RHYTHMIC STOMACH FULLNESS IN ATLANTIC SALMON (*SALMO SALAR* L.) AND THE INFLUENCING VARIABLES

Thesis submitted for the degree
of Doctor of Philosophy
at the University of Stirling

by

Claire E. Rawlings B.Sc., M.Sc.

The work presented in this Thesis is the result of my own investigations and has neither been accepted nor is being submitted for any other degree.

.....E. E. Mawling..... Candidate

.....7th October 1994..... Date

CHAPTER 3 - THE EFFECT OF SEASON ON RHYTHMIC STOMACH FULLNESS

CHAPTER 4 - RHYTHMIC STOMACH FULLNESS OF SMOLTS HELD IN SEA CAGES IN THE SUMMER

CHAPTER 7 - THE EFFECTS OF TEMPORAL DIFFERENCES IN FEEDING ON MEAL SIZE

CHAPTER 8 - GENERAL DISCUSSION

LIST OF TABLES AND PLATES

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

LIST OF FIGURES

CHAPTER 1 PAGE NO.

CHAPTER 3

CHAPTER 5

CHAPTER 6

ACKNOWLEDGEMENTS

Only one name appears on this Thesis, but I feel it would not be complete without mentioning the "support Teams". The work was largely carried out at S.O.A.F.D. Pitlochry and I would like to express my gratitude to the staff there especially to Dr. R. Morgan. Also to the staff, Mike Miles, Steve Keay and Jim Muir at Almondbank for their technical assistance. I would like to thank my supervisors, Dr. J. E. Thorpe for his untiring support and encouragement during this research, and Dr. N. Bromage for his support throughout.

I would like to thank B.P. Nutrition for its financial backing and its staff at Lismore and Lerang, but especially Rob Sinnott, Arthur Gilles and Clive Talbot (an original supervisor) for their technical assistance in carrying out experiments.

This work would not have been possible without friends and family. I would especially like to thank Lee and Mike for their friendship and frequently being there. My parents who have given me both financial and emotional support during my prolonged education for which words will not survice. Lastly I wish to mention my husband Bruce who has always been there whether hauling nets in the dead of night or wrestling with computers.

To everyone

Thank you.

ABSTRACT

A repeatable pattern of rhythmic stomach fullness was established for Atlantic salmon, Salmo salar L. held in freshwater throughout the year. Seasonal differences in rhythmic stomach fullness were recorded and were observed to fluctuate during summer, autumn and spring with a peak of fullness during these seasons occurring at 1800h. Stomach fullness in winter remained relatively constant, whilst food levels were minimal.

Gut fullness levels of postsmolts held in sea cages during the summer were monitored and a pattern similar to that in the summer in freshwater was observed. Tide and temperature had little influence whilst light levels showed a mirroring rhythmic pattern to that of gut fullness levels.

Under controlled environmental conditions stomach fullness was rhythmic whilst temperature and light changed. A correlation between temperature and foregut fullness was recorded. Simulated ambient photoperiod (i.e. the correct number of hours of daylight, but once on, unchanging) however, appeared to be insufficient to synchronise feeding peaks. Rhythmic stomach fullness was monitored under three different light regimes to establish the influence of fluctuating light levels. Only under simulated ambient light and photoperiod was a mirror pattern, of gut fullness and peaks in foregut fullness, to that of ambient light and

photoperiod recorded. Under simulated ambient photoperiod a rhythm was observed but dissimilar to that found under ambient environmental conditions. Changing light levels as opposed to specific light intensities are important in synchronising the timing of feeding peaks. Temperature was thought to be of secondary importance in the presence of fluctuating light levels.

Postsmolts held in sea cages were found to consume a larger meal size when fed a single meal in afternoon compared to the morning. When fed a meal both morning and afternoon the afternoon meal was again larger, thus indicating regardless of previous dietary history, the time of day influences the amount of food taken. It is hoped that the establishment of rhythmic stomach fullness will be useful in designing feeding regimes for both research and commercial use.

CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

One of the largest outlays on a commercial fish farm is the cost of fish feed, therefore any reduction in this expenditure is of great commercial value. Also, concern has been expressed recently concerning the amount of deposits on the seabed under seacages, largely contributed by uneaten feed pellets, and the impact that this may have on the environment. If feeding regimes could be designed to ensure more food is consumed by the fish and therefore less food is left to fall uneaten to the sea bed, this would be advantageous to all concerned.

The primary aim of the present study has been to further our understanding of basic salmonid feeding behaviour, thus helping in the design of future feeding experiments and accurate intrepretation of existing gut fullness data. The secondary aim however, has been to gather data to allow the design of new feeding regimes for use on commercial fish farms.

This research has been funded by B.P. Nutrition UK LTD (Northwich, Cheshire) in collaboration with the University of Stirling (Institute of Aquaculture) under Dr. N. Bromage and Scottish Office Agriculture and Fisheries Department, Freshwater Fisheries Laboratory (Faskally, Pitlochry) under Dr. J. E. Thorpe.

1.2 LIFE HISTORY

The life history of the Atlantic salmon, Salmo salar L., has been widely studied (Jones, 1959; Malloch, 1975; Netboy, 1980; Mills & Graesser, 1981; Shearer, 1992). Although it is not appropriate to go into this subject in depth in this study, a brief outline will be given.

Atlantic salmon are an anadromous species spawning in freshwater, where their young develop for one or more years before migrating to sea. Allan & Ritter (1975) have defined seven successive life stages: alevin, fry, parr, smolt, post-smolt, salmon and kelt. The first three stages are found only in freshwater whilst the latter four stages are common to both habitats.

In Scotland, most rivers without either natural or man-made obstructions (i.e. high waterfalls or dams) support Atlantic salmon populations. Due to problems of pollution this is not true of many rivers in the rest of Britain. Juvenile salmon usually spend 1-5 years in freshwater developing through alevin, fry and parr stages before becoming smolts - a descriptive category associated with emigration from the river in the spring. Whether or not emigration occurs in a particular spring depends on a physiological decision taken during the previous summer. This decision influences both appetite and growth and it has been suggested (Thorpe, 1986) that it depends on

developmental performance exceeding a genetically determined critical threshold. Those individuals in which the critical threshold is exceeded, maintain appetite in late July, and continue to grow through the autumn: those in which it is not, become anorexic and arrest growth thus influencing subsequent appetite and growth. However, the actual ability to perform is ultimately determined by environmental opportunity. As not all members of a population may exceed the critical threshold in any given year, the consequent divergence of growth patterns results in a bimodal length frequency distribution within the population, which becomes clear by November. The upper modal group (UMG) represents those fish that have maintained appetite and growth and which will migrate in the following May. The lower modal group (LMG) are those individuals which have reduced both their metabolic and growth rates and will remain in freshwater for at least another a year (Thorpe et al, 1992).

Scottish smolts that migrate down river in spring may travel considerable distances into the Norwegian or Greenland seas during feeding. Adult salmon return to their natal river after one or more years at sea, and then spawn between mid October and February. The female selects gravelly areas of stream bed and excavates a hollow (redd) in which to lay her eggs. A spawned salmon remaining in freshwater is termed a kelt. In Scotland, less than 5% survive to spawn a second time (Mills & Piggins, 1986).

1.3 COMMERCIAL FISH FARMING

The commercial production of Atlantic salmon under intensive culture conditions has developed greatly within the last 30 years. A few companies carry out both the freshwater and seawater stages of salmon farming, whereas others concentrate on one of these phases only. This has resulted in dividing the industry into smolt producers and market size growers, each with their own skills and technology.

The main objective of the smolt producers is to grow large smolts in the shortest possible time, usually a fish ready for sea less than 1 year after hatching. Smolt producers either maintain their own broodstock (which is costly) or buy in certified disease-free ova from other fish farms. If they produce smolts for a specific farm, brood stock from that farm's sea site are spawned artifically (stripped) to maintain the particular genetic strain.

The salmon are fed a dry pelleted diet and are grown through alevin, fry and parr to the smolt stage in freshwater tanks, or in cages in freshwater lochs. Smolting is characterised externally by 'silvering' due to the deposition of guanine in the skin obscuring the cryptic colouration associated with life in a river (parr markings). Although this is the most obvious visible change in morphology, smolting fish also become longer and thinner.

They become restless, shoaling in midwater, and fish that have been swimming against the current in a tank i.e. facing upstream, may turn and swim intermittenly downstream (Sedgwick, 1988; Thorpe et al, 1988). Physiological changes also take place: the fish become more buoyant, the number of chloride cells in the gills increases reflecting changes in osmoregulatory ability, urine production by the kidney first increases and then declines, and the ratio of porphyropsin : rhodopsin pigments in the retina changes. The amount of rhodopsin increases substantially, apparently in order to adjust to the changes in environment (Whitmore & Bowmaker, 1989). The smolts are then ready for transfer to saltwater. These physiological processes are influenced by increasing number of daylight hours and temperature and are possibly mediated by pituitary and thyroid permissive hormones. However, timing of sea entry, or transfer from freshwater to seawater rearing units varies depending on the health and size of the fish.

A good source of freshwater is essential for smolt production, so freshwater sites are often situated some distance from cage sites. Depending on the relative position of the two sites, fish can be transferred by road in trucks, by sea in well boats, or by air in helicopter buckets.

The smolts are stocked at low density in either square or round cages at sea sites in May and June and grown on for

14-20 months. In Scotland, a typical farmer will stock a $200m^3$ cage with 6,000-8,000 smolts, thinning them down to 4,000-5,000 by early winter (Laird & Needham, 1988). Few farmers handle post-smolts before the temperature has dropped below $10^{o}C$ due to the risk of extensive scale loss and susceptibility to furunculosis and vibriosis disease outbreaks. Prior to the temperature decrease the only husbandry involved is feeding, net changing, parasite and disease treatment and predator control.

1.4 FEEDING BEHAVIOUR

Feeding behaviour involves a complex of sensory and motor processes which result in patterns that can be recognised in five successive steps as defined by Pavlov & Kasumyan (1990):

1. Rest
2. Readiness to feed
3. Receipt of signal of food presence
4. Search and discovery of source of signal
5. Determination of suitability of food

The resting phase has been shown in rockling, Gaidropsarus mediterraneus, as the period after an individual has reached a definite level of fullness (Pavlov, 1962: cited Pavlov & Kasumyan, 1992) and is thought to be evident in most species of fish. As phase 2 is entered fish become receptive to signals of food presence. In phase 3 the fish perceive the

signals by one or several sensory systems including vision, olfaction, taste, hearing, lateral line, electroreception, touch and chemical sense. These senses are used to a varying degree throughout feeding (Pavlov & Kasumyan, 1990). Their use will depend on the fish's habitat, the limits of detection and the species being preyed upon. Ali (1961) noted that juvenile Atlantic salmon are predominantly visual feeders, whilst Talbot & Higgins (1983) noted that tactile and olfactory senses are also used by salmon for feeding in freshwater. Jorgensen & Jobling (1992) reported that Atlantic salmon feed at night during winter and then only when allowed access to the tank bottom. This was explained by a shift from a visual to an olfactory feeding strategy. The sense of smell has the greatest effective distance range but has a less precise orientation. Therefore, although smell may alert the fish to the presence of a food item, other senses such as vision may be needed for precise location.

In phase 4, when the fish search for and discover the food item from which the signal was received, several senses may interact. Also the order of priority of these senses may alter as the food item is approached. Vision is the most informative sense relaying information on size, shape, colour, brightness and movement, which is important for phase 5, determining the suitability of the food item.

For fish held in a tank environment phases 3-5 are

condensed into a matter of seconds once food is introduced into the tank. Metcalfe et al (1986, 1987) studied seasonal changes in feeding motivation of juvenile Atlantic salmon and described a detailed sequence of steps during phases 3-5. They distinguished initial detection of the potential prey, then an orientation followed by movement towards the prey, attack, ingestion, and swallowing. These first steps were visual responses, but after ingestion they were tactile and chemical. If one sensory component of feeding behaviour is immobilised, for example in darkness or turbid conditions, where vision is rendered useless, priority must shift to one of the alternative senses to locate food.

1.5 VISION

The retina of the fish's eye contains 2 types of light sensitive cells: cones which are used for colour vision in bright light and rods which can detect various shades of grey in dim light (Muntz & Wainwright, 1978). The fish's eye adapts for vision at different light levels by photomechanical movements. In the light the cones are aligned along the external limiting membrane freely exposed to light, whilst the rods, with elongated myoids are enveloped by pigment granules inside pigment cells. In the dark the cone myoids lengthen, moving the cones away from the external membrane. The rods contract their myoids and migrate out of the pigment towards the limiting membrane.

Pigment granules move out of the pigment extensions and concentrate inside the peripherally located cell bodies (Fig. 1.1). It is thought that the retinomotor action has an endogenous rhythm which is interrupted under continuous light (Schwassmann, 1971). Salmon may be able to locate food at night provided there is sufficient light for the rod cells to operate under. Fraser et al (1993) reported that juvenile Atlantic salmon became increasingly more nocturnal (emerging to feed at night) as the temperature dropped below 10 ^{o}C. The sensitivity of several salmonid species to low light generally increases in winter due to seasonal increase in the total amount of visual pigments in the retina (Allen et al, 1982) and increase in proportion of porphyropsin.

1.6 CHRONOLOGY OF BEHAVIOUR

The concept of the presence of periodical rhythms in biology was first introduced when an astronomer, De Mairan, investigated leaf movements more than 200 years ago in 1729. The term chronobiology was introduced later to describe the study of biology in relation to time. It has only been within the last 30-40 years that detailed research has been carried out and reported (Cloudsley-Thompson, 1961; Harker, 1964; Bunning, 1960, 1967; Schwassmann, 1971; Parker, 1984). The terminology used is often confusing, therefore the following definitions (Jenkins & Green, 1977; Monk, 1982) will be used:

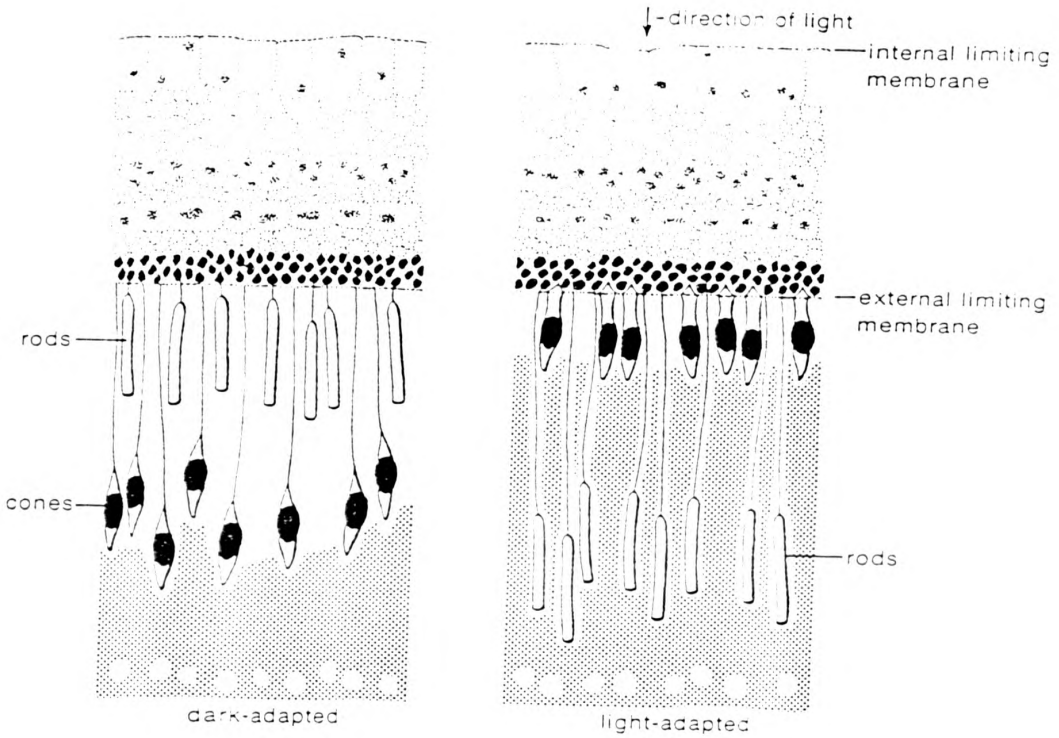


Figure 1.1. Diagram illustrating movement of rods, cones, and pigment in the retina of teleosts. In light adaptation (right) the rods are moved away from the light and are protected by forward movement of pigment.
(From: Biology Of Fishes Ed. C Bond)

A 'pattern' may be identified which shows the existence of a trait characterizing a behaviour over time. The pattern may exhibit a predictable regularity or 'periodicity' such that the sequence of observations starts repeating itself. This periodicity is termed a rhythm and each complete sequence of events is regarded as a cycle.

Reference to the homeostatic state of an organism is an oversimplification. Measurement of physiological variables indicate that the physiology of an organism is maintained in a relatively stable state (homeostasis) by rhythmic oscillation between certain limits (Minors & Waterhouse, 1986). Such a situation is apparent in the amount of food present in the intestine.

Since biological processes require a finite amount of time it would seem advantageous for an animal to be able to prepare for predictable events (Harker, 1964). Examples of such preparations are: activation of a feeding bout before nutrition depletion becomes critical and the presence of enzymes to breakdown nutrients once consumed (Armstrong, 1990). The enzymes present in the small intestine of rats show a rhythm which reflects anticipation of food ingestion, and these are still evident during food deprivation (Saito, 1972). Relatively little work on rhythms of digestive enzymes has been carried out in any fish species so we can only hypothesise that a similar system of anticipatory preparation exists. This would be most likely if fish feed

at regular and predictable times.

Observed rhythms which oscillate at approximately once per 24 hours are termed circadian (from circa=about, dies=day) rhythms, whilst rhythms which oscillate every year are termed circannual (Halberg, 1959 cited Harker 1964). The feeding rhythm is an overt rhythm and not the endogenous timing mechanism itself. An overt rhythm, if maintained under otherwise constant conditions, is the expression of the endogenous rhythm and therefore an indicator of its presence (Schwassmann, 1971).

1.7 FEEDING PATTERNS

A complex series of physiological and environmental interactions control the onset of feeding. Integral mechanisms that suppress or inhibit feeding of fish have also been implicated . Some of these factors are associated with short term regulation (i.e. the termination of a feeding bout) while others regulate long term appetite and therefore maintain body weight and condition factors (Holmgren et al, 1986). These levels of control are seen ultimately in the amount of food taken into the stomach of the fish. If there was no control then the only restraint on fish feeding would be stomach size and feeding would be initiated and terminated according to stomach fullness. Brett (1971) found considerable variation in the amount of food in a 'full' stomach of sockeye salmon, Oncorhynchus

nerka. Not all the variability could be accounted for by morphologically different stomach sizes of the fish. The variability was also attributed to physiological and behavioural factors. Hence the changes in stomach fullness with time indicate some degree of control.

Very little is known about the regulatory mechanisms of the gastro-intestinal tract in the fish: most information has been gained by studying the emptying of the mammalian stomach. Once the food has been consumed it passes from the mouth to the pharynx, oesophagus and to the stomach. Once in the stomach digestible solids are reduced to a specific particle size before they can pass through the pylorus (Meyer, 1980). The food consumed is reduced into chyme both by enzymatic action and contractions of the gastric musculature. The emptying of the food from the stomach is not a continuous smooth process but occurs in small pulses (Jobling, 1984). The timing of emptying of pulses of chyme into the intestine is determined by feedback loops and is dependent on the nutrient concentration of the gastric chyme. When the stomach contains nutrient-rich food gastric emptying is slower than when the contents are less energy-rich (Jobling, 1984). Other factors that affect gastric emptying are temperature, meal size, frequency of feeding, fish size and fish species.

1.8 TIME OF DAY

Living systems have an inherent circadian organization, which is most noticeable in the diel rhythmic nature of a large majority of physiological states. The selective advantage of this system has been defined by Schwassmann (1971) as 'the unique manner in which the system causes periodic changes in the organism's physiological state, regulating its activities which are often best performed at specific times of the daily cycle'. The reaction therefore, to a specific stimulus will result in a wide variation of biological responses dependent on time of presentation of the stimulus. This concept has an extensive range of consequences for both fish farmer and researcher alike and should be considered continually. Some examples of specific stimuli in aquaculture which may elicit different responses at different times of the day are: administering medication, hormone injection, temperature and feeding.

When medication is being administered, pathogens may also show a diel response to the medication. An optimal time of the day for treatment should occur when the host exhibits a decreased sensitivity and the pathogen an increased sensitivity to the medication (Spieler, 1977).

Benefits can be gained by specific timing of hormone injections. For example prolactin injections altered the quantity of body lipids in golden shiners, Notemigonus

crysoleucas. Lipid declined in fish injected 2 hrs after the beginning of light onset and increased in fish injected 10 hours after light onset in a 15.5L:8.5D photoperiod (Pardo & de Vlaming, 1976).

Daily husbandry practices disturb the fish and may stress them, affecting weight gain. Also lipid deposition and gonadal growth are adversely affected at some times of the day (Spieler 1977). These responses may be mediated by hormone rhythms and since these show seasonal variation the specific response will also differ seasonally (Spieler et al. 1976).

Elevated water temperature tends to enhance growth (Seymour, 1989) but heating the water is expensive. In goldfish, Carassius auratus, 4 hour periods of increased water temperature elicited different gonadal growth and body weight gain compared to either constant or no additional heat (Spieler, 1977). Trials on Atlantic salmon have also shown that increased growth rates at higher temperature can be maintained when heated water is supplied at timed intervals (Seimen & Carline, 1989). Timed heat or thermocycles may decrease heating costs whilst giving a more precise control over the life cycle.

Prey animals also show diel activity rhythms, so that prey is not constantly available to salmon (Simpson, 1993). Hence, food intake is likely to be discontinuous, so that

discontinuous meals may be a more efficient method than continuous feeding in fish culture. The response of fish to meal feeding may depend on the timing of the meal in relation to the animal's circadian rhythms, or to other periodic factors that influence these rhythms. Differences in such responses have been reported in appetite, somatic growth, condition factor and gonadal growth (Noeske et al, 1981).

1.9 ENVIRONMENTAL EFFECTS

Living organisms have evolved in a periodic environment, due to the daily rotation of the earth about its polar axis, and its annual rotation about the sun. To exist, organisms rely on their capacity to tolerate or resist changes in the environment that occur as natural diel or seasonal variations. Some organisms have developed further, to maximise the use they make of variable conditions such as increased light and temperature levels during different seasons (Schwassmann, 1980). Since photoperiod cyclicity is more reliable than thermoperiod, photoperiod is the dominant physical oscillator regulating a large number of functions including food availabilty, reproduction, growth and movement between habitats.

The link which transduces information from the external environment into the internal physiological system has been explored chiefly in higher vertebrates. In the eye of the

rat the retina relays information on light changes to the pineal gland by a complex series of neural pathways (Moore & Card, 1985). The situation in fish is not so clear, as the pineal gland, unlike in mammals, may perceive light signals directly. The pineal gland translates these into a hormonal signal, synthesising melatonin in dark periods, which may be used then by the organism to make the necessary physiological adjustments (Axelrod, 1974).

