, G. Y., Tomassy, E. and Tölg, I. (1967). The gastric digestion of living predatory fish. In: *The Biological Basis of Freshwater Fish Production* edited by S. D. Gerking. Blackwells: Oxford and Edinburgh, pp. 139-149.

Moriarty, C. M. and Moriarty, D. J. W. (1973). Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplchromis nigripinnis* in Lake George, Uganda. *Journal of Zoology,* **171,** 15-23.

Morrison, C. M. and Odense, P. H. (1978). Distribution and morphology of the rodlet cell in fish. *Journal of the Fisheries Research Board of Canada,* **35,** 101-116.

Mortensen, S. H., Evensen, O., Rodseth, O. M and Hjeltnes, B. K. (1993). The prevalence of infectious pancreatic necrosis virus (IPNV) in farmed Norwegian turbot (*Scophthalmus maximus*). *Aquaculture,* **115,** 243-252.

Moshin, S. M. (1962). Comparative morphology and histology of the alimentary canals in certain groups of Indian teleosts. *Acta Zooloogica,* **43,** 79-133.

Mourier, J. P. (1970). Structure fine de *Rhabdospora thelohani* Henneguy, protiste parasite de *Gasterosteus aculeatus* L. *Z. Parasitenk.* **34,** 198-206.

Mudarris, M., Austin, B., Segers, P., Vancanneyt, M., Hoste, B. and Bernardet, J. F. (1994). *Flavobacterium scophthalmum* sp. nov., a parasite of turbot (*Scophthalmus maximus* L) *International Journal of Systemic Bacteriology,* **44,** 447-453.

Mueller, J. F. (1968). Growth stimulating effect of experimental sparganosis in thyroidectomized and hypophysectomized rats, and comparative activity of different species of *Spirometra. Journal of Parasitology,* **54,** 795-801.

Muller-Feuga, A., Petit, J. and Sabaut, J. J. (1978). The influence of temperature and wet-weight on the oxygen demand of rainbow trout (*Salmo gairdneri* R) in fresh water. *Aquaculture,* **14,** 355-364.

Munilla-Moran, R. and Stark, J. R. (1989). Protein digestion in early turbot larvae, *Scophthalmus maximus* L., *Aquaculture,* **81,** 315-327.

Munilla-Moran, R. and Stark, J. R. (1990). Metabolism in marine flatfish - VI. Effect of nutritional state on digestion on digestion in turbot, *Scophthalmus maximus* L. *Comparative Biochemistry and Physiology B,* **95,** 625-634.

Munro, P. D., Barbour, A. and Birkbeck, T. H. (1995). Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the presence of *Vibrio anguillarium, Vibrio alginolyticus,* or a marine *Aeromonas* sp. *Applied and Environmental Microbiology,* **61,** 4425-4428.

Munro, A. L. S., McVicar, A. H. and Jones, R. (1983). The epidemiology of infectious diseases in comercially important wild marine fish. *Rapports et Procés-verbaux des Reunions Commission internationale pour l'exploration Scientifique de la Mer Méditerrané,* **182,** 21-32.

Murray, H. M., Wright, G. M. and Goff, G. P. (1994a). A study of the posterior oesophagus in the winter flounder, *Pleuronectes americanus,* and the yellow flounder, *Pleuronectes ferruginea*: morphological evidence for pregastric digestion? *Canadian Journal of Zoology,* **72,** 1191-1198.

Murray, H. M., Wright, G. M. and Goff, G. P. (1994b). A comparative histological and histochemical study of the stomach from three species of pleuronectids, the Atlantic halibut, *Hippoglossus hippoglossus,* the yellowtail flounder, *Pleuronectes ferruginea,* and the winter flounder, *Pleuronectes americanus. Canadian Journal of Zoology,* **72,** 1199-1210.

Murray, H. M., Wright, G. M. and Goff, G. P. (1996). A comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectid, the Atlantic halibut, the yellowtail flounder and the winter flounder. *Journal of Fish Biology,* **48,** 187-206.

Nash, C. (1987). Aquaculture attracts increasing share of development aid. *Fish Farming International,* **14,** 22-24.

Nelson, G. (1989). Phylogeny of major fish groups. In: *The hierarchy of life: molecules and morphology in phylogenetic analysis* edited by B. Fernholm, K. Bremer and H. Jörnvall. International Congress Series 824. Elsevier Science Publishers: Amsterdam, pp 325-336.

Nelson, J. S. (1994). *Fishes of the World.* Third edition. John Wiley and Sons Inc.: New York, Brisbane, Toronto, Singapore, 600 pp.

Ney, J. J. (1993). Bioenergetics modeling today - growing pains on the cutting edge. *Transactions of the American Fisheries Society,* **122,** 736-748.

Nielsen, J. G. (1973). Scopthalmidae, Bothidae, Pleuronectidae. In: *Check-list of the Fishes of the North-Eastern Atlantic and of the Mediterranean,* Volume 1, edited by J. C. Hureau and T. Monod,. Paris: UNESCO, pp 616-627

Nielsen, J. G. (1986). Scopthalmidae, Bothidae, Pleuronectidae. In: *Fishes of the North-eastern Atlantic and of the Mediterranean,* Volume 1 edited by P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. G. Nielsen and E. Tortonese. Paris: UNESCO, pp. 1287-1307.

Niimi, A. J. (1978). Lag adjustment between estimated and actual physiological responses conducted in flow-through systems. *Journal of the Fisheries Research Board of Canada.* **35,** 1265-1269.

Noaillac-Depeyre, J. and Gas, N. (1973). Mise en évidence d'une zone adaptée au transport des ions dans l'intestin de carpe commune (*Cyprinus carpio* L.). *Comptes Rendus des Séances de l' Académie des Sciences, Paris,* **276,** 773-776.

Noaillac-Depeyre, J. and Gas, N. (1979). Structure and function of the intestinal epithelial cells in the perch (*Perca fluviatilis* L.). *Anatomical Record,* **195,** 621-639.

Nonnotte, G. and Truchot, J. P. (1990). Time course of extracellular acid-base adjustments under hypo- or hypertonic conditions in the euryhaline fish *Platyichthys flesus*. *Journal of Fish Biology,* **36,** 181-190.

Norman, J. R. (1934). *A Systematic Monograph of the Flatfishes (Heterosomata),* Volume 1. British Museum Natural History: London, 459 pp.

Novoa, B., Nunez, S., Fernandezpuentas, C., Figueras, A. J. and Toranzo, A. E. (1992). Epizootic study in a turbot farm: bacteriology, virology, parasitology and histology. *Aquaculture,* **107,** 253-258.

Novoa, B., Figueras, A., Puentes, C. F., Ledo, A. and Toranzo, A. E. (1993a). Characterization of a birnavirus isolated from diseased turbot cultured in Spain. *Diseases of Aquatic Organisms,* **15,** 163-169.

Novoa, B., Toranzo, A. E., Dopazo, C. P. Barja, J. L. and Figueras, A. (1993b). Isolation of IPN virus VR-299 from turbot in Europe. *Diseases of Aquatic Organisms,* **17,** 61-65.

O'Shea, J. and McGuire, M. F. (1962). Determination of calorific value of feedstuffs by chromic acid oxidation. *Journal of Science Food and Agriculture,* **13,** 530-534.

Olsson, J. C., Westerdahl, A., Conway, P. L. and Kjelleberg, S. (1992). Intestinal colonization potential of turbot (*Scophthalmus maximus*) and dab (*Limanda limanda*) associated bacteria with inhibitory effects against *Vibrio anguillarium*. *Applied and Environmental Microbiology,* **58,** 551-556.

Paloheimo, J. E and Dickie, L. M. (1966). Food and Growth of fishes. III. Relations among food, body size, and growth efficiency. *Journal of the Fisheries Research Board of Canada,* **23,** 1209-1248.

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

Pandian, T. J. and Vivekanandan, E. (1985). Energetics of feeding and digestion. In: *Fish Energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London and Sydney, pp. 99-124.

Pascoe, D. and Cram, P. (1977). The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus,* L. *Journal of Fish Biology,* **10,** 467-472.

Pascoe, D. and Mattey, D. (1977). Dietary stress in parasitized and non-parasitized *Gasterosteus aculeatus* L. *Zeitschrift für Parasitenkunde,* **51,** 179-186.

Paterson, W. B. and Desser, S. S. (1981). *Rhabdospora thelohani* Laguesse, 1906 is not a member of the apicomplexa. *Journal of Parasitology,* **67,** 5, 741-744.

Pauly, D. and Christensen, V. (1995). Primary production required to sustain global fisheries. *Nature,* **374,** 255-257.

Pedersen, T. and Jobling, M. (1989). Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. *Aquaculture*, **81,** 161-168.

Pedersen, T. and Jobling, M. (1989). Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. *Aquaculture*, **81,** 161-168.

Pederson, K., Kofod, H., Dalsgaard, I. and Larsen, J. L. (1994). Isolation of oxidase-negative *Aeromonas salmonicida* from diseased turbot *Scophthalmus maximus*. *Diseases of Aquatic Organisms*, **18,** 149-154.