1.9.1 Light

Brett (1979) noted that 'studies on the influence of light on various physiological parameters have often led to complex and confusing results'. This is due mainly to the multiple properties of light (e.g. quality, quantity and periodicity) and to interactions of light with other environmental variables, mainly temperature. Many studies have investigated the effect of different light properties on fish growth. Research on the effect of different light intensities on fish growth has used constant light intensities (Wallace et al, 1988). Therefore, daily periodicity of light-dark changes were omitted. The results from such studies only indicate that fish grew better under one continuous light intensity than under another. The effect of unchanging spectral composition on fish growth has also been assessed (Stefansson & Hansen, 1989). Varying the periodicity of light has shown that photoperiod does act as a synchroniser of major developmental changes such as

maturation, reproduction and smolting (Saunders & Henderson, 1970; Wagner, 1974; Wedemeyer et al, 1980; Villarreal et al, 1988; Stefansson et al, 1989). More interesting as far as feeding is concerned are the effects of light as a temporal influence, and how light-dark changes may sychronise physiological events.

1.9.2 Temperature

Temperature governs both the fish's metabolic requirements for food, and the processing rate of that food. It is thought to act only as a minor influence in sychronising circadian rhythms (Nelson et al, 1975, but see also Fraser et al 1993). The majority of work on the effects of temperature on food intake has used constant temperature regimes without ambient fluctuations. A relationship between food and temperature has been noted for several fish species including rainbow trout, Oncorhynchus mykiss (Grove et al, 1978); eel, Anguilla anguilla, (Seymour, 1989); and Atlantic salmon (Farmer et al, 1983).

The rate of transit of food through the digestive tract is greatly affected by the water temperature in which the fish have been acclimated. The gastric evacuation rate has been shown generally to increase with increased temperature, although there are also complex interactions with other variables e.g. fish species and size, meal size and frequency of feeding. Fauconneau et al (1983) found that when the temperature was raised from 10-18 ^{o}C, it took 8

days before the transit rate of food through the gastric tract of rainbow trout had adjusted to the higher temperature. Hence, if the time to acclimate is related to temperature increase linearly it would take a whole day for the digestive tract of a rainbow trout to adjust to a 1 ^{o}C rise (a conceivable ambient increase within a couple of hours). Therefore, immediate effects on the rate of transit of food should not be observed in response to increases in ambient water temperature.

1.10 IMPORTANCE OF RHYTHMIC STOMACH FULLNESS

Salmonids have evolved in the wild for at least 100 million years, under conditions in which feeding is affected by food availability and interaction within and between fish species (Eriksson & Alanara, 1992). For example, a riverine population of juvenile chinook salmon, Oncorhynchus tshawytscha, in November, showed a decrease in dry weight after dawn and the greatest number of fresh prey in their stomachs in the afternoon (Sagar & Glova, 1988). This feeding periodicity was explained by both prey abundance and previous dietary history. Atlantic salmon and brook trout, Salvelinus fontinalis, were sampled in a Newfoundland river to assess competition for food (Thonney & Gibson, 1989). Within the salmon population competition for food was reduced by spatial segregation, but severe competition was observed between large parr and brook trout. The effects of short term temporal differences (i.e. not daily but hourly)

were excluded from this study.

Salmonids live at moderate to high latitudes, characterised by regular patterns of environmental change. These are reflected in the salmon's rhythmical temporal feeding patterns. Juvenile coho salmon, Oncorhynchus kisutch, sampled off Oregon in the summer, exhibited peaks of fullness in early morning and around dusk (Brodeur & Pearcy, 1987). Juvenile pink salmon, Oncorhynchus gorbuscha, sampled in two marine bays of British Columbia in May, showed a maximum biomass in their stomachs near dusk (Godin, 1981). Only a few temporal studies of salmon feeding in the wild have been performed due to feasibility problems: of these, none are known to have been carried out on Atlantic salmon.

Salmonids have only been subjected to intensive farming conditions for a few decades. Consequently it is unlikely, that their feeding behaviour and physiological rhythms will have adapted genetically to fish farming practices. However since there have been few studies of temporal variation of salmon feeding, farm routines and feeding regimes have been designed for human convenience rather than for conditions particularly suited to the fish. In the last 10 years two major problems have been highlighted in feeding of salmon in culture: firstly the amount of food wasted; and secondly the impact of fish farming on the environment. The problems are

linked, since as more food falls uneaten to the sea bed under the cage, more pollution may be caused. The first problem is of more concern to the fish farmers as this results in a loss of income and increasing food bills (Ingram, 1990). While the second problem is of concern to conservation organisations it is also important to fish farmers, for whom a clean environment is vital for the production of healthy fish.

One solution to both these problems depends on improving feeding efficiency by presenting the food at times when fish can use it most efficiently (Seymour & Bergheim, 1991; Thorpe & Huntingford, 1992), thus ensuring that more of the food offered will be consumed. This cuts feed costs and reduces the amount left to fall as uneaten waste, thus decreasing pollution of the environment.

If fish are offered a constant and continuous amount of food over time, but the amount of food present in the intestine does not remain constant, then the fish are showing a preference for taking food at particular times of the day. If meals are fed at these times then it is hypothesised that more food would be consumed and less would fall as uneaten waste. Investigations of feeding rhythms are therefore important for understanding the basic biology of Atlantic salmon and may be invaluable commercially in designing new and appropriate feeding regimes. It is also important to establish what influences and possibly controls

these feeding rhythms for these can be exploited to improve food intake and hence growth.

As highlighted above, although both wild and farmed Atlantic salmon are relatively abundant in both fresh and coastal waters little is known about their feeding rhythms or patterns. Early research concentrated on what they feed as opposed to when they feed. This was necessary information to ensure the maintenance of food resources for wild salmon in managed rivers and to manufacture the optimal artifical diet for cultured salmon. However, it is important to understand when the fish feed to ensure maximal feed consumption in cultured salmon. It is necessary to identify whether salmon exhibit a feeding rhythm by consuming defined meals (e.g. identified as sudden increased gut fullness) or simply maintain a constant amount of food in their guts throughout the day by nibbling (small frequent feedings). This is important in the regulation of metabolism. In rats, meal-eating compared to nibbling results in a 50 - 100% increase in body fat (Cohn & Joseph 1960). It is also important to understand how feeding patterns may alter with season as this will help to establish the role of factors such as temperature, light levels and daylength over long periods. Since light and temperature are continually varying within a 24 hour period it is also important to identify their influences on the feeding patterns of salmon over short periods.

The present study sought to address the problems and some of the gaps in our knowledge of the feeding of Atlantic salmon by testing the following null hypotheses:

1. Atlantic salmon do not show fluctuating amounts of gut fullness when held in freshwater.
2. If gut fullness does fluctuate, then the pattern is constant throughout the year.
3. Light and temperature conditions do not affect the amount of food taken.
4. Atlantic salmon smolts held in seawater cages in the summer do not show rhythmic stomach fullness.
5. If gut contents fluctuate then they do so similarly under all controlled environmental conditions.
6. If gut fullness peaks then this is influenced equally by both light and temperature levels.
7. If patterns of gut fullness are observed then they are the same under all conditions of ambient and simulated, light and photoperiod.
8. Size of a single daily meal is not affected by the time of day it is offerred.
9. Multiple meal sizes are not affected by the time of day they are offered.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.7 LABELLED FOOD

2.8 X-RAYING

2.9 MONITORING

2.10 CALCULATION OF THE AMOUNT OF FOOD PRESENT

2.1 FISH STOCK

Four research facilitites were used throughout this study. The two fresh water sites (Almondbank and Pitlochry) were used continuously throughout the seasons. The two seawater facilities (Lismore Salmon Farm and Lerang Resarch Station) were used only for specific experiments and fish were therefore not kept continuously, for our purposes, at these locations.

The Atlantic salmon used at both the fresh water facilities were derived from River Almond stock. Ripe adult fish were caught by electrofishing in the upper parts of the River Almond, a tributary of the River Tay. They were caught in the winter months, during the fish's journey upstream to spawn, especially after the river has been in a spate condition.

Female and male salmon were hand-stripped of their ova and milt respectively and standard hatchery practices used to fertilize the eggs (Huet, 1972). The fertilized eggs were transferred to hatchery baskets and kept under a low intensity red light. The light was necessary, to allow removal of dead ova thus preventing fungal infection of the live eggs. However the wavelength used was outwith the wavelength range which elicits any behavioural response from salmon (Thorpe, 1981). The light was kept permanently on to prevent the switching on and off of the additional light

entraining a rhythm. The horizontal flow trough system, with corrugated bottom trays was used to incubate the eggs (Thorpe, 1981).

After hatching, the alevins were kept in hatchery trays until the yolk sac had been absorbed and they were ready to feed on artificial dry food. Fry were then moved to 1m radial flow tanks and fed on commercial crumb fish food supplied by BP Nutrition.

2.2 ALMONDBANK HOLDING FACILITIES

The Scottish Office Agriculture and Fisheries Department (S.O.A.F.D.), Almondbank smolt rearing unit is situated north of Perth (56^{o} 24′N,3^{o} 27′W) in a converted aircraft hangar close to the River Almond.

2.2.1 Water supply

The freshwater supply to the unit was taken from the River Almond into a lade about 1km upstream of the station, from which it was piped unfiltered to individual tanks. Suspended solids were a problem only when the river was in spate, during which time the colour of the water ranged from yellow to brown. This reduced the visibility within the tanks, and increased the amount of cleaning necessary.

2.2.2 Tanks

Until required for experiments, stock fish were held in

$4m^2$ tangential-flow square tanks, in which food was introduced above the inflow and the water level controlled by an external stand pipe.

Standard 1m-diameter radial flow tanks (Plate 1) were used for experimental studies. Water and food was introduced via a central vertical pipe, which then flowed radially across a shallowly-conical floor to a peripheral drain. The water level was adjusted by two standpipes situated on either side of the tank (Thorpe, 1981).

2.2.3 Fish Husbandry

Tanks were cleaned regularly by dropping the water level to approximately 5cm and scrubbing the sides, bottom and inflow pipes with a brush. On raising the water level, floating debris was removed by net. If more thorough cleaning was necessary the fish were moved to an aerated holding bin, and the tank was cleaned with an abrasive and rinsed before returning the fish. This practice also allowed the outflow pipes to be cleared of uneaten food and detritus.

Occasionally some fish showed signs of fungal infections (Saprolegnia sp.). These were treated successfully by immersion for 1 hour in a 1ppm solution of malachite green on alternate days for a week.

2.2.4 Feeding

Fish were fed commercial fish diets (BP Nutrition Ltd,

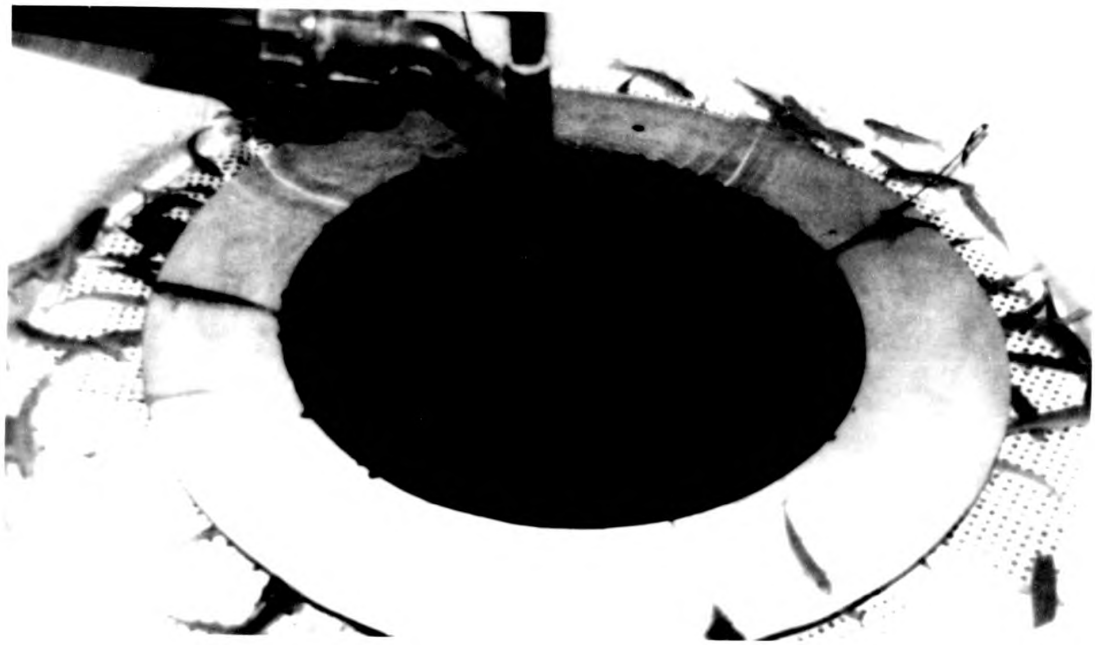


Plate 1: 1m diameter radial flow tanks were used for all experimental work at the Almondbank facility. Water and food entered simultaneously through the central vertical pipe.

Wincham, UK) of appropriate pellet size (Wankowski, 1979). Caddymatic feeders (Minaur, 1973) were used which had been calibrated to deliver the same amount of food to the individual tanks within an experiment. A Sangamo 7-day electronic time clock (RS Components, Corby, UK) was used to regulate the feeding regimes required. The feeders were refilled regularly to ensure a constant supply.

2.2.5 Lighting

Tanks within the hangar were maintained under an ambient light regime, but at intensities of reduced amplitude compared to the natural regime outside the building. Fluorescent tubes were used to supplement the light intensity in the hangar, if necessary, when experiments were not in progress. These lights were always switched off for the period between dusk and dawn.

2.2.5a Controlled Lighting

For controlled photoperiod experiments the tanks were surrounded by a black plastic tent, and illuminated by fluorescent tubes contained in a box with a movable blind. The timing of closure of the blind was controlled centrally, automatically programmed to simulate natural photoperiod (together with twilight) at 56 ^{o}N. This system could also be over-ridden to give constant continuous light.

2.2.6 Temperature

Air temperature was controlled only to keep it above

freezing during winter months. Water temperature was normally that of the River Almond and was continuously recorded.

2.2.6a Controlled Temperature

Water temperature could be controlled for specific tanks, either by raising it by a constant amount above ambient, or by heating it to a pre-set constant level. In the latter case, if the ambient water temperature exceeded this level, the heater automatically cut out and the temperature varied as ambient until the heater was reset manually. During such times the frequent cutting out of the heater resulted in reducing the ambient fluctuations although not maintaining a constant water temperature.

2.3 PITLOCHRY HOLDING FACILITY

The S.O.A.F.D., Freshwater Fisheries Laboratory, Faskally, is situated at Pitlochry (56^{o} 43′N, 3^{o} 43′W). The tanks here were contained in three separate rooms and were set up specifically for the present experiments during March and April 1990.

2.3.1 Water Supply

Water was pumped from Loch Faskally through a header tank and supplied to the tanks as needed.

2.3.2 Tanks

Two tank types were used: $1m^2$ tangential flow square

fibreglass tanks, with a central drain covered with a fixed perforated cover (Plate 2), and single standpipes to control the water level; and 1m - diameter radial-flow tanks as described previously (Thorpe, 1981).

2.3.3 Fish Husbandry

Tanks were cleaned regularly by lowering the water level as described previously (section 2.2.3). Some fish showed signs of Saprolegnia infection and were treated as before. Also fish showing signs of flashing (diving at the perforated drain cover) resulting in surface abrasion were treated by 1 hour immersion in 1 ppm Chloramine T (BDH, Glasgow, Scotland) in water once a week.

2.3.4 Feeding

The $1m^2$ square tanks were fitted with 25cm diameter open disc feeders (Gearing, Isle of Skye, Scotland) in which the food gathered in front of an adjustable barrier and was trickled into the water as the disc moved. Food was placed around the disc so the fish were fed at a rate of 3% of their body weight per day continuously throughout the day and night. The fish were monitored regularly to assess body weight and so to determine the 3% ration.

Radial flow tanks were fitted with spinning disc feeders (Ewos Ltd, Bathgate, Scotland), which dropped feed into the hopper of the supplementary water supply. Both types of feeders were activated for a 5 minute period every hour by a

Plate 2: $1m^2$ tangential flow tanks were used for experimental work at the Pitlochry facility. Water entered via a peripherally situated inflow pipe. Food was dropped into the water before the inflow pipe therefore ensuring maximum despersal.

timing clock (RS Components, Corby, UK). During this time the feeders were centrally controlled (Gearing, Isle of Skye, Scotland) to allow activation frequency and length of time per activation to be altered.

2.3.5 Lighting

Tanks under ambient light received supplementary red fluorescent light continuously day and night. This gave sufficient light for night work without disturbing the fish. The spectral composition of these lights (Thorn) fell outside the action spectrum of the Atlantic salmon retina (Ali, 1961), and so did not influence the normal feeding patterns. This is the conclusion that was reached since the same feeding rhythm was attained whether the additional lights were used or omitted.

2.3.5a Controlled Lighting

Tanks with controlled lighting were enclosed in black plastic tents. Each tank had two tungsten lights positioned directly above it whose intensity was controlled by a central programming unit. The period of increasing light levels was specified up to a maximum pre-determined limit, at which intensity they remained constant until the decreasing period commenced. The period of time that the light level was increasing and decreasing could also be controlled.

2.3.6 Temperature

The loch water temperature, recorded regularly, fluctuated less then that of the river at Almondbank and therefore no temperature control was incorporated into the system.

2.4 LISMORE SALMON FARM

Lismore is an island approximately 5 miles off the West coast of Scotland (56^{o} 32′N 5^{o} 25′W) beside the Benderloch Peninsula. The salmon cages were situated in the Oscair inlet on the North-East side of the island (Plate 3). The fish farm, owned by BP Nutrition, was a commercial production site with limited research facilties.

2.4.1 Cages

Access to the cages was via work boats from Port Appin - approximately a 30 minute boat ride. The majority of cages at this site were 12m square Kames cages (Kilmelford, Oban, Scotland) linked by metal walkways. A 5m square net was suspended in a large cage for experimental work. X-raying (see below) was carried out in the housing block, a purpose-built pine house on a cement floating block accessible only by sea.

2.4.2 Fish Husbandry

Post-smolts were held in nets which were not treated with anti-fouling chemicals and were therefore changed regularly

Plate 3: Lismore Salmon Farm, a commercial farm, consisted of a walk-way surrounded by 20 cages. The farm was serviced from a housing barge which had a generator to supply limited electricity.

and cleaned with high-pressure hoses. Seawater temperature was recorded automatically every hour (Squirrel Logger, Grant Instruments, Cambridge, UK). Sea lice, Lepeophtheirus salmonis and Caligus elongatus, a major problem on older fish, were not seen on these fish.

2.4.3 Feeding

The feeders were a spinning disc type below a bucket food hopper (Aquatess, Ullapool, Scotland), calibrated to deliver 65g of feed per activation. The feeders were set to deliver food throughout the day and night.

2.5 LERANG RESEARCH STATION

The Lerang Research Station is situated at Forsand, east of Stavanger, Norway (59^{o} $5'$N, 6^{o} $2'$E).

2.5.1 Cages

The seawater facilities which consisted of 5m square cages were used for experimental work (Plate 4). Again the cages were linked by metal walk-ways and access to the cages was via small motor boats. X-raying was carried out on the cage site.

2.5.2 Fish Husbandry

Post-smolts were held in nets treated with anti-fouling chemicals, so net changes were less frequent than at Lismore. The frequency of net changes was dependent on the build-up of algae, which in turn depended on temperature

Plate 4: Lerang Research Station was situated at the head of a fjord, the sea cages were approximately a 5 minute boat trip from shore. The farm was serviced from land, although a temporary sampling station was set up on the cage walk ways for this experiment.

levels. Temperature, oxygen and salinity were recorded automatically every hour on a centrally controlled computer.

2.5.3 Feeding

The feeders were a spinning disc type with a bucket container to hold the food (Ewos, Ltd, Bathgate, Scotland). The frequency of feeding and the amount of food delivered was controlled by a central computer to make control and adjustments more accurate.

2.6 X-RADIOGRAPHICAL TECHNIQUE

An x-radiographical technique was used to determine the feeding patterns of Atlantic salmon. The fish were fed a diet labelled with an inert radio-opaque marker. The fish were sampled frequently and x-rayed (see below). From the developed x-ray plates the amount of food present at each sampling time for each fish was calculated. The amount of food present in each portion of the gut i.e. fore- ,hind- and whole-gut (see p. 50 and Appendix 1), was expressed as the percent dry weight to give an index of gut fullness. Frequent and consecutive sampling gave a pattern of change of gut fullness for the population of fish sampled. Such changes were determined over a 4-5 day periods. When meals were temporally separated, meal sizes were assessed.

2.7 LABELLED FOOD

Fish food was labelled with one of two types of radio-

opaque inert markers, either iron powder (BDH) or glass ballotini spheres (Jencons Ltd, Leighton Buzzard, UK). The markers were sieved to obtain the required size range, depending on the size of fish being fed the labelled food. The food was made up in 1 kg or smaller batches (to ensure uniform mixing of the marker). The marker was included at concentrations between 0.5 and 5 % by weight, depending on fish size, marker size and season. Commercial starter diet (Mainstream '00', B.P. Nutrition, Wincham, UK) was mixed with the correct amount of sieved marker using a Kenwood Catering Mixer. Water was added to form a thick paste, which was then forced through a mincer attachment. This was spread on wire mesh trays and dried overnight in a hot air oven (60 ^{o}C). The food could be stored in this form in sealed bags for up to 3 months. Food was pelleted, by sieving, to provide the optimal size range for maximal growth, diameter approximately 2.5% of fish length (Wankowski & Thorpe, 1979). The pelleted labelled diet was coated in oil provided by BP Nutrition and dried before being fed to the fish.

Quantities of labelled food were weighed to the nearest 0.01mg and x-rayed. The amount of marker present on the developed plate was counted using a light box (GEC, London, UK) and electronic counter (Monostat, RS Components, Corby, UK) to determine the amount of marker present per unit weight of food.

2.8 X-raying

At Almondbank, a static Todd Research Triton 300s x-ray machine (Chelmsford, UK) was set up to expose the plates for 0.4 seconds, at 75mv and 100mA. At the other sites a Todd Research Portable x-ray machine TR 80/20 (Chelmsford, UK), with an x-ray Collimator S120 (Todd Research, Chelmsford, UK) camera lens was used. This was set up to expose the plates for 2 seconds, at 100mv and 20mA.

X-ray cassettes (GEC, London, UK) were loaded with Kodak Industrex CX film (Hemel Hempstead, UK), anaesthetised fish were placed on the cassette, exposed, and the fish were returned to their respective tanks. All exposed plates were developed in a red light dark room using developer (Kodak, LX 24, Hemel Hempstead, UK) and left to dry in hot air drying cabinets. The plates were viewed using a light box and the number of radio-opaque particles present in the intestine of the fish were counted using a hand-operated electronic counter.

2.9 MONITORING

Fish were monitored at regular intervals to calculate the average fish weight per tank, to adjust the amount of food fed and to establish the relationships between fish weight and fork length.

In some of the experiments, particularly when a feeding

rhythm was being monitored, sampling was relatively frequent (i.e. every 4 hrs) for long periods of time (i.e. upto 5 consecutive days). It was decided in these circumstances not to weigh these fish individually at each sampling, to reduce the length of time the fish were kept under the anaesthetic and therefore to reduce stress. For experiments that required less frequent sampling individual weights were taken for each fish x-rayed. It is not possible to determine the fork length from the x-ray plates, as the resolution was not precise enough to resolve the tip of the tail, only cranial length can be determined from the x-ray plates. Two relationships (cranial length to fork length, and fork length to fish weight) were used to establish the wet weight of the fish x-rayed, this process was carried out at the end of each experiment.