Pennycuick, L. (1971a). Seasonal variations in the parasite infections in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L. *Parasitology*, **63** 373-388.

Pennycuick, L. (1971b). Differences in the parasite infections in the three-spined sticklebacks (*Gasterosteus aculeatus* L.) of different sex, age and size. *Parasitology*, **63,** 407-418.

Pennycuick, L. (1971c). Frequency distribution of parasites in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L. *Parasitology*, **63,** 389-406.

Pennycuick, L. (1971d). Quantitative effects of three species of parasites on a population of three-spined sticklebacks, *Gasterosteus aculeatus*. *Journal of Zoology*, **165,** 143-162.

Perlmutter, A. Sarot, D.A., Uy, M., Fillozola, R. J. and Seeley, R. J. (1973). The effect of crowding on the immune response of the blue gourami *(Trichogaster trichopterus)* to infectious pancreatic necrosis (IPN) virus. *Life Sciences* **13,** 363-375.

Person-Le-Ruyet, J. (1990). Sole and turbot aquaculture. In: *Aquaculture* Volume 2 edited by G. Barnabé. Ellis Horwood: New York, London, Toronto, Sydney, Tokyo, Singapore, pp 687-734.

Pfuderer, P., Williams, P. and Francis, A. A. (1974). Partial purification of the crowding factor from *Carassius auratus* and *Cyprinus carpio*. *Journal of Experimental Zoology*, **187,** 357-382.

Pickering, A.D. (Editor) (1981). *Stress and Fish*, London and New York: Academic Press, 367 pp.

Pickering, A. D. (1993). Husbandry and Stress. In: *Recent Advances in Aquaculture IV*, edited by J. F. Muir and R. J. Roberts. Blackwell Scientific Press: Oxford. pp 155-169.

Pickering, A.D. and Pottinger, T.G. (1989). Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry*, 7, 253-258.

Pierce, R. J. and Wissing, T. E. (1974). Energy cost of food utilization in the bluegill *Lepomis macrochirus*. *Transactions of the American Fisheries Society*, **103,** 38-45.

Pillay, T V. R. (1993). *Aquaculture: Principles and Practices*. University Press: Cambridge. 575 pp.

Pitcher, T. J. and Hart, P. J. B. (1982). *Fisheries Ecology.* Croom Helm: London, New York, 414 pp.

Plehn, M. (1906a). Uber eigentumliche Drusenzellen im Gefassystem und in anderen Organen bei Fischen. *Anat. Anz.* **28,** 192-203.

Plehn, M. (1906b). Drusenzellen oder Parasiten? *Anat. Anz. 29,* 152-156.

Plumb, J. A. (1993). Viral diseases of marine fish. In: Advances in Fisheries Science, *Pathobiology of marine and estuarine organisms* edited by J. A. Couch and J. W. Fournie. CRC Press: Boca Raton, London, Tokyo, pp 25-52.

Pooley, A. (1995). Aeration for fish in transit. *Fish Farmer,* **18,** 8-9.

Pratap, H. B. and Bonga, S. E. W. (1993). The effect of ambient and dietary cadmium on pavement cells, chloride cells and Na^+/K^+-ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water. *Aquatic Toxicology,* **26,** 133-149.

Priede, I. G. (1985). Metabolic scope in fishes. In: *Fish Energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London and Sydney, pp. 33-63.

Propp, M. V., Garber, M. R. and Ryabuschko, V. I. (1982). Unstable processes in the metabolic rate measurements in flow-through systems. *Marine Biology,* **67,** 47-49.

Prosser, C. L. (1973). Comparative Animal Physiology edited by C. L. Prosser, third edition. W. B. Sanders: Philadelphia, London, Toronto.

Pulsford, A., Fauge, R. and Zapata, A. G. (1991). The thymic environment of the common sole, *Solea solea. Acta Zoologica* (Stockholm), **72,** 4, 209-216.

Purdom, C. E. (1974). Variation in fish. In: *Sea Fisheries Research,* edited by F. R. Harden Jones. London: Elek Science.

Purdom, C. E., Jones, A. and Lincoln, R. F. (1972). Cultivation trials with turbot (*Scophthalmus maximus*) *Aquaculture,* **1,** 213-230.

Quantz, G. (1985). Use of endogenous energy sources by larval turbot *Scophthalmus maximus. Transactions of the American Fisheries Society,* **114,** 558-563.

Randall, D. J., Perry, S. F. and Heming, T. A. (1982). Gas transfer and acid/base regulation in salmonids. *Comparative Biochemistry and Physiology,* **73B,** 93-103.