A stock solution of anaesthetic was prepared by dissolving benzocaine (BDH, Glasgow, Scotland) (4g) in alcohol (100ml). 10ml of this stock solution was placed in 5L of water in a small tank. The fish were removed from an aerated holding bin, into the tank containing anaesthetic and anaesthetised, wet weight and fork length were recorded on a computer (Epson HX 20) and the fish were returned to their respective tanks. The data were analysed to obtain a linear regression and correlation coefficients of weight on fork length.

The linear regression of cranial length on fork length (which can be related to the fish weight) was also

rhythm was being monitored, sampling was relatively frequent (i.e. every 4 hrs) for long periods of time (i.e. upto 5 consecutive days). It was decided in these circumstances not to weigh these fish individually at each sampling, to reduce the length of time the fish were kept under the anaesthetic and therefore to reduce stress. For experiments that required less frequent sampling individual weights were taken for each fish x-rayed. It is not possible to determine the fork length from the x-ray plates, as the resolution was not precise enough to resolve the tip of the tail, only cranial length can be determined from the x-ray plates. Two relationships (cranial length to fork length, and fork length to fish weight) were used to establish the wet weight of the fish x-rayed, this process was carried out at the end of each experiment.

A stock solution of anaesthetic was prepared by dissolving benzocaine (BDH, Glasgow, Scotland) (4g) in alcohol (100ml). 10ml of this stock solution was placed in 5L of water in a small tank. The fish were removed from an aerated holding bin, into the tank containing anaesthetic and anaesthetised, wet weight and fork length were measured, and recorded on a computer (Epson HX 20) and the fish were returned to their respective tanks. The data were analysed to obtain a linear regression and correlation coefficients of weight on fork length.

The linear regression of cranial length on fork length

(which can be related to the fish weight) was also calculated. A sample of fish was anaesthetised, the fork length measured, the fish were x-rayed and returned to the aerated holding bin to allow recovery before returning the fish to their respective tanks. The cranial length was determined from the x-ray plates by measuring the distance from tip of the snout to the otolith using a micrometer (Vernier Caliper, RS Components, Corby, UK).

The two relationships (cranial length and fork length and fork length and fish weight) were used to relate cranial length (measured from the x-ray plates) to fish weight. From this measurement dry weight of the fish, taken as 25 % of wet fish weight was calculated (Higgins & Talbot, 1985).

2.10 CALCULATION OF THE AMOUNT OF FOOD PRESENT

A calibration curve for each batch of food was prepared by x-raying known weights of food and counting the amount of marker present. The marker present in the fish's intestine was counted, therore allowing the dry weight of food present in the intestine of the fish to be calculated. The ratio of dry weight of food/dry weight of fish x 100 (% DBW) was calculated as an index of gut fullness for each part of the intestine. The amount of marker present in the foregut was counted separately from that in the hindgut, the total amount of food present was calculated as the sum of the two, also referred to as wholegut fullness.

CHAPTER 3

THE EFFECT OF SEASON ON RHYTHMIC STOMACH FULLNESS

3.1 INTRODUCTION

Atlantic salmon are known to consume more food by day than by night (Higgins & Talbot, 1989; Jorgensen and Jobling, 1992). Hence, their feeding is not constant but discontinuous. However, it is not known that a feeding pattern exhibited at any one time of the year will be exhibited without alteration throughout the year, because appetite is not constant (Thorpe et al, 1992). A study of seasonal changes in feeding motivation of juvenile Atlantic salmon observed that the lower modal group (LMG) have a suppressed appetite in their first summer resulting in no growth during the winter (Metcalfe et al, 1986). The overall effect this may have on the feeding pattern of salmon has as yet to be investigated.

Environmental parameters also show rhythmic changes both during a 24 hour period associated with the earth's revolution about its axis, and during the year associated with the earth's revolution about the sun. Since the light intensity rhythm (photoperiod) is determined by these geometrical revolutions, light rhythms provide the most dependable environmental signals of season. Several investigations of photoperiod regulation show that photoperiod rhythms act as synchronizers of internal timing of biological processes (Wagner, 1974; Clarke et al, 1978; Eriksson and Lunqvist, 1982; Saunders et al, 1982; Saunders et al, 1987; Saunders & Harmon, 1988; Thorpe et al, 1990).

The light-dark cycle on a daily basis may act as a temporal cue for both physiological processes and overt rhythms like feeding (Moore-Ede, 1981). The increase and decrease in light intensity also indicates a change in biotic environment i.e. a change in food availability (Manteifel, 1978).

Temperature rhythms may sometimes act as indicators of time (Nelson et al, 1975). Ambient fluctuations are often limited within 24 hours, variable and not dependable, but temperature variations are more important and consistent over a longer temporal period. Temperature may have a more direct effect on food intake than does light, by influencing metabolic and locomotor activity, and thereby influencing the amount taken during feeding. Grove et al (1978) estimated that rainbow trout, O. mykiss, consumed food in proportion to their body weight, but ration increased proportionally with temperature.

The difference in feed intake with season has been investigated for Arctic charr, Salvelinus alpinus, (Jorgensen & Jobling, 1989). If quantities and patterns of food intake can be established for Atlantic salmon, then feeding regimes in culture might be altered seasonally to accommodate these changes. Such feeding patterns could be established if the fish are offered a constant amount of food in excess of their normal requirements, over several

days, and are sampled frequently to determine intake.

An investigation into seasonal changes in feeding involves either following a group of fish throughout a complete annual cycle or using different groups of fish of the same size at different times (Jorgensen & Jobling, 1989). However, as different groups of fish may have had a dissimilar growth history, it is preferable to sample the same group of fish at different seasons throughout a year.

The present study was designed to investigate the following null hypotheses:

1. Atlantic salmon do not show fluctuating amounts of gut fullness throughout the day when held in freshwater in tanks.
2. If gut fullness fluctuates during the day then the pattern established remains constant regardless of season.
3. The amount of food taken does not vary with season.
4. Environmental parameters do not effect the amount of food taken.

3.2 MATERIALS AND METHODS

3.2.1 Fish Stocks

Sibling LMG salmon (see p. 4) (mean weight 4.2g, mean length 7.3cm) (i.e. committed to a second year in freshwater) were transported to Pitlochry from Almondbank, in plastic lined bins containing aerated water, on 30.04.91. One hundred fish were placed in each of 2 square ($1m^2$) tanks. An extra one hundred fish were held in a separate square tank to provide replacements if necessary.

The fish were maintained under natural daylight (sky lights), additionally two red strip lights were on to allow night work to proceed. Red lights with spectral emmission range below 650 nm were used, as it is thought not to be perceived by the retina of salmon (Ali, 1961).

3.2.2 Feeding Regime

The fish were fed 3% of their body weight per 24 hours, delivered continuously throughout the day and night (i.e. ad libitum). This was in excess of the ration level recommended by the food manufacturers, BP Nutrition (Wincham, Cheshire). This allowed the fish to have constant access to the same amount of food throughout the experiment. Fish were fed a commercial Mainstream diet until 48 hours before the onset of sampling, thus preventing a period of pre-prandial starvation prior to sampling. The standard was then replaced

by a diet labelled with iron powder (180-250 um) at 5% w/w. The iron powder was included as an inert marker that travelled with the food without affecting palatability or digestion of the food (Talbot and Higgins 1983). The marker was also radio-opaque so that when the fish were sampled and x-rayed the amount of food present in the gut could be assessed for that particular time of day.

3.2.3 Sampling

The two replicate tanks were sampled every 4 hours from 0200h for 116 consecutive hours (i.e. 0600h, 1000h, 1400h, 1800h, 2200h, on each of 5 days). This resulted in 6 samples for each of the 5 days, for each single sampling period during summer, autumn, winter and spring. Ten to twenty fish were anaesthetised, x-rayed and returned to their respective tanks. This allowed the feeding rhythm for each season to be established over 5 consecutive days and therefore an average feeding cycle for 24 hours to be calculated.

The x-ray plates were developed and the amount of food present in the fore-, hind- and wholegut for each fish at each sampling time was calculated from iron particle counts. The foregut was taken to be from the beginning of the digestive tract to just below the swim bladder, on the x-ray plate, and the hindgut from the cut off point to the end of the digestive tract. The wholegut (or total gut fullness) is defined as the entire length of the fish gut and therefore

the total marker count (Rawlings, 1989). Average gut fullness was expressed as a percentage of dry body weight (% DBW). Light and temperature levels were recorded every 4 hours, at the time of sampling.

3.2.3a Summer

Ten to 15 fish (mean weight 10.5g $^{+}$/_0.2, mean length 9.6cm $^{+}$/_0.05) were sampled from replicate tanks from 0200h on 24.06.91 until 2200h on 28.06.91. Fish were weighed and measured on 04.07.91 to establish weight / fork length and fork length / cranial length relationships. This allowed dry weight of the fish to be calculated from length measurements obtained from the radiographs, since it was decided that the fish should not be stressed further by individually measuring each fish at the time of x-raying.

3.2.3b Autumn

Ten to fifteen fish were sampled from replicate tanks (mean weight 19.5g $^{+}$/_0.3, mean length 11.8cm $^{+}$/_0.06) from 0200h on 16.09.91 to 2200h on 20.09.91, and monitored on the 25.09.91 as before on the 04.07.91.

3.2.3c Winter

Ten to fifteen fish were sampled from replicate tanks (mean weight 23.8g $^{+}$/_0.4, mean length 12.6cm $^{+}$/_0.08) from 0200h on 09.12.91 to 2200h on 13.12.91, and monitored on 16.12.91.

3.2.3d Spring

Ten to fifteen fish were sampled from replicate tanks (mean weight 32.0g $^{+}$/_0.7, mean length 14.4cm $^{+}$/_0.1) from 0200h on 09.03.92 to 2200h on 13.03.92, and were monitored on the 25.03.92.

3.2.4 CALCULATIONS

The amount of food present in fore- hind- and wholegut was calculated for each fish, and average gut fullness was calculated, at each sampling time to give a pattern of feeding over 5 days for that season.

To give an indication of the pattern of fullness and light levels for one 24 hour period, for each season, the average deviation (n=5) from the overall mean (n=30) was calculated, for each of the variables - foregut fullness and light levels, at each of the sampling times (e.g. 0200h, 0600h etc).

The mean foregut fullness value for each sampling time was calculated. The first cycle was omitted for summer and autumn due to the uncharacteristically high initial values observed, which were thought to be because of the disturbance of the fish (see below). The mean value and standard error were calculated to assess the significance of differences between gut fullness values at different times of the day.

3.2.5 Statistical Analysis

To test whether there was an association between the 2 variables fore- and hindgut fullness, the correlation coefficient for the 2 variables was calculated and tested for significance. This was used to assess the degree of association of the 2 variables given that the average relationship was linear (Campbell 1989).

A two sample t-test was used to test that replicate samples were not significantly different. The null hypothesis that the 2 means did not differ was tested using the following equation (Campbell 1989):

$$t = \frac{x_1 - x_2 - B}{S_p\,(1/n_1 + 1/n_2)}$$

where:

x_1 = mean of sample 1

x_2 = mean of sample 2

B = hypothesized difference between the means

S_p = pooled standard deviation

If the test hypothesis was not rejected, then the replicate samples were not significantly different and could be pooled.

A two sample t-test was also used to assess whether the mean foregut fullness taken at one specific time compared to the next sample time (i.e. all samples at 1400h compared to 1800h etc) for each season was significantly different.

Two-way analysis of variance (ANOVA Campbell, 1989) was used to test the effect of day and time on gut fullness. Pooled data from the replicate samples were used as appropriate. Where significant differences were found between replicates separate tests were carried out on the 2 individual data sets. The 2 F-ratios were tested for significance at the 5% ($p<0.05$), 1% ($p<0.01$) and 0.1% levels ($p<0.001$).

3.3 RESULTS

Over the 116 hour sampling periods, for each of the seasons investigated, the amount of food present fluctuated with time. These fluctuations in fullness occurred in the fore-, hind- and wholegut and indicated differences in the balance between food intake and evacuation rates. Peaks in the amount of food present in the foregut, in particular, can be observed at specific times of the day for some seasons. Maximum and minimum values of gut fullness are summarized in Table 3.1. Temperature readings were taken every 4 hours, however for each of the seasons temperature fluctuated very little over the 4 hr periods or even between days. Therefore the temperature is expressed as a range for each season.

3.3.1 Summer

During the summer sampling, sunrise was at 0330h and sunset at 2300h and the average water temperature was 13.08 ^{o}C ($^{+}/_{-}$ 0.10). For both tanks the first reading was disregarded when comparing maximum and minimum values of fullness as they were unusually high for that time of day (see below). The maximum foregut fullness for tank 1 was 0.662 %DBW ($^{+}/_{-}$ 0.053) at 1800h, and the minimum foregut fullness was 0.238 %DBW ($^{+}/_{-}$ 0.037) at 1400h. For tank 2 maximum foregut fullness was 0.663 %DBW ($^{+}/_{-}$ 0.062) and minimum 0.215 %DBW ($^{+}/_{-}$ 0.053) at 1800h and 0600h respectively. The maximum wholegut fullness for tank 1 was

Table 3.1 : Maximum and minimum values of gut fullness for replicate tanks for each season

Season & Tank Number	Gut Portion	Minimum			Maximum		
		Mean	s.e.	Time	Mean	s.e.	Time
Summer							
Tank 1	Foregut	0.238	0.04	1400	0.662	0.05	1800
	Hindgut	0.663	0.08	1400	1.745	0.13	2200
	Wholegut	0.901	0.11	1400	2.265	0.18	2200
Tank 2	Foregut	0.215	0.05	0600	0.663	0.06	1800
	Hindgut	0.792	0.08	0600	2.037	0.18	1800
	Wholegut	1.008	0.12	0600	2.603	0.21	1800
Autumn							
Tank 1	Foregut	0.271	0.04	1400	0.722	0.11	1800
	Hindgut	0.644	0.09	0600	1.173	0.10	2200
	Wholegut	0.937	0.13	0600	1.871	0.19	2200
Tank 2	Foregut	0.192	0.04	1400	0.735	0.07	2200
	Hindgut	0.597	0.09	0600	1.349	0.08	2200
	Wholegut	0.839	0.13	1400	2.084	0.14	2200
Winter							
Tank 1	Foregut	0.021	0.01	1000	0.322	0.04	1800
	Hindgut	0.135	0.04	1800	0.606	0.08	2200
	Wholegut	0.205	0.05	1800	0.926	0.07	0200
Tank 2	Foregut	0.007	0.007	0600	0.265	0.06	2200
	Hindgut	0.037	0.03	1400	0.335	0.08	2200
	Wholegut	0.046	0.03	1400	0.513	0.12	0600

Table 1: Continued

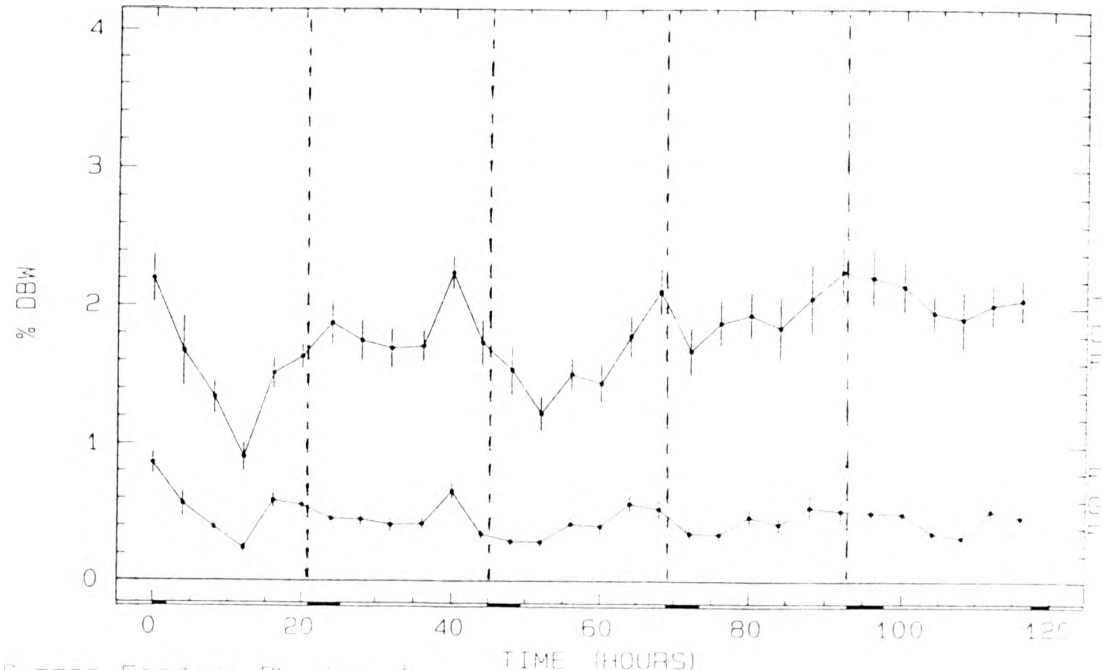
Season & Tank Number	Gut Portion	Minimum			Maximum		
		Mean	s.e.	Time	Mean	s.e.	Time
Spring							
Tank 1	Foregut	0.094	0.03	0200	0.432	0.07	2200
	Hindgut	0.151	0.03	0200	0.867	0.16	2200
	Wholegut	0.270	0.04	0200	1.086	0.13	2200
Tank 2	Foregut	0.100	0.01	0200	0.294	0.04	2200
	Hindgut	0.201	0.03	0200	0.762	0.10	2200
	Wholegut	0.378	0.08	0200	1.008	0.14	2200

2.265 %DBW ($^{+}$/_ 0.175) at 2200h and minimum fullness was 0.901 %DBW ($^{+}$/_ 0.107) at 1400h. For tank 2 maximum wholegut fullness was 2.603 %DBW ($^{+}$/_ 0.214) and minimum fullness was 1.008 %DBW ($^{+}$/_ 0.122) at 1800h and 0600h respectively.

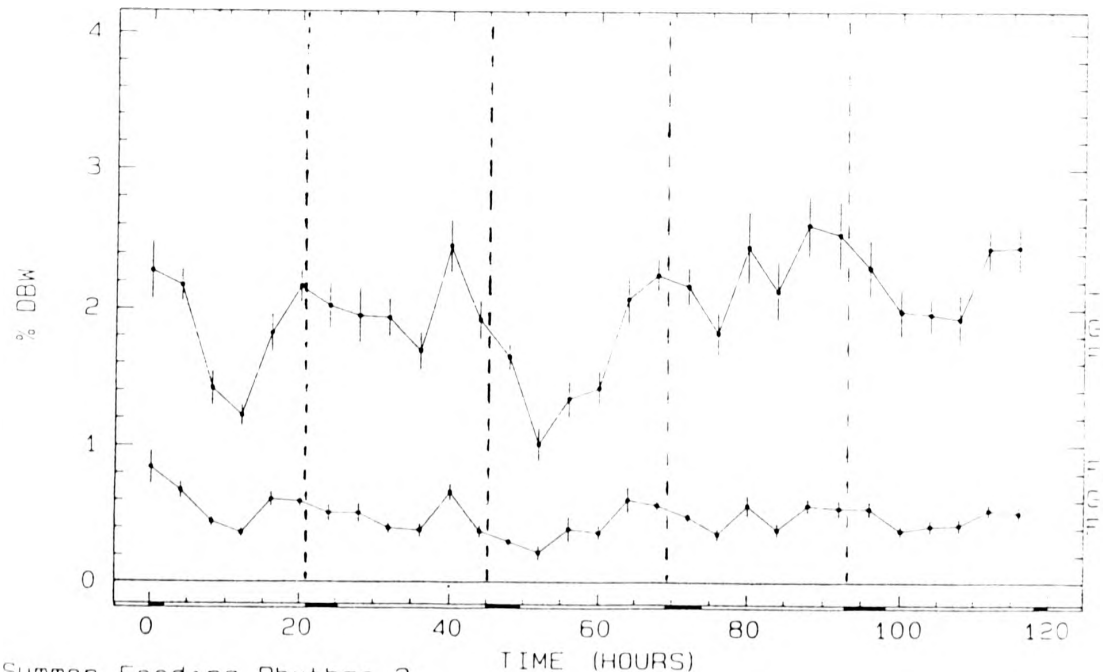
Fore- and wholegut fullness fluctuated throughout the day with a repeatable decrease at the beginning of the dark period each day (Fig. 3.1). The fore- and wholegut fullnesses for tanks 1 and 2 were not significantly different and therefore these data, for replicate tanks, were pooled. However, hindgut fullness was different and the data sets were treated separately. Two-way analysis of variance showed that foregut fullness varied significantly between days ($p<0.05$) and between times ($p<0.001$). For wholegut fullness, variation both between days and times was significant at the 0.1% level (Table 4.2).

Fore- and hindgut fullness fluctuated over time although not necessarily together. A decrease in foregut fullness was not always followed by a decrease in hindgut fullness. Initially the hindgut was at a relatively low level although its fullness increased after the first 24 hours of sampling. The foregut fullness rose and fell over the entire sampling time (Fig. 3.2). In tank 1 no significant correlation was seen between fore- and hindgut fullness and in tank 2 the correlation was only significant at the 5% level (Table 4.3, Fig. 3.3).

Figure 3.1: Summer feeding rhythms 1 and 2 show fore- and total (or whole) gut fullness for tanks 1 and 2 respectively. Each point shows the mean gut fullness for the fish sampled and vertical lines indicate the error bars. The fish were sampled every 4 hrs continuously over 5 days. The horizontal bar below the axis indicates periods of darkness.



Summer Feeding Rhythms 1



Summer Feeding Rhythms 2

Table 3.2 : Two way analysis of variance for gut fullness, to test the differences between days and times for each season

Season & Gut Portion	Day (4 d.f.)			Time (5 d.f.)		
	Tank 1	Tank 2	Pooled	Tank 1	Tank 2	Pooled
Summer						
FG	--	--	*	--	--	***
HG	**	***	--	N.S.	**	--
WG	--	--	***	--	--	***
Autumn						
FG	--	--	***	--	--	***
HG	--	--	***	--	--	N.S.
WG	--	--	***	--	--	**
Winter						
FG	***	***	--	N.S.	N.S.	--
HG	***	***	--	N.S.	N.S.	--
WG	***	***	--	N.S.	N.S.	--
Spring						
FG	--	--	***	--	--	**
HG	--	--	***	--	--	N.S.
WG	--	--	***	--	--	N.S.

FG - Foregut fullness

HG - Hindgut fullness

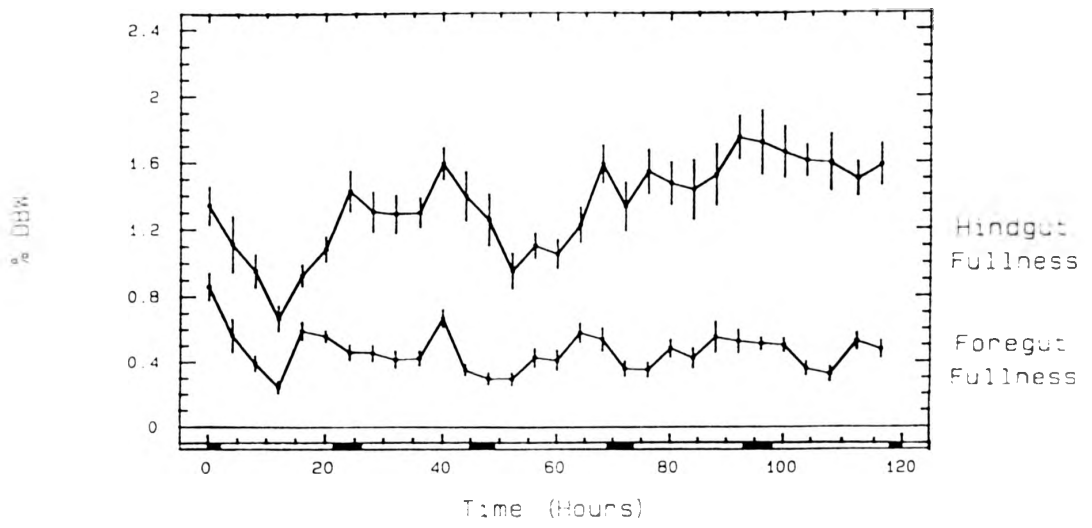
WG - Wholegut fullness

* p<0.05

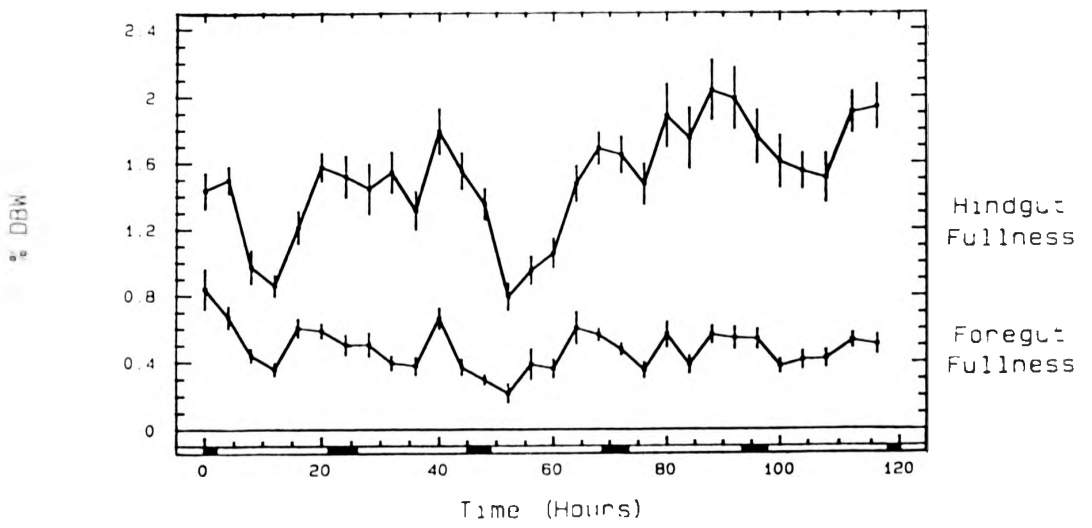
** p<0.01

*** - p<0.001

Figure 3.2: Summer feeding rhythms 1 and 2 show hind- and foregut fullness as %DBW for tanks 1 and 2 respectively. Each point is the average gut fullness for the fish sampled, the vertical bars indicate error bars. The horizontal bar below the axis shows periods of light and darkness.



Summer Feeding Rhythms 1

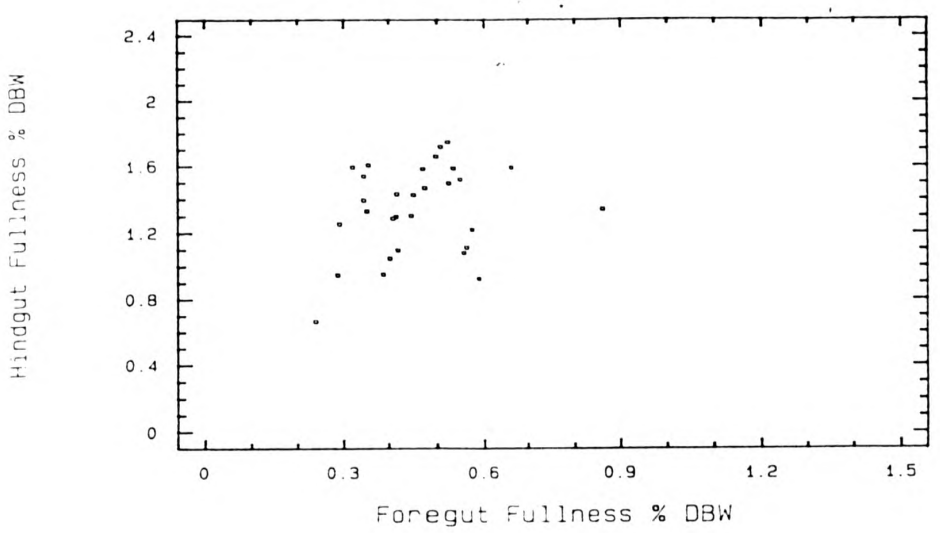


Summer Feeding Rhythms 2

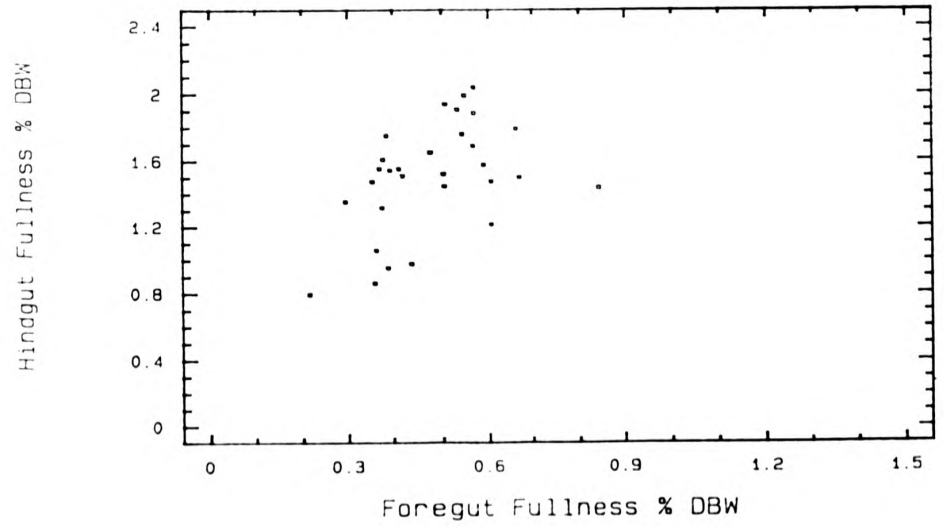
Table 3.3 : Correlation of fore- and hindgut fullness during each season

Season	Tank Number	Significance
Summer	1	N.S.
	2	*
Autumn	1	***
	2	***
Winter	1	***
	2	***
Spring	1	*
	2	N.S.

Figure 3.3: Each point indicates the degree of correlation for average hind- and foregut fullness for tanks 1 and 2 respectively. For tank 2 a correlation can be seen, significant at the 5% level.



Summer Feeding Rhythms 1



Summer Feeding Rhythms 2

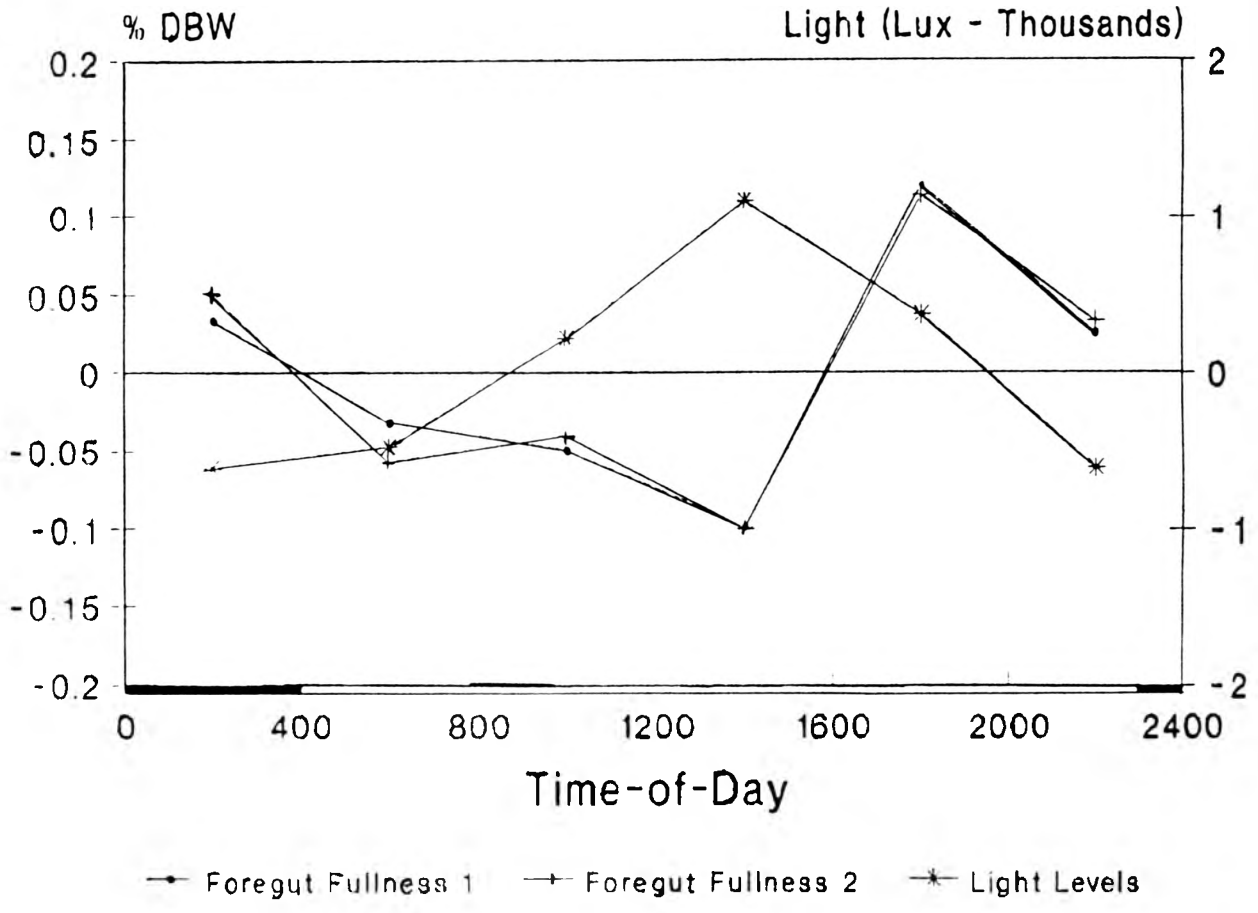
The deviation from the mean showed that out of the average 6 samples per day the highest recorded light level was at 1400h (Fig. 3.4). The deviation from the mean for foregut fullness reflected the maximum values already reported in that average levels also peaked at 1800h. Therefore the highest foregut fullness levels were observed at the first sampling after light levels had peaked.

The mean foregut fullness cycle, excluding the first 24 hours, showed that for tank 1 the mean sample at 1800h was significantly different from the mean values taken prior to this sample but not from the sample at 2200h. In tank 2 the mean sample at 1800h was significantly different from those taken after 0600h (but not those taken at 0200h and 2200h) (Table 3.4, Fig. 3.5).

3.3.2 Autumn

During the autumn sampling sunrise was at 0600h and sunset at 2000h and average water temperature was 14.5 ^{o}C ($^{+}$/_ 0.071). As in the summer the first reading was disregarded. The maximum foregut fullness for tank 1 was 0.722 %DBW ($^{+}$/_ 0.108) at 1800h and the minimum was 0.271 %DBW ($^{+}$/_ 0.042) at 1400h. For tank 2 maximum foregut fullness was 0.735 %DBW ($^{+}$/_ 0.072) and the minimum was 0.192 %DBW ($^{+}$/_ 0.042), at 2200h and 1400h respectively. The maximum wholegut fullness for tank 1 was 1.871 %DBW ($^{+}$/_ 0.185) at 2200h and the minimum 0.937 %DBW ($^{+}$/_ 0.125) at 0600h. For tank 2 maximum wholegut fullness was 3.320 %DBW ($^{+}$/_ 0.440) and the minimum

Figure 3.4: The data has been calculated to show the deviation from the mean, this indicates the average cycle of light and foregut fullness levels seen in tanks 1 and 2. The horizontal bar below the axis shows the period of darkness.



Summer Feeding Rhythms

Table 3.4: Summary of significant differences between mean foregut fullness at 1800h and all other times sampled

Season	Tank No.	Times				
		1800h & 0200h	1800h & 0600h	1800h & 1000h	1800h & 1400h	1800h & 2200h
Summer	1	*	*	**	**	N.S.
	2	N.S.	*	*	***	N.S.
Autumn	1	N.S.	N.S.	N.S.	N.S.	N.S.
	2	N.S.	N.S.	N.S.	N.S.	N.S.
Winter	1	N.S.	N.S.	N.S.	N.S.	N.S.
	2	N.S.	N.S.	N.S.	N.S.	N.S.
Spring	1	*	*	*	***	N.S.
	2	**	N.S.	N.S.	*	N.S.

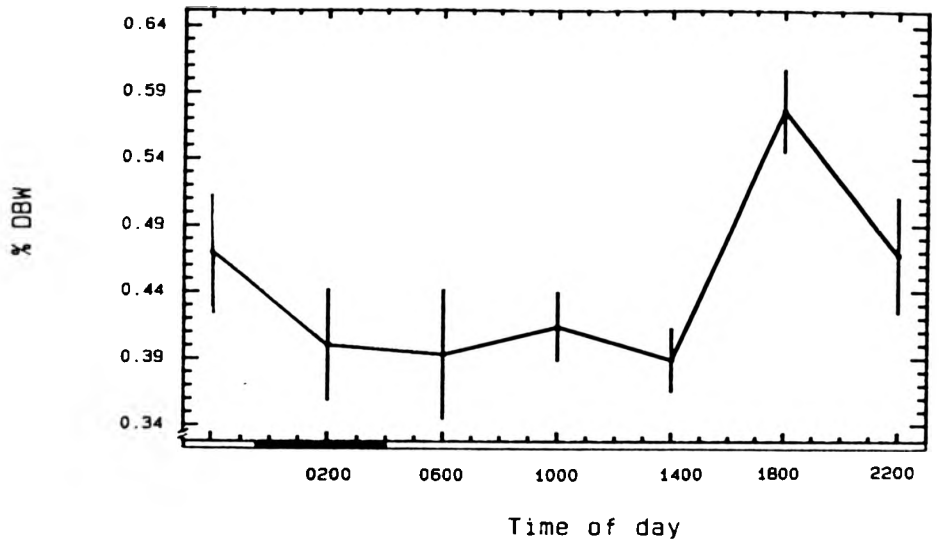
N.S. - Not Significant

* - $p<0.05$

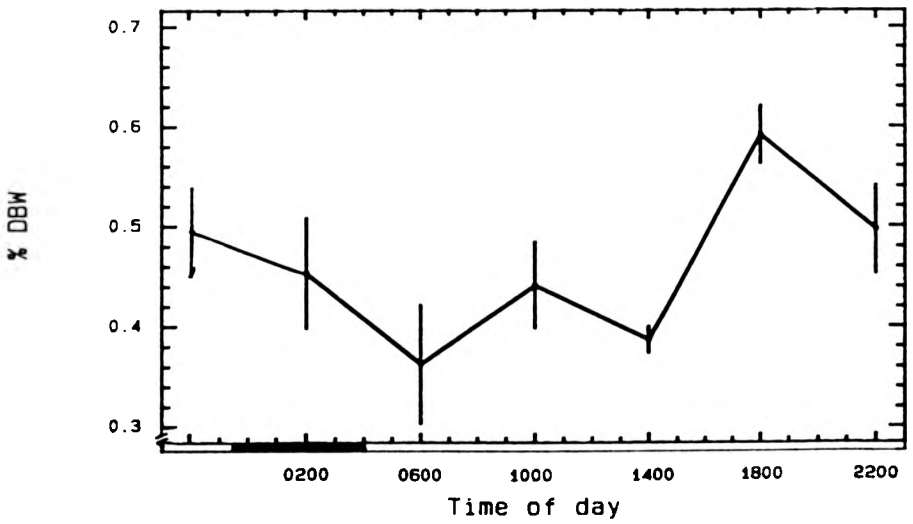
** - $p<0.01$

*** - $p<0.001$

Figure 3.5: Summer foregut fullness 1 and 2 indicate foregut fullness levels for tanks 1 and 2 respectively. Each point is the average value of foregut fullness for the fish sampled at that time over the 5 days. The vertical bars indicate standard error.



Summer foregut fullness 1



Summer foregut fullness 2

0.839 %DBW ($^{+}$/_ 0.128) at 0600h and 1400h respectively.

Fore- and wholegut fullnesses fluctuated throughout the day (Fig. 3.6). Replicate samples for all portions of the gut showed no significant differences, so the data were pooled. Foregut fullness varied significantly between both days and times ($p<0.001$). Hindgut fullness varied significantly only between days and wholegut fullness varied significantly between days ($p<0.001$) and times ($p<0.01$, Table 3.2).

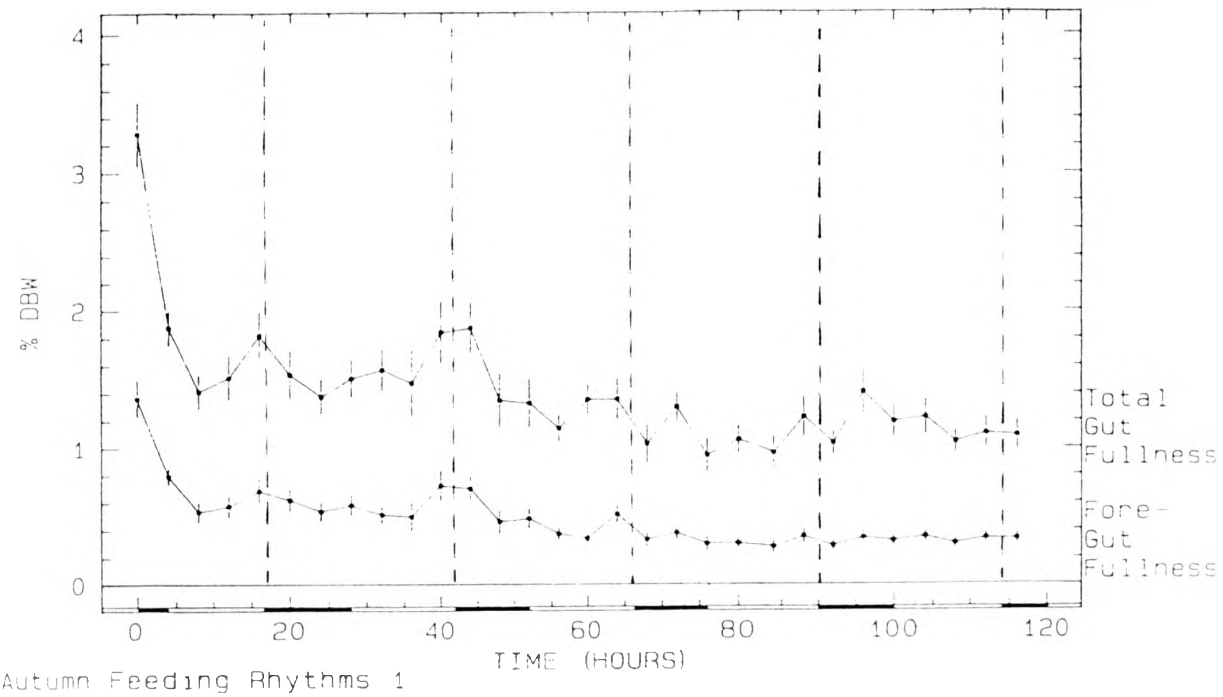
Fore- and hindgut fullnesses fluctuated together over time and appeared to mimic one another. A decrease in foregut fullness was nearly always accompanied by a decrease in hindgut fullness (Fig. 3.7). Neither the fore- nor hindgut altered their holding capacity for food over a prolonged period and the 2 variables showed a highly significant correlation ($P<0.001$, Table 3.3; Fig. 3.8).

The deviations from the overall mean revealed two peaks of foregut fullness, at 0200h and 1800h (Fig. 3.9). However, when the first 24 hours of data were excluded, due to high values as in summer, no peaks were observed (Fig. 3.10). The mean values of foregut fullness were not significantly different (Table 3.4), therefore statistically no peaks were exhibited in the average cycle.

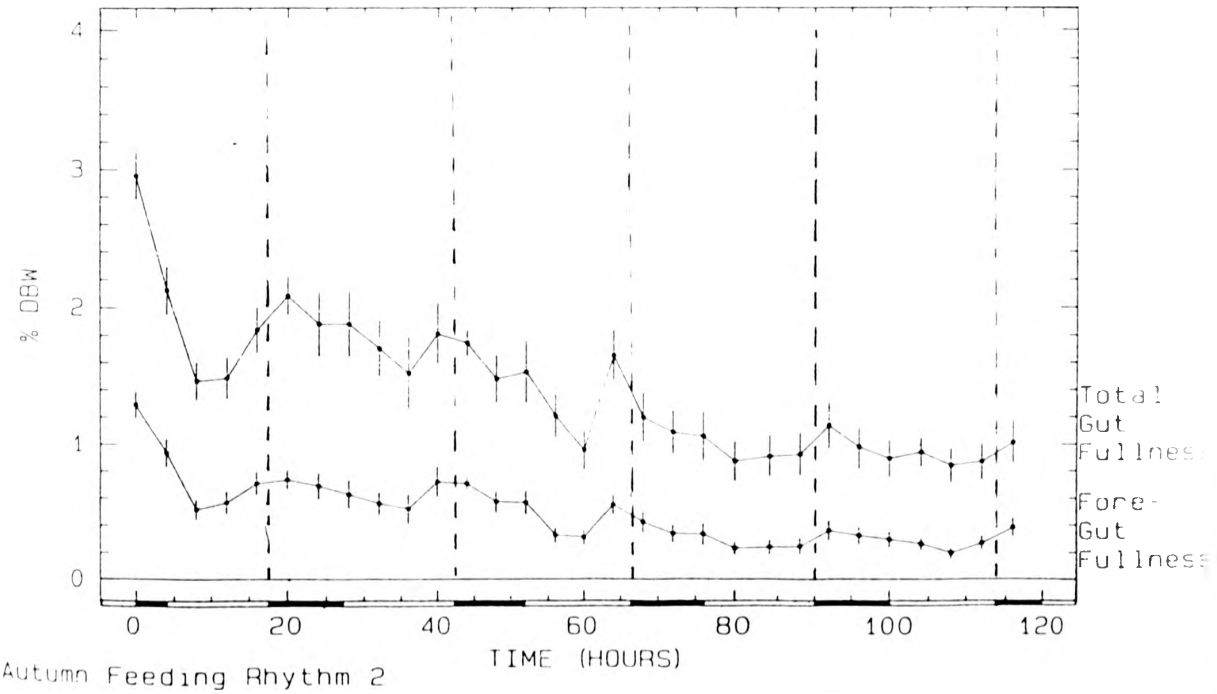
3.3.3 Winter

During the winter sampling sunrise was at 0800h and sunset

Figure 3.6: Autumn feeding rhythms 1 and 2 show fore- and total (or whole) gut fullness for tanks 1 and 2 respectively. Each point indicates the mean gut fullness for the fish sampled at each sampling time. The vertical bars show standard error and the horizontal bar below the axis indicates the periods of darkness.