Rees, G. (1958). A comparison of the structure of the scolex of *Bothriocephalus scorpii* (Müller 1776) and *Clestobothrium crassiceps* (Rud. 1819) and the mode of attachment of the scolex to the intestine of the host. *Parasitology,* **48,** 468-492.

Rees, G. (1967). Pathogenesis of adult cestodes. *Helminthological Abstracts,* **36,** 1-23.

Renaud. F., Blanquer, A. and Pasteur, N. (1983). Le complexe *Bothriocephalus scorpii* (Mueller, 1776) différenciation par électophorèse enzymztique des espèces parasites du Turbot

(*Psetta maxima*) and de la Barbue (*Scophthalmus rhombus*). *Comptes Rendus de l'Académie des Sciences,* Série III, Sciencies de la Vie, **296,** 127-129.

Renaud, F., Gabrion, C. and Pasteur, N. (1986). Geographical divergence in *Bothriocephalus* (Cestoda) of fishes demonstrated by enzyme electrophoresis. *International Journal of Parasitology,* **16,** 553-558.

Renaud, F., Blanquer, A. and Gabrion, C. (1990). Evolution of host parasite associations. Palcogegraphic movements of teeostean (*Psetta*) populations: an hypothesis on the genetic divergence of their paraites (*Bothriocephalus*). *International Journal of Parasitology,* **20,** 637-643.

Rhodin, J. A. G. (1974). *Histology: A text and Atlas.* Oxford University Press: New York, London, Toronto, 803 pp.

Richards, D. T., Hoole, D., Lewis, J. W., Ewens, E. and Arme, C. (1994). Rodlet cells: normal fish cells or parasites? *British Society for Parasitology.* Abstract.

Ricker, W. E. (1979). Growth rates and models. In: *Fish Physiology, Vol. VIII, Bioenergetics and Growth* edited by W. S. Hoar, D. J. Randall and J. R. Brett. Academic Press: New York and London, pp 678-743.

Robert, F., Renaud, F., Mathieu, E. and Gabrion, C. (1988). Importance of the paratenic host in the biology of *Bothriocephalus gregarius* (Cestoda, Pseudophyllidea) a parasite of the turbot. *International Journal of Parasitology,* **18,** 611-621.

Robert, F., Boy, V. and Gabrion, C. (1990). Biology of parasite populations: Population dynamics of bothriocephalids (Cestoda-Pseudophyllidea) in teleost fish. *Journal of Fish Biology,* **37,** 327-342.

Roberts, R. J. (Editor) (1989). *Fish Pathology,* Second edition. Ballière Tindall: London, Philadelphia, Tokyo, Toronto, 467 pp.

Rocha, E., Monteiro, R. A. F. and Periera, C. A. (1994). The presence of rodlet cells in the intrahepatic biliary ducts of the brown trout, *Salmo trutta fario* Linnaeus, 1758 (Teleostei, salmonidae). *Canadian Journal of Zoology,* **72,** 1683-1687.

Rosenberg, A. A. and Haugen, A. S. (1982). Individual growth and size-selective mortality of larval turbot reared in enclosures. *Marine Biology,* **72,** 73-77.

Rozin, P. and Mayer, J. (1964). Some factors influencing short-term food intake in goldfish. *American Journal of Physiology,* **206,** 1430-1436.

Rudstam, L. G., Binkowski, F. P. and Miller, M. A. (1994). A bioenergetics model for the analysis of food consumption patterns of bloater in Lake Michegan. *Transactions of the American Fisheries Society,* **122,** 344-357.

Santos, Y., Bandín, I., Núñez, S., Gravningen, K. and Toranzo, A. E., (1991). Protection of turbot, *Scophthalmus maximus* (L.) and rainbow trout, *Oncorhynchus mykiss* (Richardson), against vibriosis using two different vaccines. *Journal of Fish Diseases,* **14,** 409-414.

Sarokon, J. (1975). Feeding frequency, evacuation, absorption, growth and energy balance in rainbow trout, *Salmo gairdneri, Ph.D. Thesis* Colorado, Boulder.

Schaperclaus, W. (1979). *Fischkrankheiten.* **4.** Aufl. Akademic Verlag Berlin.
Scherrer, P. (1984). Influence de la température et de la salinité sur la croissance et la consommation d'oxygène du juvenile de turbot, *Scophthalmus maximus* L. (phase nurserie). Thèse troisième, Université de Bretagne Occidentale, 151 pp.

Schmid, T. H. and Murru, F. L. (1994). Bioenergetics of the bull shark, *Carcharhinus leucas*, maintained in captivity. *Zoological Biology,* **13,** 177-185.