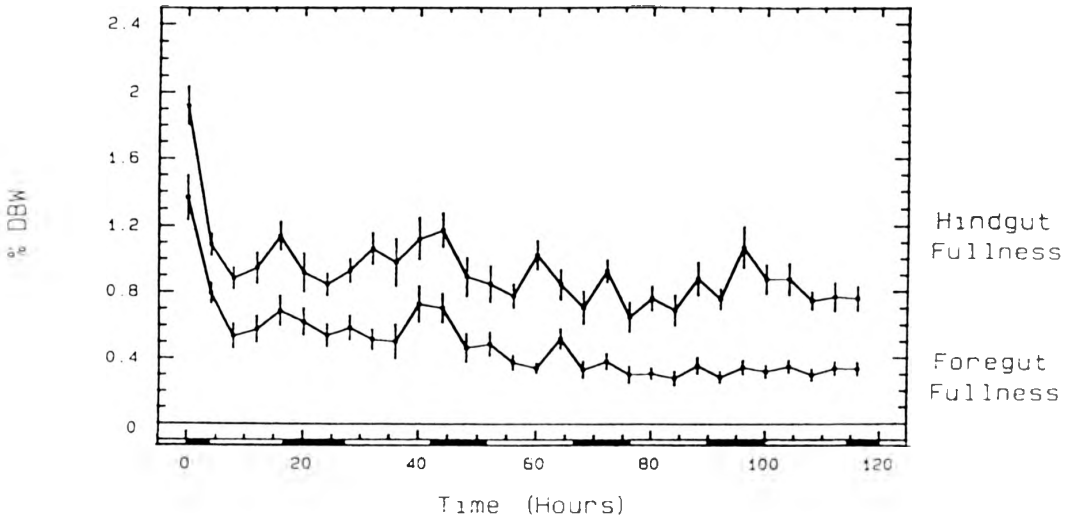


Autumn Feeding Rhythms 1

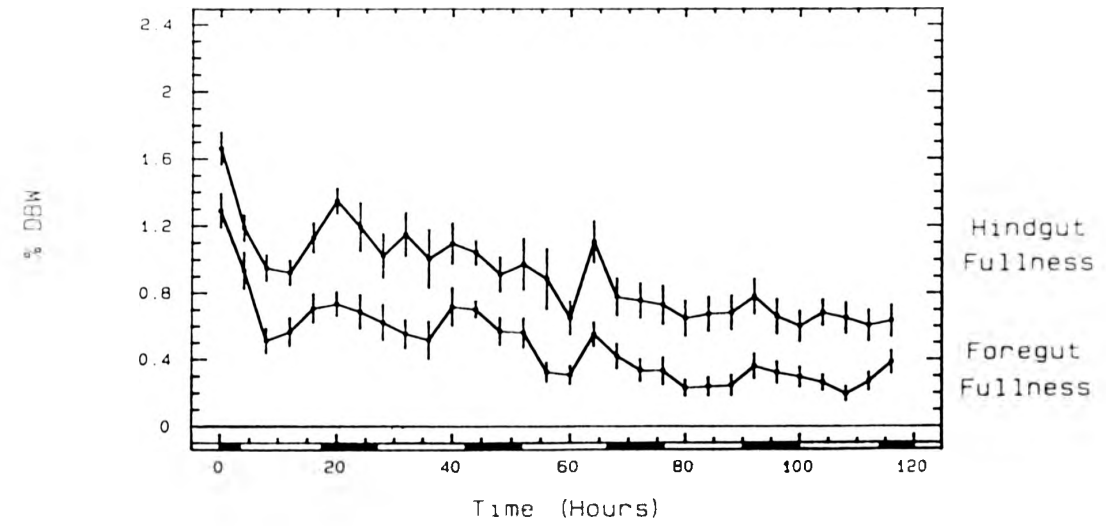


Autumn Feeding Rhythm 2

Figure 3.7: Autumn feeding rhythms 1 and 2 show hind- and foregut fullness levels expressed as %DBW for tanks 1 and 2 respectively. Each point represent the average gut fullness for the fish sampled at each time, the vertical bars show standard error. The horizontal bar below the axis indicates periods of darkness.

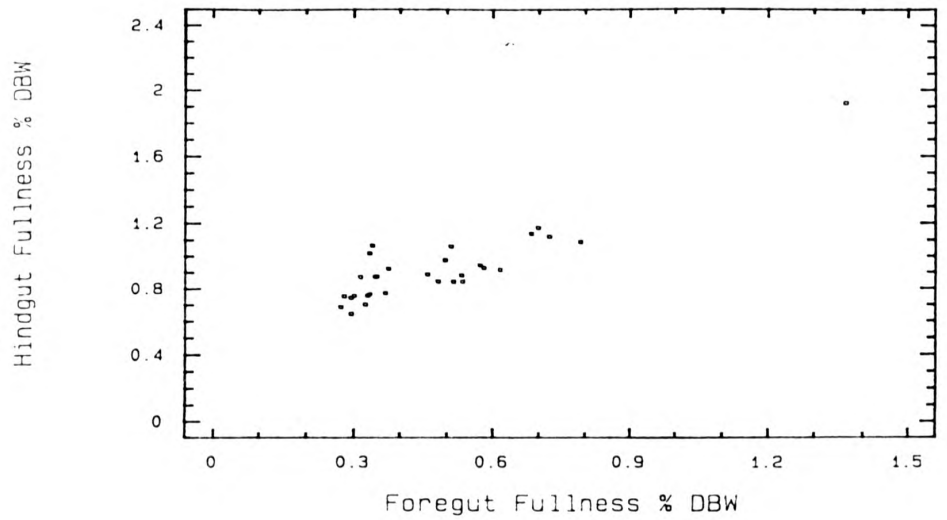


Autumn Feeding Rhythms 1

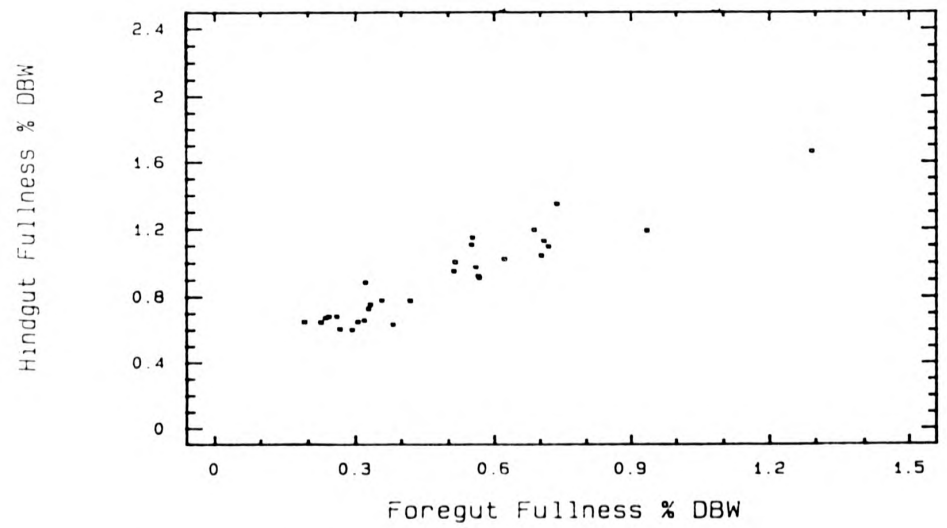


Autumn Feeding Rhythms 2

Figure 3.8: The correlation between hind- and foregut fullness is shown for fish in both tanks 1 and 2. A high degree of correlation for fish held in both tanks was observed.

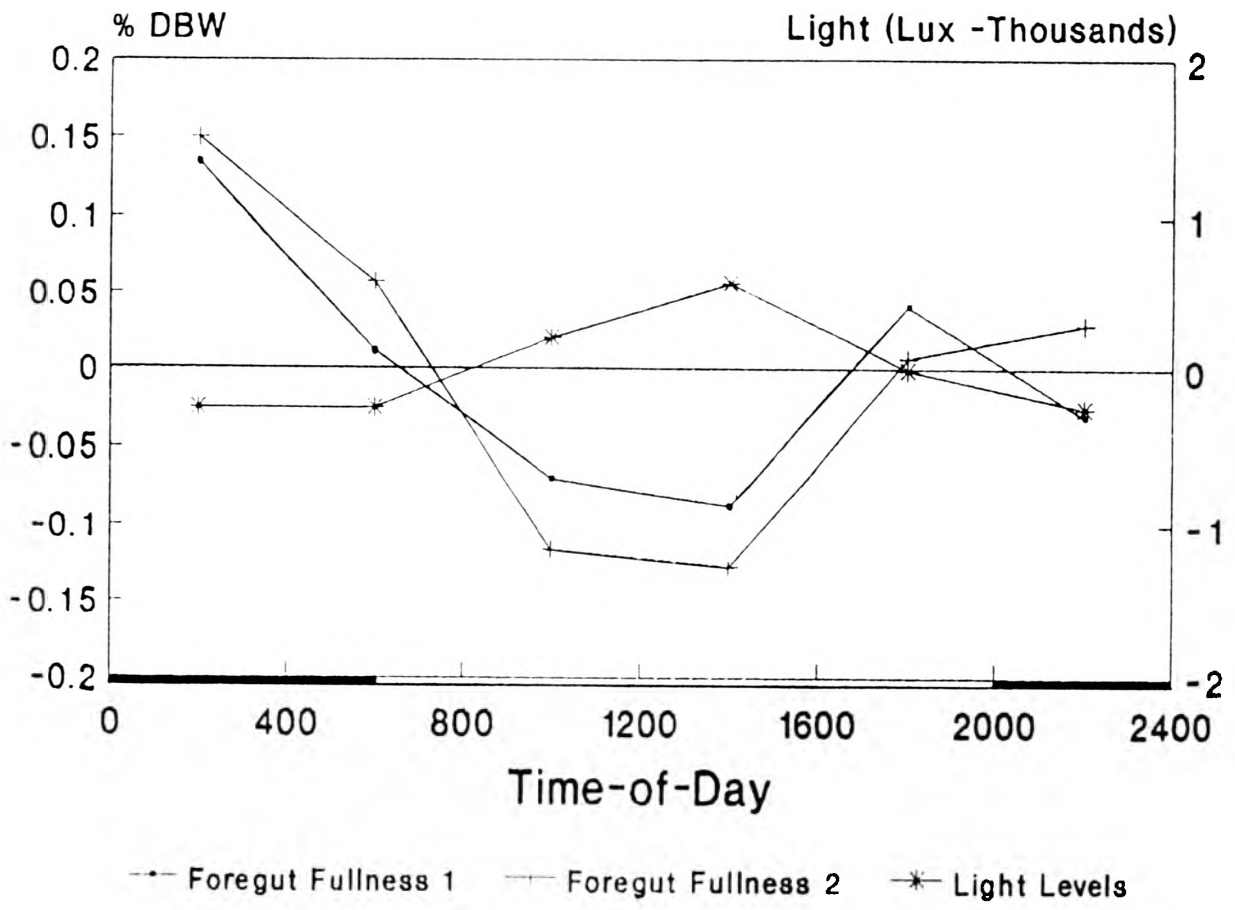


Autumn Feeding Rhythms 1



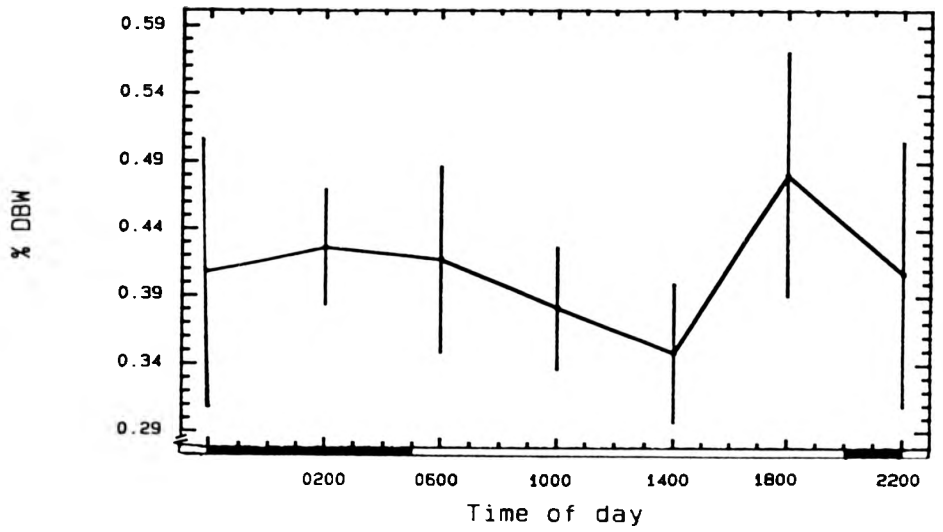
Autumn Feeding Rhythms 2

Figure 3.9: The deviation from the mean shows an average cycle of foregut fullness during autumn over a 24 hr period for both tanks 1 and 2. The cycle of light levels during that period is also shown. The horizontal bar below the axis indicates the period of darkness.

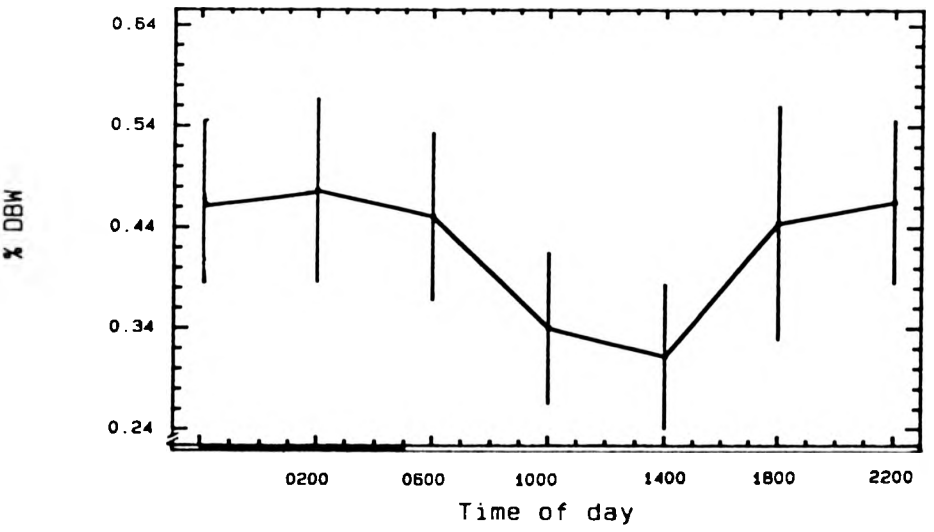


Autumn Feeding Rhythm

Figure 3.10: The average cycle for foregut fullness over a 24 hr period during autumn is shown for both tanks 1 and 2. The vertical bars indicate standard error and the horizontal bar below the axis shows periods of darkness.



Autumn foregut fullness 1



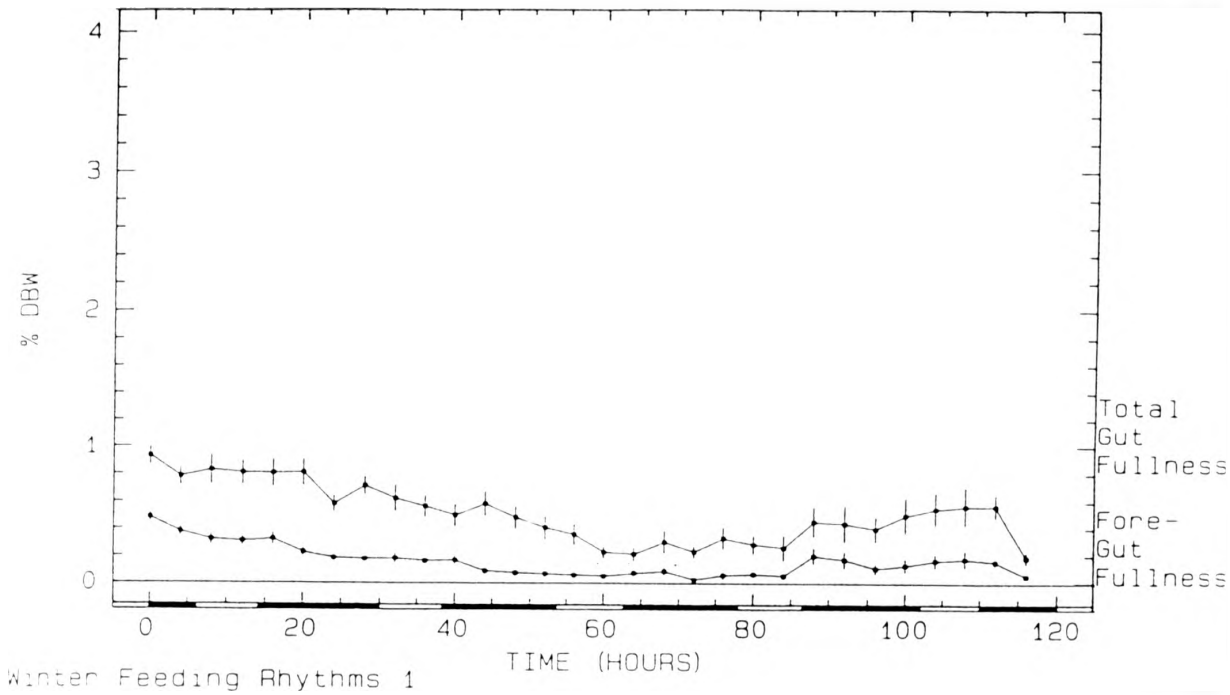
Autumn foregut fullness 2

at 1600h and average water temperature was 4.9 ^{o}C ($^{+}/_{-}$ 0.06). The maximum foregut fullness for tank 1 was 0.322 %DBW ($^{+}/_{-}$ 0.044) at 1800h and the minimum 0.0206 %DBW ($^{+}/_{-}$ 0.0106) at 0200h. For tank 2 maximum foregut fullness was 0.265 %DBW ($^{+}/_{-}$ 0.06) at 0600h and the minimum 0.0072 %DBW ($^{+}/_{-}$ 0.0072), also at 0600h. The maximum wholegut fullness for tank 1 was 1.333 %DBW ($^{+}/_{-}$ 0.292) at 1400h and the minimum 0.205 %DBW ($^{+}/_{-}$ 0.047) at 1800h. For tank 2 maximum wholegut fullness was 0.513 %DBW ($^{+}/_{-}$ 0.118) at 0600h and the minimum 0.0456 %DBW ($^{+}/_{-}$ 0.032) at 1400h.

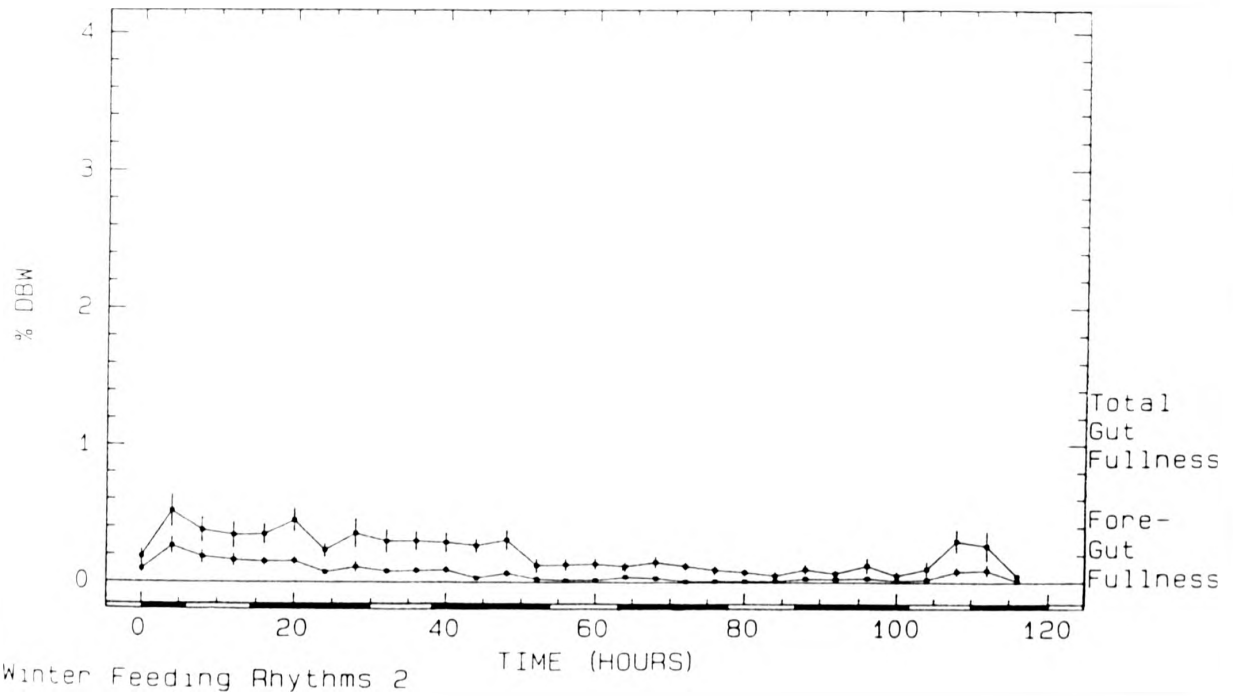
The ranges over which the various portions of the gut changed were very reduced compared to other seasons. There were no repeatable peaks in foregut fullness and several times the values were very close to zero. Wholegut fullness also altered very little, but declined gradually with a brief increase on day 5 (Fig. 3.11). The replicates were significantly different and could not be pooled. Separate two-way analyses of variance showed that in both replicates all portions of the gut varied significantly between days ($p>0.001$, Table 3.2) but not between times.

Although in other seasons all the fish fed, on 2 occasions in winter less than 20% of the fish had food in their intestines (Fig. 3.12). However, even excluding the data from non-feeding fish, changes in fore- and wholegut fullness were still relatively slight (Fig. 3.13).

Figure 3.11: Winter feeding rhythms 1 and 2 show the fore- and total (or whole) gut fullness expressed as % DBW for tanks 1 and 2 respectively. Each point represents the average gut fullness for the fish sampled at that time. The vertical bars show standard error and the horizontal bar below the axis indicates the periods of darkness during the sampling.

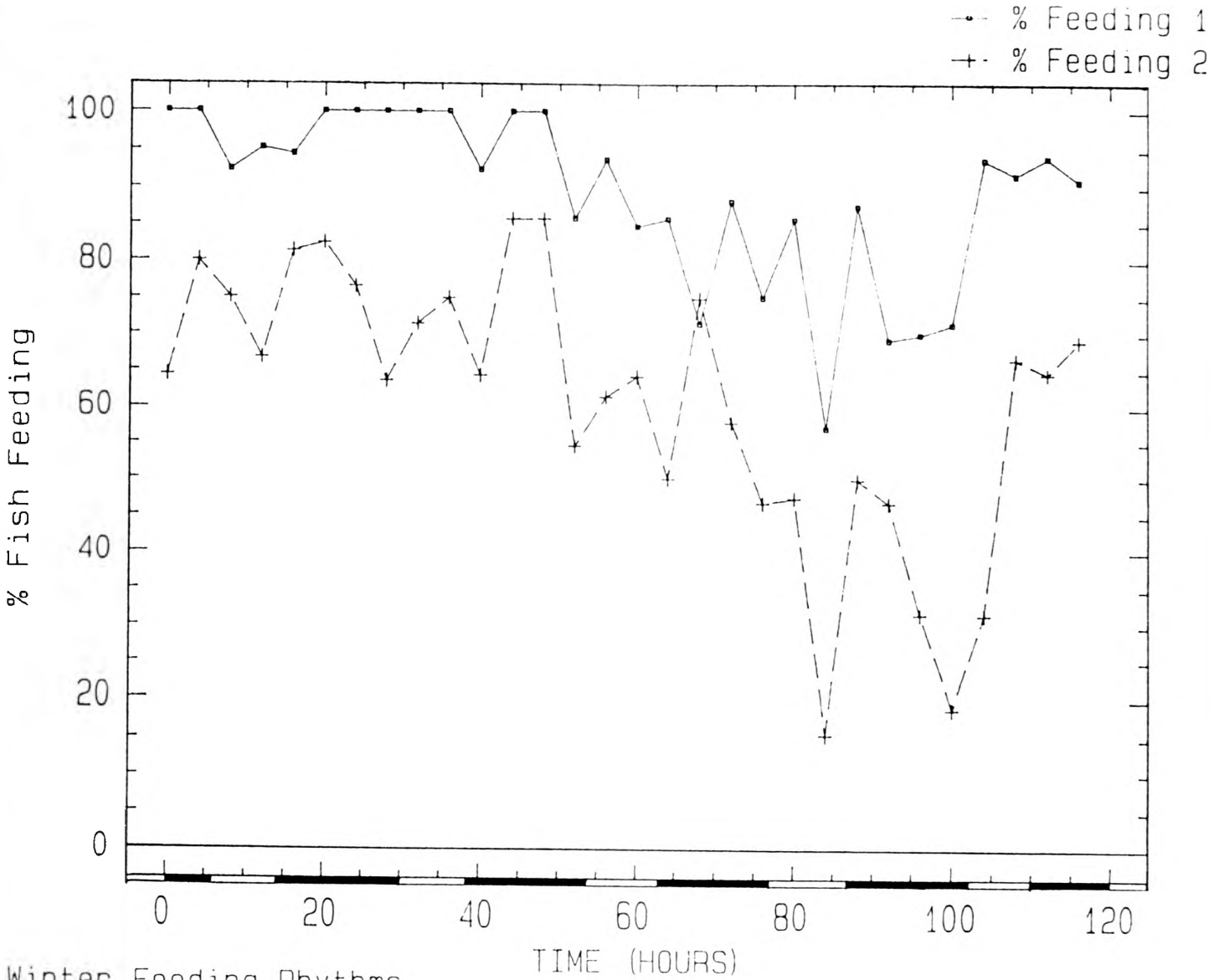


Winter Feeding Rhythms 1



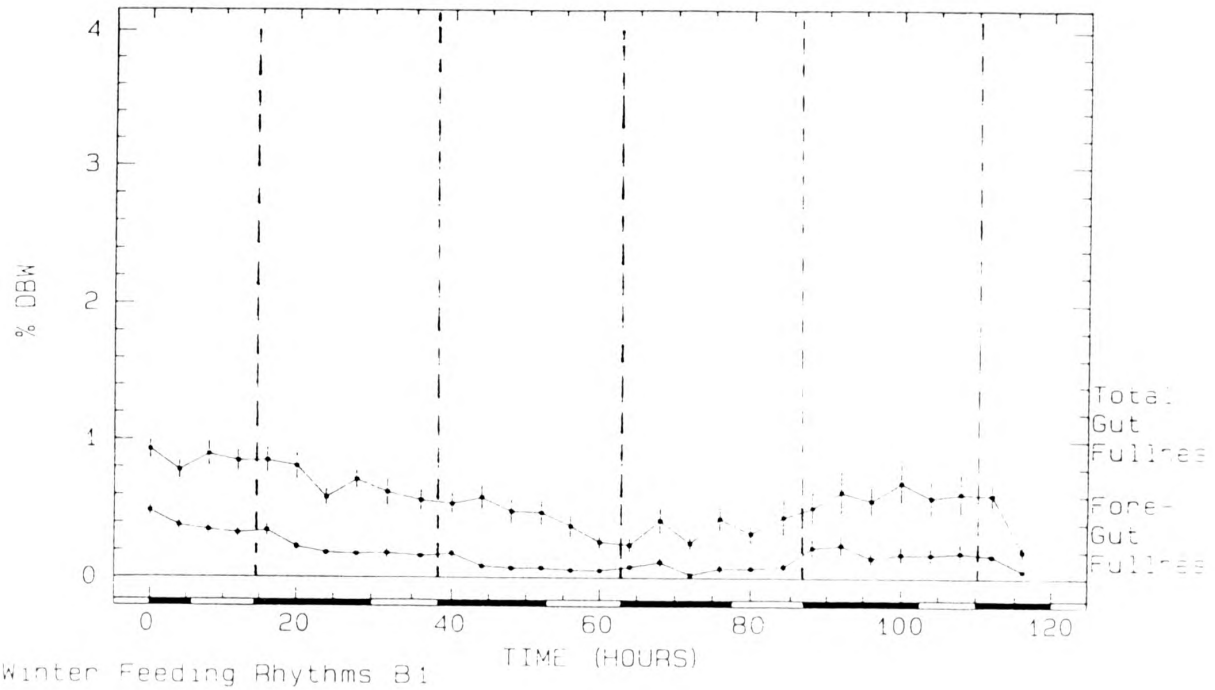
Winter Feeding Rhythms 2

Figure 3.12: The percentage of fish feeding at each sampling time was recorded for both tanks 1 and 2 during winter. The horizontal bar below the axis represents periods of darkness during sampling.

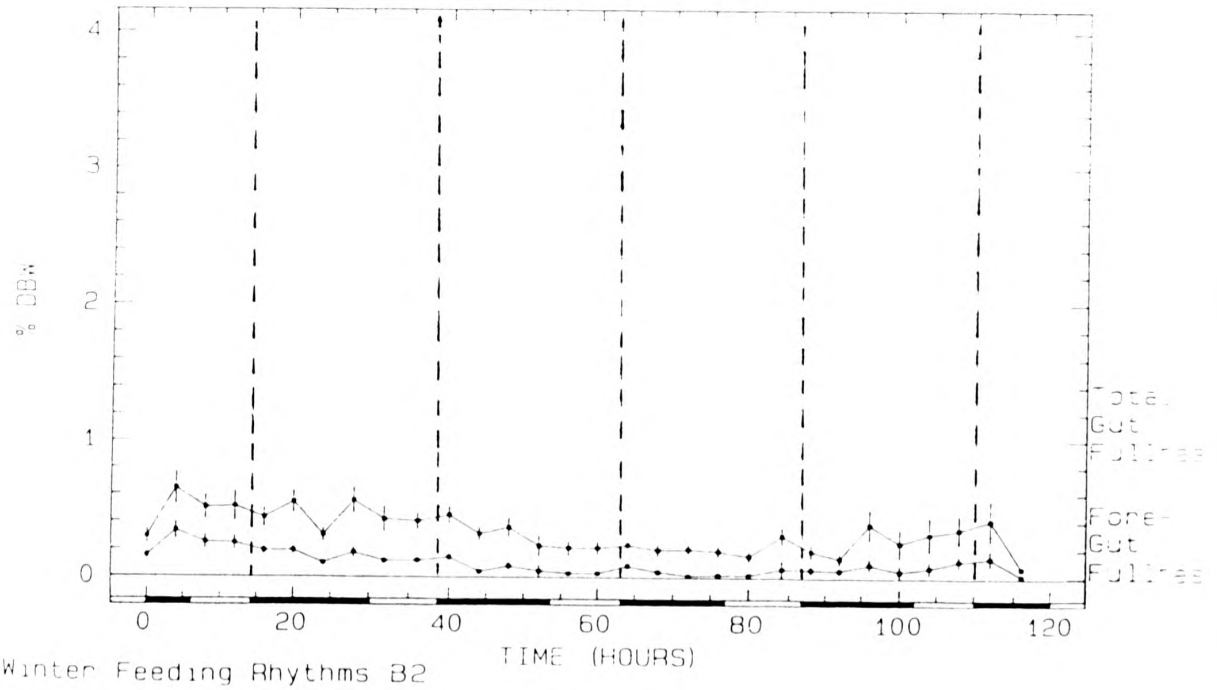


Winter Feeding Rhythms

Figure 3.13: Winter feeding rhythms b1 and b2 show fore- and total (or whole) gut fullness levels for the fish feeding in both tanks 1 and 2 respectively. Each point indicates the average gut fullness for the fish sampled at that time. The vertical bars show standard error and the horizontal bar below the axis represents periods of darkness during sampling.



Winter Feeding Rhythms B1



Winter Feeding Rhythms B2

Fore- and hindgut fullnesses were at times equal, at other times some mirroring of fullness changes were observed (Fig. 3.14). Fore- and hindgut fullnesses were significantly correlated in both tanks ($P<0.001$, Table 3.3) (Fig. 3.15).

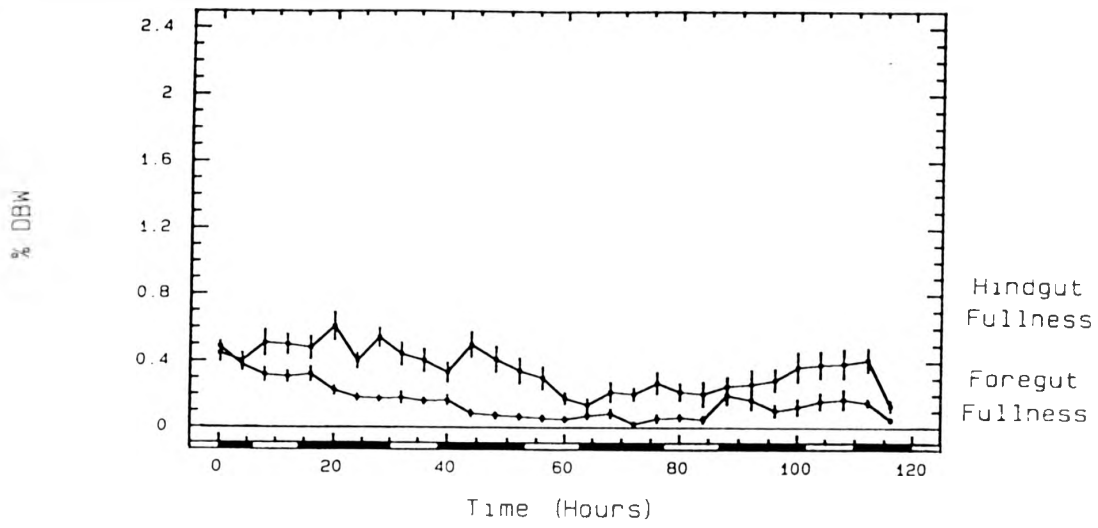
The average cycle as expressed by deviation from the mean reaffirmed the visual observation that foregut fullness altered little over a 24 hour period as did light levels (Fig. 3.16). The mean values for each sampling time, including all the cycles, showed no significant differences between foregut fullness for each sampling time (Fig. 3.17, Table 3.4).

3.3.4 Spring

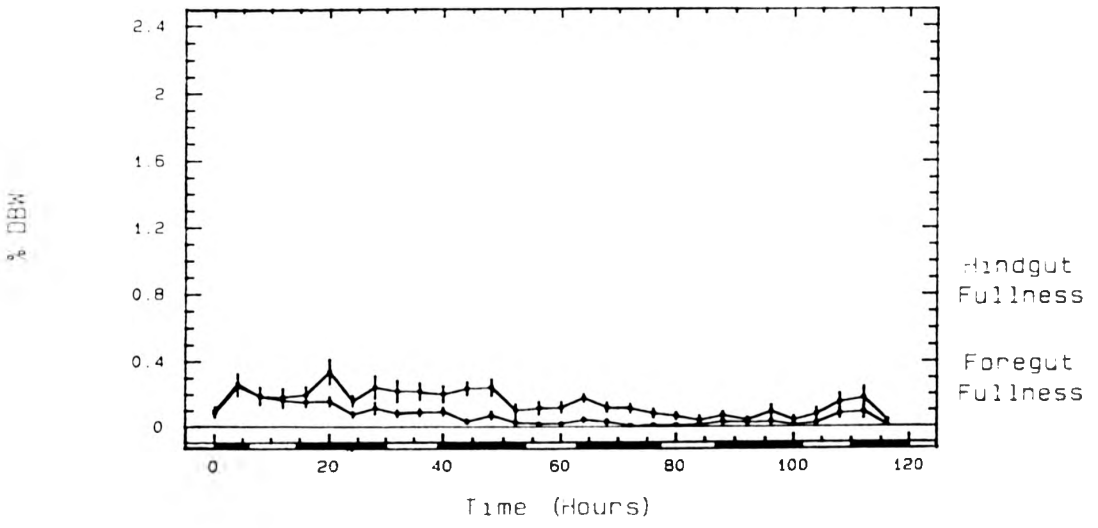
During the spring sampling sunrise was at 0700h and sunset at 2000h and average water temperature was 4.6 ^{o}C ($^{+}/_{-}$ 0.2). The maximum foregut fullness for tank 1 was 0.432 %DBW ($^{+}/_{-}$ 0.069) at 2200h and the minimum 0.0942 %DBW ($^{+}/_{-}$ 0.026) at 0200h. For tank 2 maximum foregut fullness was 0.294 %DBW ($^{+}/_{-}$ 0.043) at 2200h and the minimum 0.100 %DBW ($^{+}/_{-}$ 0.014) at 0200h. The maximum wholegut fullness for tank 1 was 1.086 %DBW ($^{+}/_{-}$ 0.127) at 2200h and the minimum 0.270 %DBW ($^{+}/_{-}$ 0.043) at 0200h. For tank 2 maximum wholegut fullness was 1.008 %DBW ($^{+}/_{-}$ 0.142) at 2200h and the minimum 0.378 %DBW ($^{+}/_{-}$ 0.077) at 0200h.

Foregut fullness fluctuated relatively little over time although several peaks occurred just before sunset. Wholegut

Figure 3.14: Winter feeding rhythms 1 and 2 show hind- and foregut fullness levels for tanks 1 and 2. Each point shows the average gut fullness of the fish sampled at each time. The vertical bars indicate standard error and the horizontal bar shows periods of darkness during sampling.

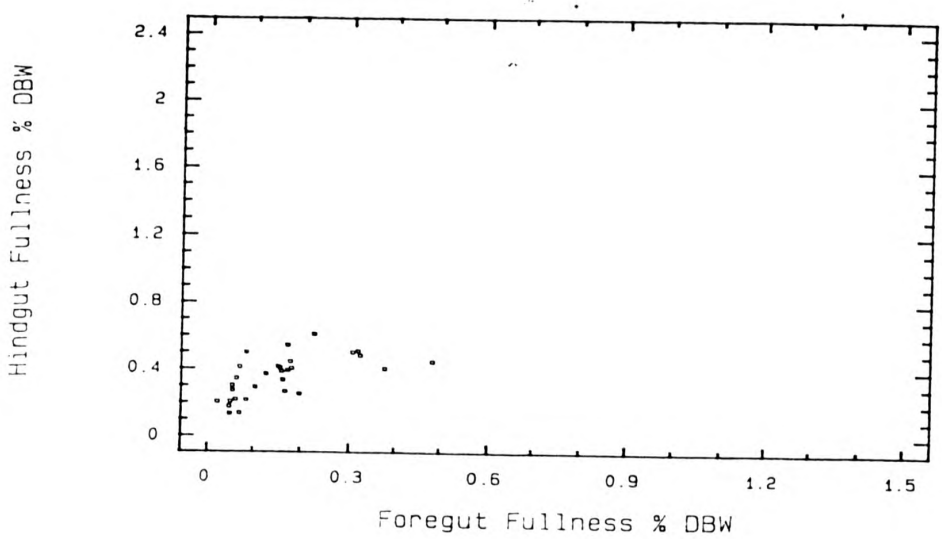


Winter Feeding Rhythms 1

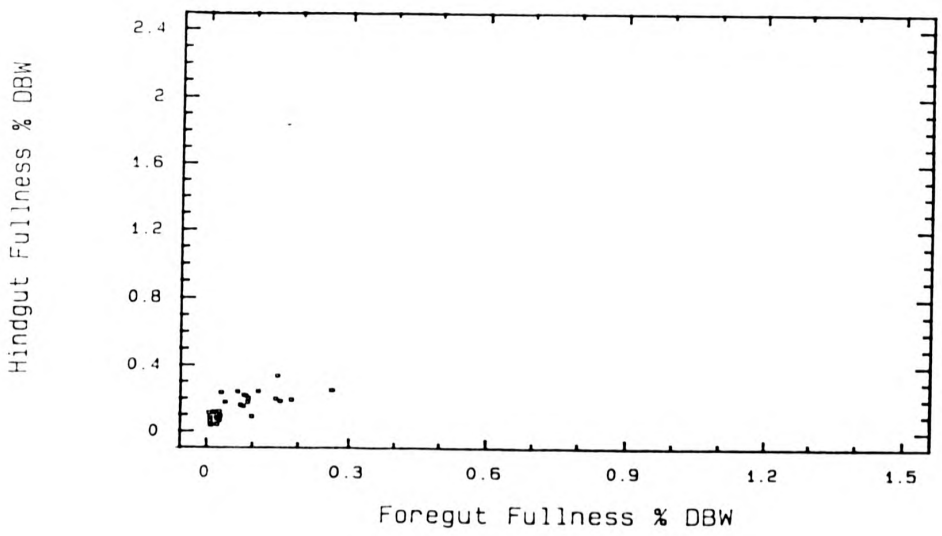


Winter Feeding Rhythms 2

Figure 3.15: The correlation between fore- and hindgut fullness for fish in tanks 1 and 2 is shown. A high degree of correlation for gut fullness is observed in both tanks.

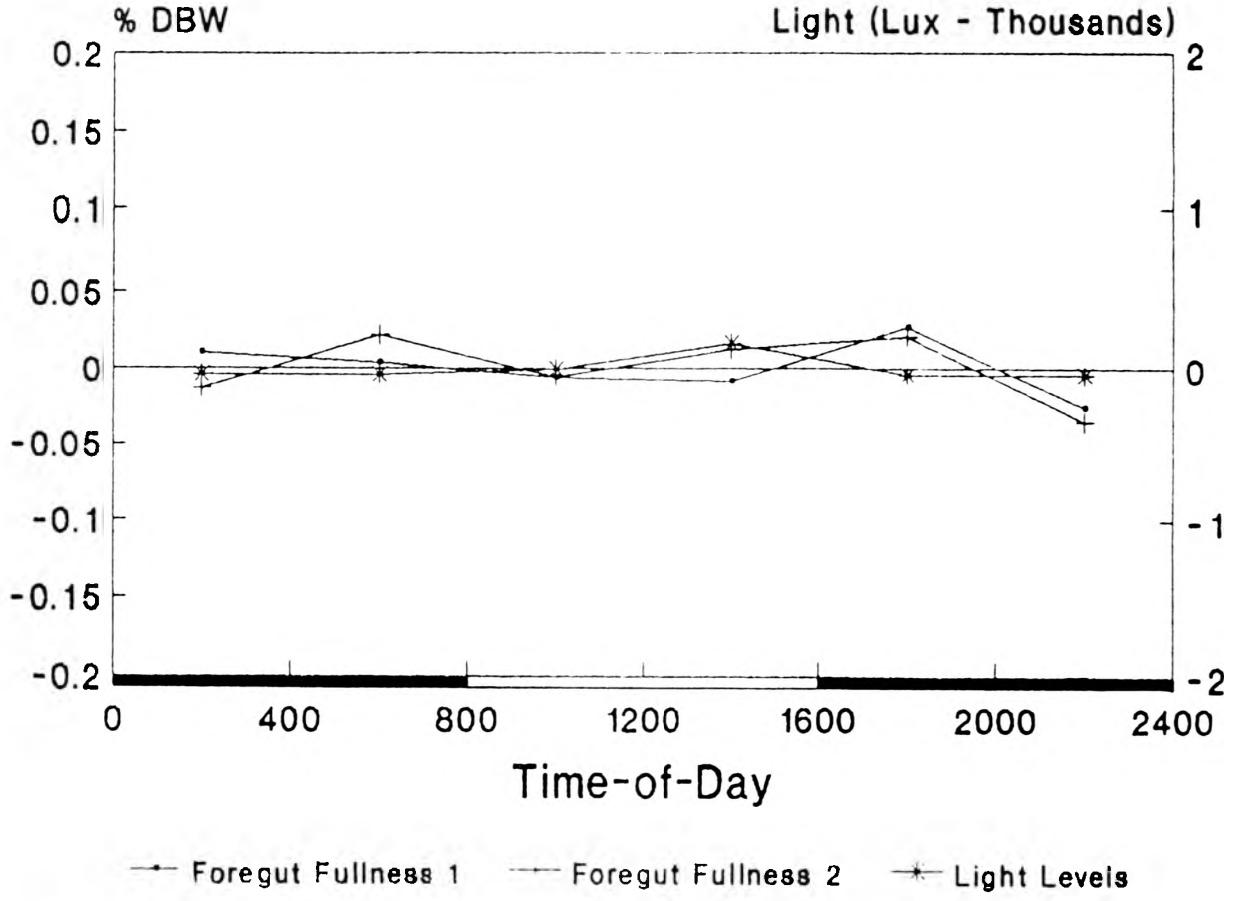


Winter Feeding Rhythms 1



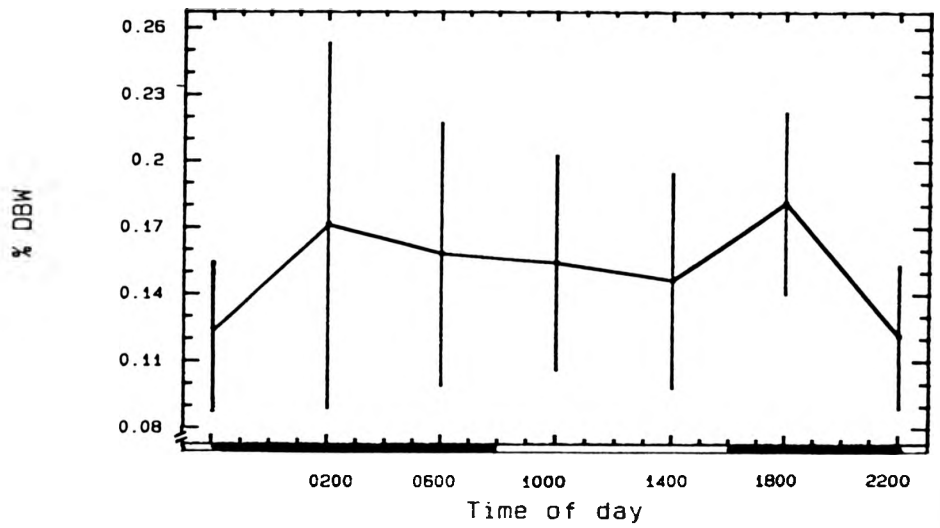
Winter Feeding Rhythms 2

Figure 3.16: The deviation from the mean for light and foregut fullness levels for fish in tanks 1 and 2 is shown, indicating and average cycle over 24 hrs. The horizontal bar below the axis indcates the period of darkness.

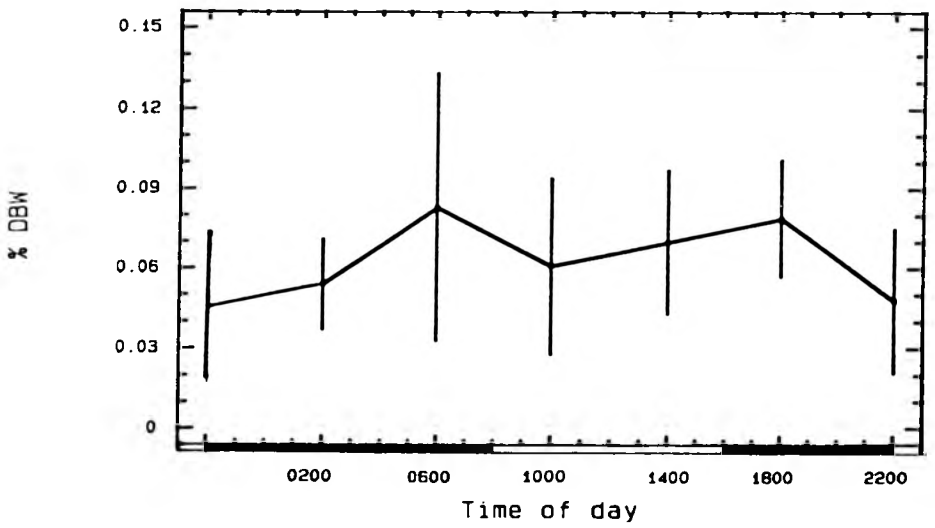


Winter Feeding Fish Only

Figure 3.17: Winter foregut fullness 1 and 2 shown an average foregut fullness cycle over 24 hrs for both tanks 1 and 2. The vertical bars indicate standard error and the horizontal bar below the axis shows the period of darkness.