Schmidt, G. D. (1986). *Handbook of tapeworm Identification.* CRC Press: Boca Raton, Florida, 675 pp.

Schmidt, G. D. and Roberts, L. S. (1989). *Foundations of Parasitology.* Fourth edition. Times Mirror/Mosby College Publishing: St. Louis, Toronto, Boston.

Schreck, C. B. (1981). Stress and compensation in teleostean fishes: responses to social and physical factors. In: *Stress and Fish* edited by A. D. Pickering. Academic Press: London, New York, pp. 295-321.

Scott, A. L. and Grizzle, J. M. (1979). Pathology of cyprinid fishes caused by *Bothriocephalus gowkongensis* Yea, 1955 (Cestoda: Pseudophyllidea). *Journal of Fish Diseases,* **2,** 69-73.

Scott, D.B.C. and Currie, C.E. (1980). Social hierarchy in relation to adrenocortical activity in *Xiphophorus helleri* Heckel. *Journal of Fish Biology.* **16,** 265-277.

Seyle, H. (1950). Stress and the general adaptation syndrome. *British Medical Journal,* **1,** 1383-1392.

Sharp, G. D. (1995). It's about time: new beginnings and old good ideas in fisheries science. *Fisheries Oceanography,* **4,** 324-341.

Sharp, L., Pike, A. W. and McVicar, A. H. (1994). Parameters of infection and morphometric analysis of sea lice from sea trout (*Salmo trutta,* L.) in Scottish waters. In: *Parasitic Diseases of Fish* edited by A. W. Pike and J. W. Lewis. Samara Publishing Ltd.: London, pp. 151-170.

Shepherd, C. J. and Bromage, N. P. (Editors) (1989). Intensive Fish Farming. BSP Professional Books: Oxford, London, Edinburgh, Boston, Melbourne, 404 pp.

Sindermann, C. J. (1986). Effects of parasites on fish populations: Practical considerations. In: *Parasitology - Quo vadit? Proceedings of the Sixth International Congress of Parasitology,* edited by M. J. Howell, pp 371-382.

Sindermann, C. J. (1990). *Principal Diseases of Marine Fish and Shellfish,* Volume 1, second edition. Academic Press: New York, London, Tokyo, 521 pp.

Skiftesvik, A.B. and Bergh, O. (1993). Changes in behaviour of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) yolk-sac larvae induced by bacterial infections. *Canadian Journal of Fisheries and Aquatic Sciences,* **50,** 2552-2557.

Smart, G.R. (1981). Aspects of water quality producing stress in intensive fish culture. In: *Stress and Fish* edited by A. D. Pickering. Academic Press: London New York, pp. 277-289.

Smit, H. (1967). Influence of temperature on the rat of gastric juice secretion in the brown bullhead (*Ictalurus nebulosus*). *Comparative Biochemistry and Physiology,* **21,** 125-132.

Smith, H. D. (1973). Observations on the cestode *Eubothrium salvelini* in juvenile sockeye salmon (*Onchorhynchus nerka*) at Babine Lake, British Columbia. *Journal of the Fisheries Research Board of Canada,* **30,** 947-964.

Smith, S. A., Caceci, T., Marei, H. E-S. and El-Habback, H. A. (1995). Observations on rodlet cells found in the vascular system and extravascular space of angelfish (*Pterophyllum scalare scalare*). *Journal of Fish Biology,* **46,** 241-254.

Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. *Journal of Fish Biology,* **6,** 197-208.

Soivio, A., Nyholm, K. and Huhti, M. (1977). Effects of anaesthesia with MS222 neutralized MS222 and benzocaine on the blood constituents of rainbow trout, *Salmo gairdneri. Journal of Fish Biology,* **10,** 91-101.

Solomon, D. J. and Brafield, A. E. (1972). The energetics of feeding, metabolism and growth of perch, (*Perca fluviatilis* L.) *Journal of Animal Ecology,* **41,** 699-718.

Soofiani, N. M. and Hawkins, A. D. (1982). Energetic cost at different levels of feeding in juvenile cod , *Gadus morhua* L. *Journal of Fish Biology,* **21,** 577-592.

Steele, J. H. (1974). *The Structure of Marine Ecosystems.* Harvard University Press: Cambridge, Massachusetts (cited in Pitcher and Hart, 1982).

Steffensen, J. F. (1989). Some errors in respirometry of aquatic breathers: how to avoid and correct for them. *Fish Physiology and Biochemistry,* **6,** 49-59.

Stehbens, W. E. (1966). The ultrastructure of *Lankesterella hylae. Journal of Protozoology,* **13,** 63-73.