Winter foregut fullness 1



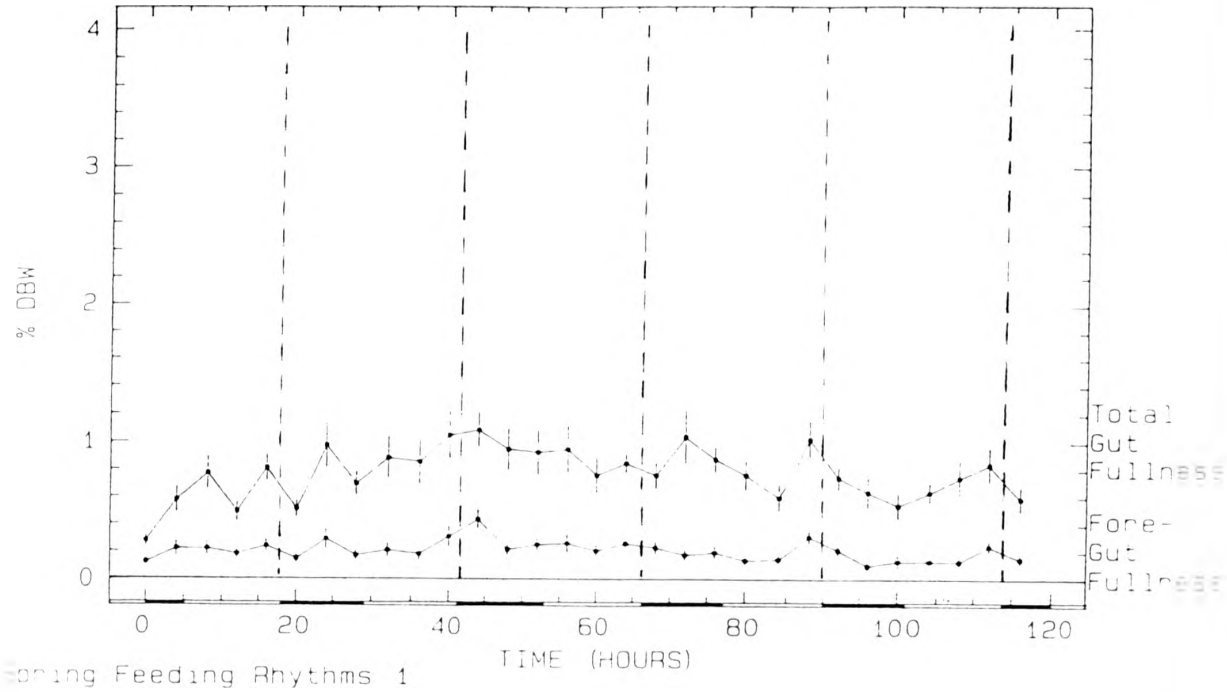
Winter foregut fullness 2

fullness fluctuated more frequently, although relatively erratically (Fig. 3.18). As the replicates did not differ, the data were pooled. Foregut fullness varied significantly between days ($p<0.001$) and times ($p<0.01$), but both hind- and wholegut fullness varied significantly only between days ($p<0.001$, Table 3.2).

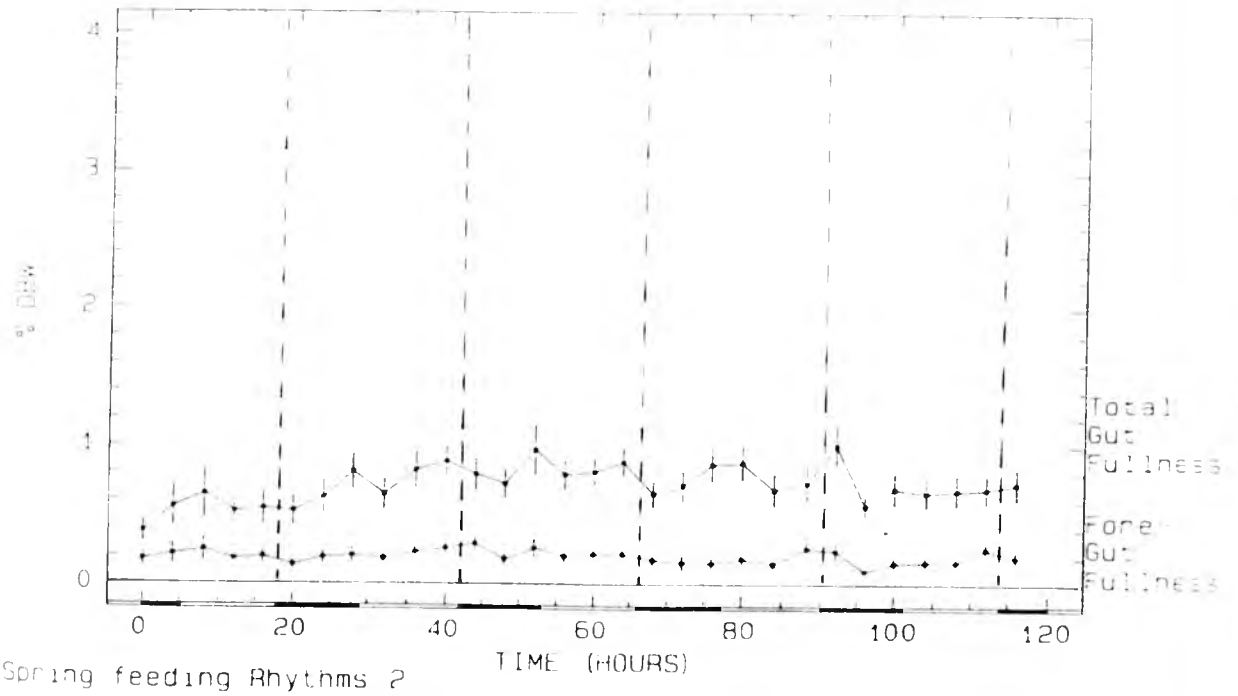
Fore- and hindgut fullness mirrored each other infrequently, often an increase in foregut fullness was accompanied by a decrease in hindgut fullness e.g. tank 1 at 44 hours (Fig. 3.19). Only tank 1 showed a small correlation between fore- and hindgut fullness ($p<0.05$, Table 3.3) (Fig. 3.20).

The deviations from the overall mean showed that foregut fullness peaked in late afternoon at the first sampling after the light level peak (Fig. 3.21). The mean 24 hour cycle of foregut fullness showed that in tank 1 the mean sample at 1800h was significantly different from those samples taken prior to this. However, the mean samples at 1800h and at 2200h were not significantly different (Table 3.4), thus indicating that there was no increase in foregut fullness after 1800h (Fig. 3.22). In tank 2 the mean foregut fullness at 1800h was significantly different from those at 1400h and 0200h. This tank therefore shows an increase in fullness as in tank 1 after 1400h.

Figure 3.18: Spring feeding rhythms 1 and 2 show fore- and total (or whole) gut fullness for tanks 1 and 2 respectively. Each point shows the average gut fullness for the fish sampled at every sampling time. The vertical bars indicate standard error and the horizontal bar shows periods of darkness during sampling.

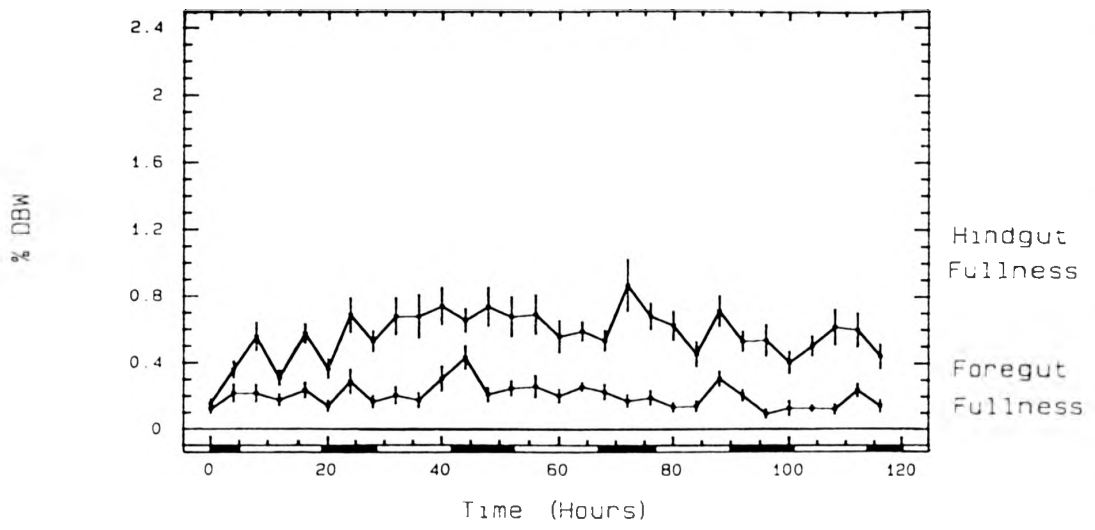


Spring Feeding Rhythms 1

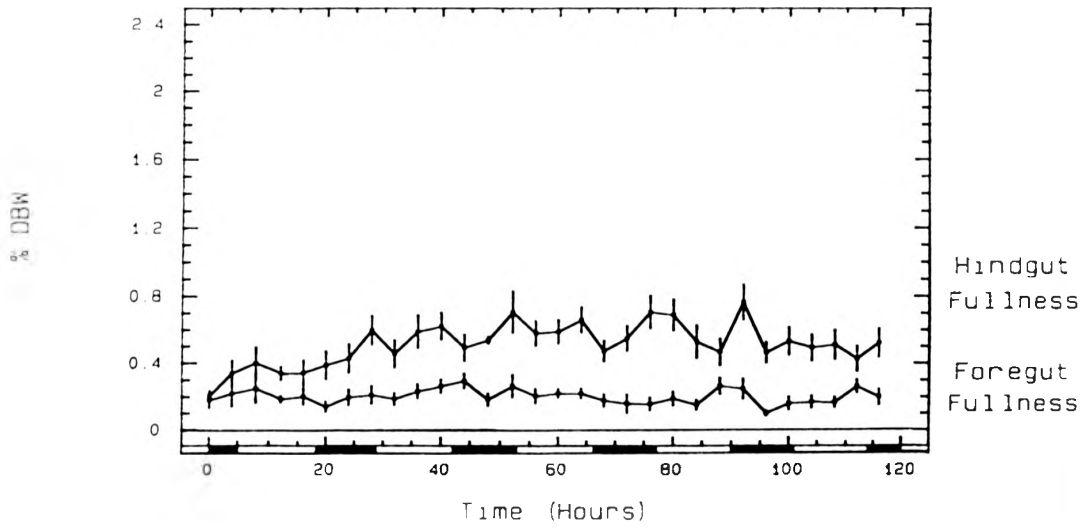


Spring feeding Rhythms 2

Figure 3.19: Spring feeding rhythms 1 and 2 shows hind- and foregut fullness for tanks 1 and 2. Each point represents the average gut fullness levels for the fish sampled at that time. The vertical bars show standard error and the horizontal bar below the axis shows the periods of darkness during sampling.

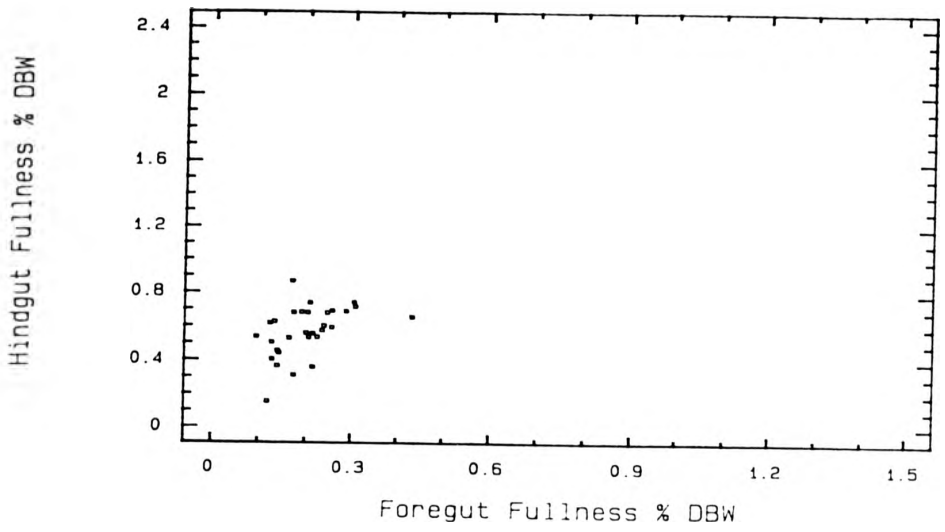


Spring Feeding Rhythms 1

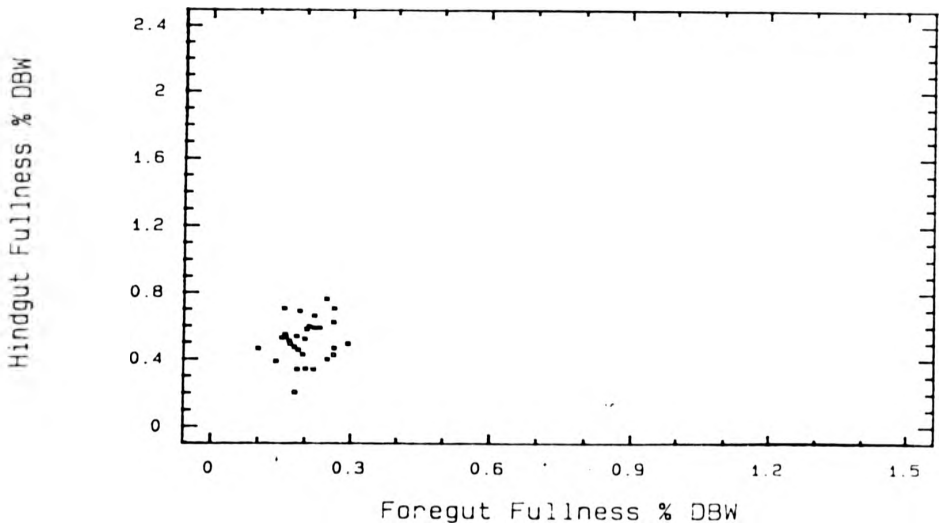


Spring Feeding Rhythms 2

Figure 3.20: The correlation between fore- and hindgut fullness is shown for tanks 1 and 2. A small degree of corrlation was observed between fore- and hindgut fullness for fish in tank 1.

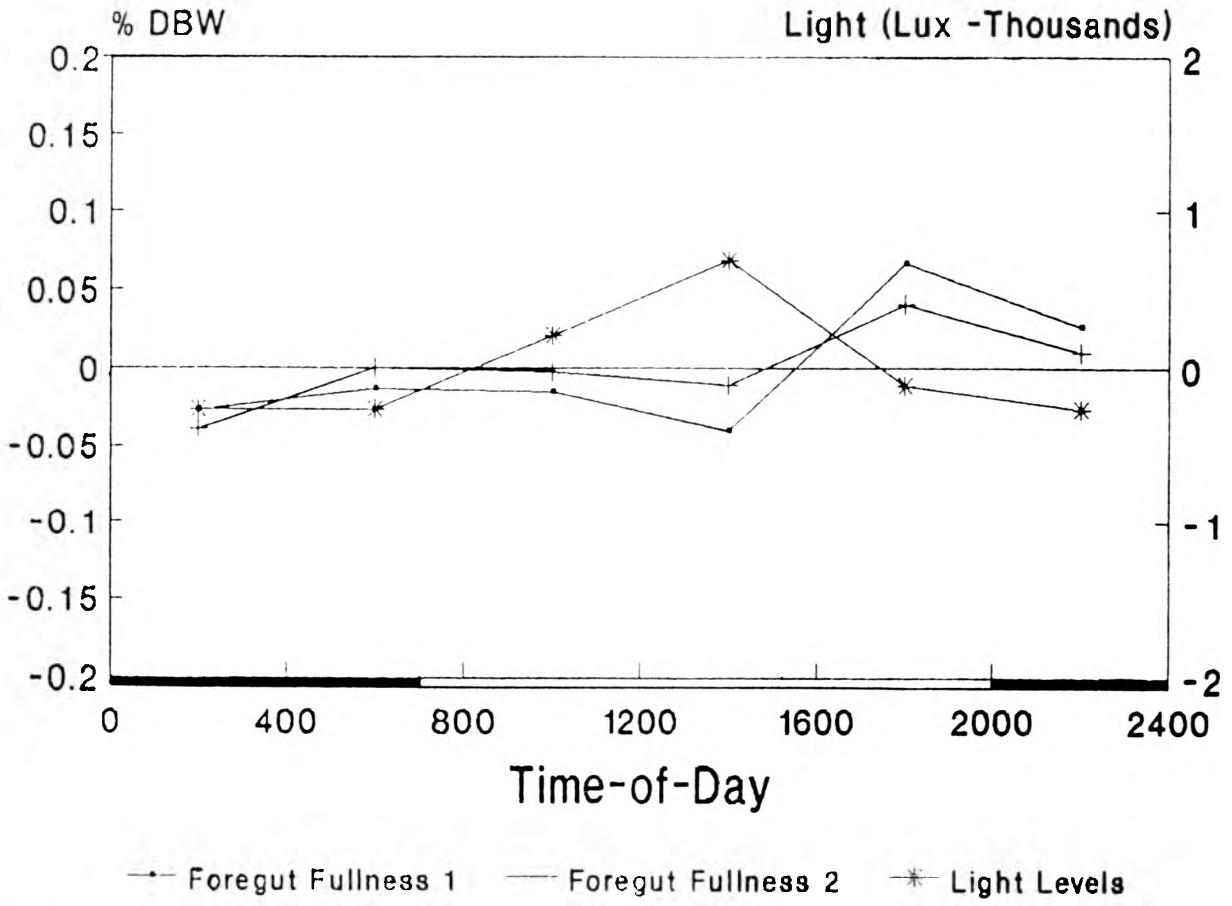


Spring Feeding Rhythms 1



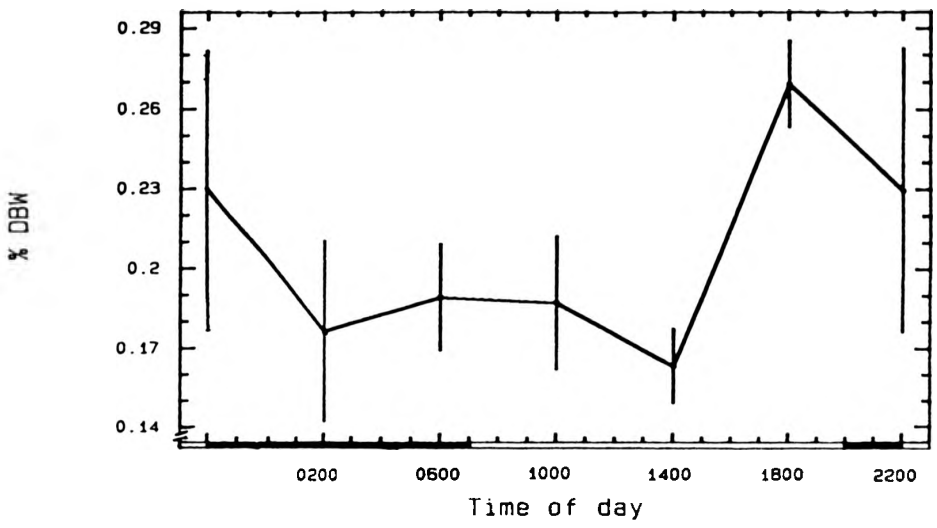
Spring Feeding Rhythms 2

Figure 3.21: The deviation from the mean indicates the average foregut fullness and light level cycle over a 24 hr period, for tanks 1 and 2. The horizontal bar below the axis indicates the periods of darkness during the 24 hr period.

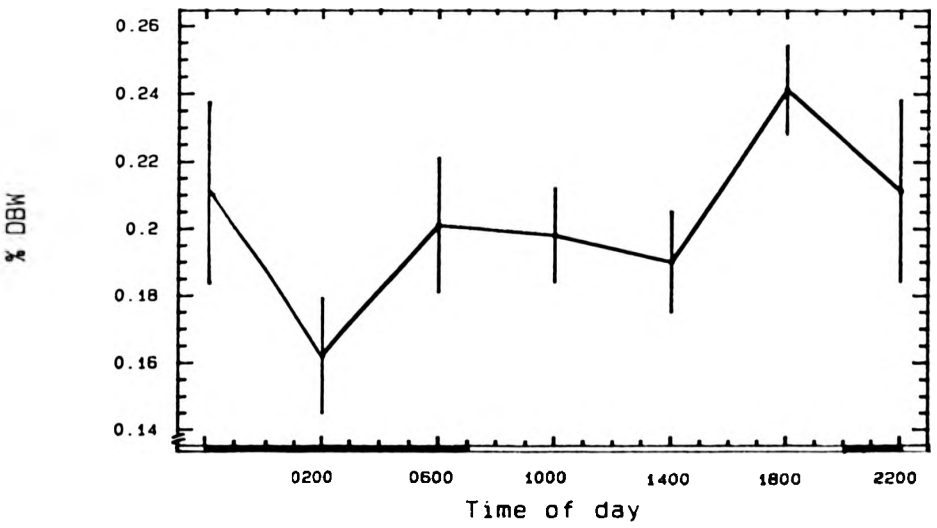


Spring Feeding Rhythm

Figure 3.22: The average foregut fullness cycle during spring over the 5 days sampled is shown for tanks 1 and 2. The vertical bars show standard error and the horizontal bar indicates the period of darkness.



Spring foregut fullness 1



Spring foregut fullness 2

3.4 DISCUSSION

The feeding of Atlantic salmon changed with season in 2 major ways: the amount of food taken altered dramatically, as did the timing of the peaks in the feeding patterns.

In both the summer and autumn foregut fullness was high at the start of sampling, then fell sharply, before a relatively stable rhythmic pattern was established after 24 hours. This settling period was not evident at the start of the winter and spring sampling. During the latter period the tanks were cleaned daily, prior to and during sampling. However, during summer and autumn, weekend cleaning was suspended, so that at the start of the sampling on Monday the fish had been undisturbed for more than 2 days. It is probable that this change of husbandry regime accounted for the qualitative difference in pattern between the first two and last two seasonal datasets.

Comparisons of the maximum and minimum values for the four seasons indicated that food intake was vastly reduced during winter (December 1991). Higgins & Talbot (1985) reported similar low food intakes in December. This was to be expected, since it is known that in juvenile Atlantic salmon feeding motivation decreases from July to September (Metcalfe *et al*, 1986) and remains at low level after December.

The summer data were similar to those obtained in a

preliminary experiment (Rawlings, 1989). However, as this preliminary experiment was undertaken without supplementary red lighting, it is concluded that this lighting did not influence feeding patterns in the 1991-92 experiments. This was expected on Ali's (1961) evidence that, juvenile Atlantic salmon are insensitive to wavelengths < 650nm.