Steigenberger, L. W. and Larkin, P. A. (1974). Feeding activities and rates of digestion of northern squawfish (*Ptychoeilus oregonensis*). *Journal of the Fisheries Research Board of Canada,* **31,** 411-420.

Stinson, A. L. W. and Calhoun, M. L. (1993). Digestive system. In: *Textbook of Veterinary Histology* edited by H. D. Dellmann. Lea and Febiger: Philadelphia, pp. 153-193.

Strange, C. D. and Kennedy, G. J. A. (1981). Stomach flushing of salmonids: a simple and effective technique for the removal of stomach contents. *Fish Management,* **12,** 9.

Stroband, H. W. J. (1977). Growth and diet dependant structural adaptations of the digestive tract in juvenile grass carp (*Ctenopharyngodon idella,* Val.). *Journal of Fish Biology,* **11,** 167-174.

Stroband, H. W. J. and Debets, F. M. H. (1978). The ultrastructure and renewal of the intestinal epithelium of the juvenile grasscarp, *Ctenopharyngodon idella* (Val.). *Cell and Tissue Research,* **187,** 181-200.

Swenson, W. A. and Smith, L. L. Jr. (1973). Gastric digestion, food consumption, feeding periodicity and food conversion efficiency in walleye (*Stizostedion vitreum vitreum*). *Journal of the Fisheries Research Board of Canada,* **30,** 1327-1336.

Talbot, C. (1985). Laboratory methods in fish feeding and nutritional studies. In: *Fish Energetics: New Perspectives* edited by P. Tytler and P. Calow. Croom Helm: London and Sydney, pp. 124-154.

Thélohan, P. (1892a). Sur quelques coccidies nouvelles parasites des poissons. *J. Anat. Physiol. Norm. Pathol. Homme Anim.* **28,** 151-162.

Thélohan, P. (1892b). Sur des Sporozoaires indetermines parasites de poissons. *J. Anat. Physiol. Norm. Pathol. Homme Anim.* **28,** 163-171.

Thorpe, J. E. (1977). Daily ration of adult perch, *Perca fluviatilis* L. during summer in Loch Leven, Scotland. *Journal of Fish Biology,* **11,** 55-68.

Threadgold, L. T. and Hopkins, C. A. (1981). *Schistocephalus solidus* and *Ligula intestinalis*: pinocytosis by the tegument. *Experimental Parasitology,* **51,** 444-456.

Tierney, J. F. (1994). Effects of *Schistocephalus solidus* (Cestoda) on the food intake and diet of the three-spined stickleback, *Gasterosteus aculeatus*. *Journal of Fish Biology,* **44,** 731-735.

Toranzo, A. E. and Barja, J. L. (1990). A review of the taxonomy and seroepizootiology of *Vibrio anguillarium,* with special reference to aquaculture in the northwest of Spain. *Diseases of Aquatic Organisms,* **9,** 73-82

Toranzo, A. E., Santos, Y., Bandin, I., Romalde, J. L., Ledo., Fou, B. and Barja, J. L. (1990). Five-year survey of bacterial infections in continental and marine aquaculture in North-west Spain. *World Aquaculture,* **21,** 91-94.

Toranzo, A. E., Romalde, J. L., Bandin, I., Santos, Y. and Barja, J. L. (1992). Evaluation of the sensitivity of bacterial fish pathogens to different antimicrobial compounds. In: C. Michel and D. J. Alderman (Editors) *Chemotherapy in Aquaculture: from theory to reality.* Office International des Epizooties (OIE), Paris, France, pp. 315-325.

Toranzo, A. E., Novoa, B., Romalde, J. L., Nunez, S., Devesa, S., Marino, E., Silva, R., Martinez, E., Figueras, A. and Barja, J. L. (1993a). Microflora associated with healthy and diseased turbot (*Scophthalmus maximus*) from 3 farms in northwest Spain. *Aquaculture,* **114,** 189-202.

Toranzo, A. E., Barja, J. L. and Devesa, S. (1993b). An overview of the main infectious problems in cultured turbot: present status and future necessities. *Proceedings in World Aquaculture,* **19,** 614.

Tyler, A. V. (1970). Rates of gastric emptying in young cod. *Journal of the Fisheries Research Board of Canada,* **27,** 1177-1189.

Ugolev, A. M. (1972). Membrane digestion. Polysubstrate processes. *Organization and regulation.* Leningrad: Nauka (cited in Kuperman and Kuz'mina (1994)).

Ugolev, A. M. (1985). *Digestion Evolution and Principles of an Evolution of the Function.* Leningrad: Nauka (cited in Kuperman and Kuz'mina (1994)).

Ulanowicz, R.E. (1978). Modeling environmental stress. In: *Energy and Environmental Stress in Aquatic Ecosystems* edited by J.H. Thorpe and J.W. Gibbons. Technical Information Center, U.S. Department of Energy, pp. 1-18.

Ursin, E. (1967). A mathematical model of some aspects of fish growth, respiration and mortality. *Journal of Fisheries Research Board of Canada,* **13,** 2355-2453.

van Dobben, W. H. (1937). Über den Kiefermechanismus der Knochenfische. *Archs néerl. Zool.* **2,** 1-72.

Vickerman, K. (1960). Structural changes in mitochondria of *Acanthamoeba* at encystation. *Nature,* **188,** 248.

Vickerman, K. (1962). Patterns of cellular organization in limax amoeba. *Experimental Cell Research,* **26,** 497-519.

Vickers, T. (1962). A study of the intestinal epithelium of the goldfish *Carassius auratus*: its normal structure, the dynamics of cell replacement, and the changes induced by salts of cobalt and manganese. *Quarterly Journal of Microscopical Science,* **103,** 93-110.

Viehberger, G. and Bielek, E. (1982). Rodlet-cells: Gland cell or Protozoan? *Experientia,* **38,** 1216-1218.

Vignuelle, M. and Laurencin, F. B. (1991). Uptake of *Vibrio anguillarium* bacterin in the posterior intestine of rainbow trout *Oncorhynchus mykiss,* sea bass *Dicentrarchus labrax* and *Scophthalmus maximus* after oral administration or anal intubation. *Diseases of Aquatic Organisms,* **11,** 85-92.

Von Bondsdorff, B. (1977). *Diphyllobothriasis in man.* Academic Press: London, 189 pp.

Vonk, H. J. (1941). Die Verdauung bei den nierderen Vertebraten. In: *Advances in Enzymology,* Volume I. Interscience: New York, pp. 371-417.

Von Oertzen, J. A. (1984). Influence of steady-state and fluctuating salinities on the oxygen consumption and activity of some brackish water shrimps and fishes. *Journal of Experimental Marine Biology and Ecology,* **80,** 29-46.

Walkey, M. and Meakins, R. H. (1970). An attempt to balance the energy budget of a host-parasite system. *Journal of Fish Biology,* **2,** 361-372.

Waller, U. (1992). Factors influencing routine oxygen consumption in turbot, *Scophthalmus maximus. Journal of Applied Ichthyology,* **8,** 62-71.

Ware, D. M. (1975). Growth metabolism and optimal swimming speed of a pelagic fish. *Journal of the Fisheries Research Board of Canada,* **32,** 33-41.

Waring, C.P., Stagg, R. M. and Poxton, M. G. (1996). Physiological responses to handling in the turbot. *Journal of Fish Biology,* **48,** 161-173.

Warren, C. E. (1971). *Biology and Water Pollution Control.* Saunders: Philadelphia, Pennsylvania (cited in Brett and Groves, 1979).

Warren, C. E. and Davis, G. E. (1967). Laboratory studies on the feeding, bioenergetics and growth of fish. In: *The biological basis of freshwater fish production* edited by S. D. Gerking. Blackwell: Oxford, pp. 175-214.

Wayne, C. M. and Mayberry, L. F. (1994). Lack of rodlet cells in *Catastomus commersoni* larvae suggest that the cell is not of fish origin. Abstract no.176 from The American Society of Parasitologists 69th annual meeting.

Weatherly, A. H. and Gill, H. S. (1987). *The Biology of Fish Growth.* Academic Press: London, 443 pp.

Webb, P. W. (1978). Partitioning of energy into metabolism and growth. In: *Ecology of Freshwater Fish Production,* edited by S. D. Gerking. Blackwell Scientific Publications: Oxford, London, Edinburgh, Melbourne, pp.184-214.

Wedemeyer, G. A. and McLeay, D. J. (1981). Methods for determining the tolerance of fishes to environmental stressors. In: *Stress and Fish,* edited by A. D. Pickering. Academic Press: London. pp 247-275.

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

Weisel, F.G. (1962). Comparative study of the digestive tract of a sucker, *Catastomus catastomus,* and a predaceous minnow, *Ptychocheilus oregonense. American Midland Naturalist,* **68,** 334-346.

Westerdahl, A., Olsson, J. C., Kjelleberg, S. and Conway, P. L. (1991). Isolation and characterization of turbot (*Scophthalmus maximus*) associated bacteria with inhibitory effects against *Vibrio anguillarium. Applied and Environmental Microbiology,* **57,** 2223-2228.

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

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

Western, J. R. H. and Jennings, J. B. (1970). Histochemical demonstrations of hydrochloric acid in the gastric tubules of teleosts using an *in vivo* prussian blue technique. *Comparative Biochemistry and Physiology,* **35,** 879-884.

Wheeler, A. (1992a). A list of the Common and Scientific Names of Fishes of the British Isles. *Journal of Fish Biology,* **41,** Supplement A. 37 pp.

Wheeler, A. (1992b). *The pocket guide to saltwater fishes of Britain and Europe.* Dragon's World Ltd.: London, 156 pp.

Wiklund, T and Bylund, G. (1991). A cytochrome oxidase negative bacterium (presumtively an atypical *Aeromonas salmonicida*) isolated from ulcerated flounders (*Platichthys flesus* L.) in the northern Baltic sea. *Bulletin of the European Association of Fish Pathology,* **11,** 74-76.

Williams, H. H. (1963). Parasitic worms in marine fishes: a neglected study. *New Scientist,* **12,** 156-159.

Williams, H. H. and Jones, A. (1994). Parasitic worms of Fish. London: Taylor and Francis, 593 pp.

Wilson, J. A. and Westerman, R. A. (1967). The fine structure of the olfactory mucosa and nerve in the teleost *Carassius carassius. L. Z. Zellforsch Mikrosk. Anat.* **83,** 196-206. (Cited in Morrison and Odense, 1978).

Winberg, G. G. (1956). Rate of metabolism and food requirements of fishes. Belorussian State University, Minsk. 251 pp. [*Fisheries Research Board of Canada* Translated Series No. 194].

Winberg, G. G. (1961). New information on the metabolic rate in fishes. From Voprosy Ikhtologii, Volume 1, Number 1 (18), p 157-165. [*Fisheries Research Board of Canada,* Translated Series, No, 362].

Windell, J. T. (1966). Rates of digestion in the bluegill sunfish. *Invest. Indiana Lakes Streams,* 7, 185-214.

Windell, J. T. (1978). Digestion and the daily ration of fishes. In: *Ecology of Freshwater Fish Production* edited by Gerking, S. D. Blackwell Scientific Publications: Oxford, London, Edinburgh, Melbourne, pp. 139-183.

Windell, J. T., Norris, D. O., Kitchell, J. F. and Norris, J. S. (1969). Digestive response of rainbow trout, *Salmo gairdneri,* to pellet diets. *Journal of the Fisheries Research Board of Canada,* **26,** 1801-1812.

Windell, J. T., Kitchell, J. F., Norris, D. O., Norris, J. S. and Foltz, J. W. (1976). Temperature and rate of gastric evacuation by rainbow trout, *Salmo gairdneri. Transactions of the American Fisheries Society,* **6,** 712-717.

Wolf, K. and Markiw, M. E. (1984). Biology contravenes taxonomy in myxozoa: New discoveries show alteration of invertebrate and vertebrate hosts. *Science,* **225,** 1449-1452.

Wooten, R. J., Evans, G. W. and Mills, L. A. (1978). Annual cycle in female three-spined sticklebacks (*Gasterosteus aculeatus* L.) from an upland and lowland population. *Journal of Fish Biology,* **12,** 331-343.

WWW. (1997). Turbot dispute. *http://gurukul.ucc.american.edu/ted/turbot1.htm*

Xie, X. and Sun, R. (1990). The bioenergetics of the southern catfish (*Siluris meridionalis* Chen). I. Resting metabolic rate as a function of body weight and temperature. *Physiological Zoology,* **63,** 1181-1195.

Xie, X. and Sun, R. (1992a). Maximum ration level in the southern catfish (*Siluris meridionalis* Chen): in relation to body weight and temperature. *Acta Ecologica Sinica*, **12,** 225-231.

Xie, X. and Sun, R. (1992b). The bioenergetics of the southern catfish (*Siluris meridionalis* Chen): growth rate as a function of ration level, body weight and temperature. *Journal of Fish Biology*, **40,** 719-730.

Xie, X. and Sun, R. (1993). Pattern of energy allocation in the southern catfish (*Siluris meridionalis*). *Journal of Fish Biology*, **42,** 197-207.

Yamamoto, M. (1978). Morphological changes in the oesophageal epithelium of the eel, *Anguilla japonica*, during adaptation to seawater. *Cell and Tissue Research*, **192,** 25-38.

Young, H. K. (1994). Do non-clinical uses of antibiotics make a difference? *Infection Control and Hospital Epidemiology*, **15,** 484-487.