No evacuation rates were investigated during these seasonal samplings. However, Higgins (1985) estimated evacuation rates at different temperatures for juvenile Atlantic salmon. The fish were fed labelled food between 0900h-1200h at various times of the year. He found that the evacuation rate decreased with decreasing temperature, but did so faster when daylength was increasing than when it was decreasing. Reading from his curves one can estimate evacuation rates for the four seasons sampled. For autumn at a temperature of 14.5 ^{o}C and a decreasing photoperiod the wholegut was evacuated in 7 hours; for winter, at 4.9 ^{o}C and a decreasing photoperiod, evacuation rate was 26 hours; for spring at 4.6 ^{o}C and an increasing photoperiod, it was 13 hours; and for midsummer at 13.1 ^{o}C the wholegut was emptied in c. 4 hours.

In autumn the maximum wholegut fullness (1.871-2.084 % DBW) was relatively high as was estimated evacuation rate. Fore- and hindgut fullnesses were significantly correlated ($p<0.001$). In summer wholegut fullness was slightly higher

(2.265-2.603 % DBW) than in autumn, but estimated evacuation rate was much higher which was reflected in a poor correlation between fore- and hindgut fullnesses. During winter maximum wholegut fullness was low (0.513-0.926 % DBW) and evacuation time was very slow resulting in a significant correlation ($p<0.001$) between the 2 portions of the gut. In spring, however, maximum wholegut fullness (1.008-1.086 %DBW) had increased slightly but evacuation time had halved, in relation to winter, and so fore- and hindgut fullnesses were unrelated. Talbot et al (1984) found that different sized test meals were evacuated at similar rates, when discrete meals were taken. Higgins (1985) proposed that seasonal changes in evacuation rates may be due to neural and hormonal modifications of peristalsis mediated by photoperiod effects.

In summer, autumn and spring the foregut showed significantly different levels of fullness between the times of sampling over the 5 day sampling period, thus indicating that varying amounts of food were taken at different times of the day (Table 3.2). The fish therefore exhibited a periodic variation in the amount of food present. In winter however the foregut fullness was not significantly different between sampling times. During this season the amount of food in the foregut was often negligible, and up to 20% of the fish had empty foreguts.

For all seasons the mean foregut fullness and standard

error was calculated. For summer and autumn the first 24 hours were omitted since this cycle contained uncharacteristically high values. The foregut fullness in summer was still observed to peak in the late afternoon (1800h). However, the 2 peaks thought to be apparent in autumn were due to a wide spread of values and hence no significant differences were observed in gut fullness at the times sampled. In winter the levels of fullness were relatively low and no pattern was observed. During spring foregut fullness increased in the latter part of the day. The distinct peak observed in summer was not apparent, however, the period of increased feeding was carried on until later in the day. These results show that a definite change in feeding occurred throughout the year. This has been reported in several fish species: Arctic charr have been shown to alter their feeding behaviour with season. Specific peaks in feeding were identified in both spring and summer whilst in autumn and winter the feeding was spread more during the day (Jorgensen & Jobling, 1989); in rainbow trout the overall patterns of feeding behaviour changed with season (Landless, 1976; Grove *et al*, 1978); and channel catfish, *Ictalurus punctatus*, showed 15% feeding activity 2400-0600h, 6% between 0600-1200h, 36% between 1200h-1800h and 43% between 1800h-2400h during June and July. However, from December to March no significant differences were obtained between time periods. Feeding was spread equally

throughout the day (Hastings et al, 1972). Eriksson and Alanara (1990) noted 'that the timing of feeding in brown trout and Atlantic salmon fits well with the timing of organic drift in northern streams, both dielly and seasonally'. The timing of drift patterns in natural streams has also been shown to be clearly dependent on light-dark changes (Muller, 1978a).

Several authors have reported a direct relationship between food intake and temperature (Grove et al, 1978; Gwyther and Grove, 1981; Farmer et al, 1983). However if the seasons were ranked in decreasing order according to highest average temperature, that order would be autumn, summer, winter, spring. But if the seasons were ranked according to highest maximum wholegut fullness recorded, the order would be summer, autumn, spring, winter. Hence although temperature may affect food intake, season or photoperiod is a stronger influence. This also highlights the possible interactive effects on gut contents of the 2 variables, temperature and light.

Hourly variations of water temperature were minimal all year and would therefore be unlikely to affect gut fullness, since the gut is thought to take a day to respond to changes of 1-2 ^{o}C in ambient water temperature (Fauconneau, 1989). Temperature is also unlikely to have a large role in synchronizing the timing of peaks of gut fullness as no obvious daily changes in temperature level were observed. As

noted by Reynolds & Casterlin (1980) temperature tends to have a greater effect on the physicochemical nature of biological processes than as a synchronizer of events. Light levels, however, show a great degree of rhythmic change within a 24 hour period. Decreasing light levels during late afternoon may synchronize feeding to a set and repeatable rhythm. During summer and spring an afternoon peak in the amount of food present in the gut occurred while light levels were decreasing. Either the diel change in light perceived by the fish, or the duration of the light period may affect the occurrence of feeding peaks. Some fish species can utilise unchanging light levels to indicate favourable times to feed. For example light onset was staggered for 6 groups of goldfish, Carassius auratus, (i.e. 1500h, 1900h, 2300h, etc) but a daily meal was fed to all groups simultaneously at 1500h (Noeske et al, 1981). Thus each group of fish was fed at a different subjective time of the day (i.e. 0, 4, 8 etc after light onset). Differences in appetite were observed between the groups of fish.

3.5 SUMMARY

1. The amount of food present in all portions of the gut of Atlantic salmon, in freshwater tanks, fluctuated throughout the day.
2. The pattern of rhythmic stomach fullness changed with season. The feeding pattern fluctuated rhythmically in

summer, autumn and spring.

3. The amount of food present in the gut of the fish sampled was not constant with season. During winter the amount of food present was minimal while the highest levels were recorded during summer.

4. Fore- and hindgut fullness correlations altered with season.

5. Peaks in foregut fullness were observed at the same time (1800h) for two seasons - summer, and spring.

6. Temperature may affect the amount of food present in the gut, but should be used as an indicator of expected gut fullness only when related to season or photoperiod.

7. Light may be a synchronizer of feeding peaks although further work is necessary to support this.

8. The degree of interaction between the 2 environmental parameters of temperature and light that may affect gut fullness is unknown and requires further investigation.

CHAPTER 4

RHYTHMIC STOMACH FULLNESS OF SMOLTS HELD IN SEA CAGES IN THE SUMMER

4.1 INTRODUCTION

There has been a growing awareness amongst fish farmers and associated conservation groups of the problem of the environmental impact of aquaculture on the marine ecosystem (Gowen et al, 1988). Problems arise in the enrichment of the seabed and therefore the water and associated changes in water quality. In some instances ecological changes may become a risk factor to the aquaculture industry itself (Rosenthal et al, 1988). Sites with a low tidal flow experience a greater build up of waste on the seabed beneath the cages than do sites with high flow (Gowen & McLusky, 1988).

The waste produced by fish held in cage sites can be divided into two categories - particulate material in the form of uneaten food and faeces and soluble material released as excretory waste. The former leads to a build up of sediment on the seabed and the latter affects the water column not necessarily in the immediate vicinity of the sediment. Uneaten food pellets arrive at the seabed with their original composition, since the time taken to settle is not sufficient for leaching or microbial decomposition. It is this sediment or organic waste that affects the ecology of the benthic macrofauna. The degree of pollution can be assessed by the disturbance of the macrofauna community in zones surrounding fish farms. At sites where

there is a high level of waste deposition the sediment is devoid of macrofauna (Gowen et al, 1988). The benthic fauna can be disrupted for considerable distances. At a salmon farm in Loch Spelve, Isle of Mull, Scotland, marked changes were observed up to a distance of 15m, but no effects were observed at a distance of 120m (Brown et al, 1987). A high localized input to the sediment can result in deterioration of the environment and consequently the viability of the farm. In these cases there is a net production of the by-products of anaerobic metabolism (methane, CH_4; ammonium, NH_4^+; and hydrogen sulphide, H_2S), from the sediment (Braaten et al, 1983). The most important anaerobic metabolite is hydrogen sulphide which is toxic to fish (Fayette & Hanes, 1980). Its release from the enriched sediments has been related to deterioration in the health of fish at some farms in Norway (Braaten et al, 1983).

Another problem is the need to reduce costs in producing marketable sized salmon. Feed is the largest operating expense of a salmon farm and is an obvious place to attempt reduction of costs, provided it does not adversely affect production (Furnell, 1990). Feed costs have been calculated as 35-40 percent of a farm's running costs. Reduction of food wastage can mean the difference between survival and bankruptcy (Murphy, 1992).

A simple answer to both problems (as discussed in Chapter 1) is a change in husbandry practice, in particular a change

in feeding ensuring that more food is consumed by the fish leaving less to fall as waste. It is believed that if feeding regimes which favoured more food consumption could be designed these problems may be minimised (Seymour & Bergheim, 1991).

To design suitable feeding regimes it must first be established whether salmon in sea cages exhibit visible patterns of feeding. Atlantic salmon parr in freshwater tanks show a rhythmic feeding pattern with peaks of foregut fullness (Chapter 3). This is probably due to salmonids having evolved in a constantly changing environment. The feeding rhythm exhibited can be seen as an evolutionary compromise between taking advantage of an ever changing habitat and merely coping or surviving in that habitat. For stream resident salmonids relative food abundance is highly predictable, both daily and yearly. For sea dwelling salmon, however, the problem of food availability is an combination of both spatial and temporal factors (Eriksson & Alanara, 1990). For sea dwelling salmonids in captivity the spatial problem is removed due to their restricted movements and the temporal aspect is controlled by the fish farmer.

A peak in foregut fullness when fish are given the opportunity to feed continuously, illustrates the time of day fish are responsive to feed. Times of feeding are important since animals are different physiological organisms

at different times of the day. This will result in the organism exhibiting a diversified range of biological responses to specific stimuli e.g. food, depending on the time of day at which the stimuli are received (Spieler, 1977). As discussed in Chapter 1 the timing of a single daily meal can affect a variety of physiological processes including weight gain per unit of food intake and possibly food conversion efficiency (Noeske & Spieler, 1984).

Up until recently the use of sea cages to carry out trials has been limited, firstly due to the small number of available sea sites for trials, and secondly due to the feasibility of working in often hostile weather conditions with limited manpower and large numbers of fish. So the work has been limited mainly to growth trials, some being carried out under various conditions of additional light (Krakenes et al, 1991; Hansen et al, 1992), of manipulated photoperiods (Saunders et al, 1985) and after altering the spectral composition of the light (Stefansson & Hansen, 1989). To overcome some of these problems a few studies have been carried out in pumped seawater tanks (Usher, 1988; Thorpe et al, 1990a).

Thorpe et al (1990b) were the first to use an x-ray technique to estimate food intake of salmon in sea cages. Kadri et al (1991) also worked in sea cages to relate swimming activity with feeding behaviour. Obviously, where possible, sea cage work is needed to be able to demonstrate

clearly the problems and solutions of commercially cultivating Atlantic salmon. One way of overcoming the problem of large scale work and vast quantities of manpower is to use smaller cages but ones with similar stocking densities of the larger cages.

The present investigation was designed to test the following null hypotheses:

1. Gut fullness is constant.
2. If it is not, then there are no differences between feeding rhythms of Atlantic salmon held in freshwater tanks and seawater cages.
3. Feeding rhythms are not influenced by environmental parameters.

4.2 MATERIALS AND METHODS

4.2.1 Fish Stocks

Atlantic salmon smolts were transported in tanks loaded on a truck to Port Appin, Argyll, Scotland, on May 13th 1990. A roll-on roll-off boat was used to take the truck to the sea cages of Lismore Salmon Farm, Argyll, Scotland. The fish were then pumped into the seacages, 2000 were stocked in a 5x5x5m Kames cage (Kimelford, Oban, Scotland). At the onset of the investigation the average fish weight was 70.2g ($^{+}$/_ 2.1) and average fork length was 193.6mm ($^{+}$/_ 1.6).

4.2.2 Feeding Regime

A spinning disc (50L Aquatess, Ullapool, Scotland) feeder was activated every 10 minutes to deliver 65g of food at each activation. The feeder was normally light sensitive, but during this investigation this was over-ridden so food was delivered day and night. The fish were fed Mainstream (BP Nutrition) diet until 24 hours before the onset of sampling. The food was then changed to a diet labelled commercially with ballotini (Jencons Ltd, Leighton Buzzard, UK) 300-350 um at 3% w/w.

4.2.3 Sampling

The fish were sampled every 4 hours from 0100h on 4th July 1990 (i.e. 0100h, 0500h, 0900h, 1300h, 1700h and 2100h) to

1300h on 8th July 1990. The sampling times were altered to accommodate normal working hours of the fish farm. At each sampling time the net was raised to allow a sample of fish to be removed. The fish were transported by boat in plastic aerated bins to the housing barge to be anaesthetised and x-rayed. The fish were allowed to recover before they were returned to the cage. Due to adverse weather conditions on the 6th July 1990 it was impossible to reach the cages, and therefore the 0500h sample had to be omitted.

Light meter readings were also taken every 4 hours but as the meter was found to be unreliable theoretical light levels were calculated (see below). This gave the pattern of light intensities observed over the period of sampling.

4.2.4 Calculations

The following equation (pers. com. A. Shanks) was used to calculate the missing value of fore-, hind- and wholegut fullness at 0500h 06.07.90.

$$x_{ij} = \frac{rx_{j.} + cx_{.j} - x_{..}}{(r-1)(c-1)}$$

where x_{ij} is the required estimate of each variable of fore-hind- and wholegut fullness

r and c are rows (24 hour cycles) and columns (times of sampling) respectively

$x_{j.}$ is the sum of observations in the same rows as the missing values

$x._j$ is the sum of observations in the same columns as the missing values

x.. is the sum of all recorded observations

Theoretical light intensities at the water surface were calculated every 4 hours for the days of the trial. The theoretical amount of daylight at latitude 56 ^{o}N, assuming a cloudless sky, was calculated using an algorithm to assess ground illumination in lux (Yallop, 1986).

Weighed amounts of labelled food were x-rayed and the amount of marker per gram of food was calculated from the plate. The amount of food present in the fore-, hind- and wholegut of each fish at each sampling time could then be calculated. The dry weight of food, expressed as a percentage of the dry weight of the fish, was defined as an index of gut fullness. The averages of gut fullness and their standard errors were calculated for each sample of 20 fish.

Mean fullness data for fore- and hindgut, and light intensity data at each sample time were also calculated as deviations from the overall mean, to obtain average cycles for 24 hour periods.

4.2.5 Statistical Analysis

Two way analysis of variance (ANOVA; Campbell, 1989) was used to test the effect of the non-metric variables of day

and time on a single dependent response variable, of gut fullness. Both F-ratios were tested for significance at the 5% ($p>0.05$), 1% ($p>0.01$), and 0.1% levels ($p>0.001$).

A two sample t-test was used to assess whether mean fore and hindgut differed significantly between sampling times (Campbell 1989).

4.3 RESULTS

Gut contents fluctuated rhythmically throughout the sampling period (Fig. 4.1). Wholegut contents were minimal (1.08 %DBW $^{+}/_{-}$ 0.17) at 0900h and maximal (2.35 %DBW $^{+}/_{-}$ 0.19) at 0100h. Foregut contents were minimal (0.33 %DBW $^{+}/_{-}$ 0.05) at 0100h and maximal (0.95 %DBW $^{+}/_{-}$ 0.13) at 2100h. Hindgut contents were minimal (0.71 %DBW $^{+}/_{-}$ 0.10) at 0900h and maximal (1.75 %DBW $^{+}/_{-}$ 0.15) at 0500h.

Two way analysis of variance for foregut fullness indicated no significant differences between days, but there were significant differences between times ($p<0.05$, Table 4.1). Hindgut fullness was significantly different between days ($p<0.05$, Table 4.1), but not between times. The average hindgut fullness for day 1 differed from the corresponding averages of the third and fourth days.

In all but the first cycle the highest foregut values were recorded at 1700h, but the average fullness values at 1700h and 2100h were closely similar. The hindgut was maintained throughout at a higher level of fullness than the foregut. From the deviation from the mean cycle, hindgut fullness peaked at 0100h whilst the foregut peaked between 1700h and 2100h (Fig.4.2).

The mean foregut fullness values show that the level of fullness at 1700h is significantly different from the samples taken at 0500h, 0900h and 1300h (Table 4.1) prior to

Figure 4.1: The rhythm shown by open triangles indicates the foregut fullness expressed as %DBW and the rhythm of closed triangle symbols shows the total (or whole) gut fullness levels. Each point represents the average gut fullness for all the fish sampled at that time. The vertical bars show standard error and the horizontal bar below the axis indicates periods of darkness during the sampling.

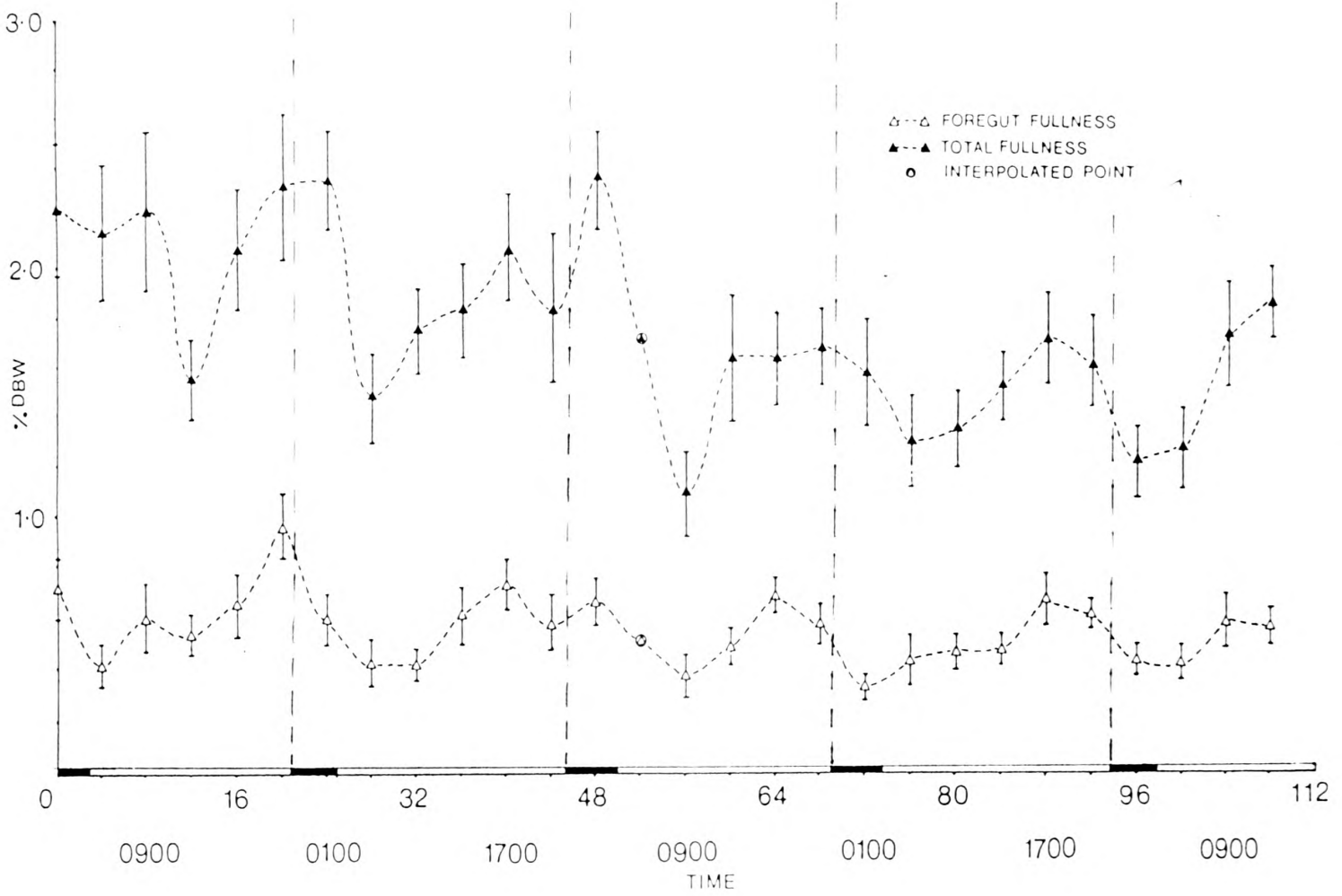

3·0
2·0
1·0
% DBW
△--△ FOREGUT FULLNESS
▲--▲ TOTAL FULLNESS
o INTERPOLATED POINT
0
16
32
48
64
80
96
112
0900
0100
1700
0900
0100
1700
0900
TIME

Table 4.1 : Differences in fore- and hindgut fullness of Atlantic salmon in sea cages between different:

a, sampling times

Sample Times	Foregut Fullness Significance Level	Sample Times	Hindgut Fullness Significance Level
1700h & 0100h	N.S.	0100h & 0500h	N.S.
1700h & 0500h	***	0100h & 0900h	N.S.
1700h & 0900h	**	0100h & 1300h	*
1700h & 1300h	**	0100h & 1700h	N.S.
1700h & 2100h	N.S.	0100h & 2100h	*

b, cycles/days

Cycles	Foregut Fullness Significance Level	Hingut Fullness Significance Level
1 & 2	N.S.	N.S.
1 & 3	N.S	***
1 & 4	N.S.	***
2 & 3	N.S.	N.S.
2 & 4	N.S.	*
3 & 4	N.S.	N.S.

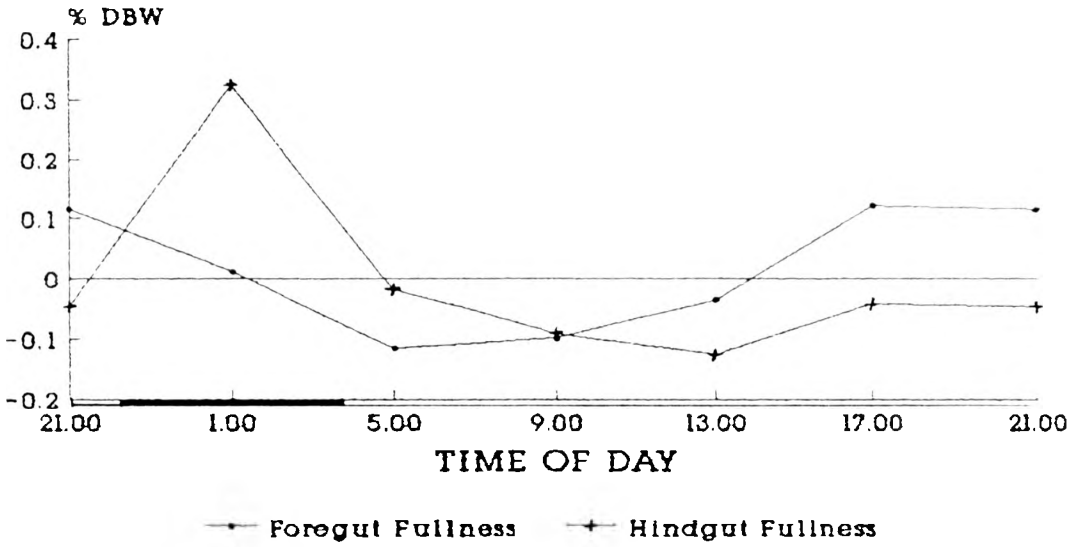
N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

*** - $p<0.001$

Figure 4.2: The data calculated as deviation from the mean shows an average cycle of fore- and hindgut fullness over a 24 hr period. Thw horizontal bar below the axis shows the period of darkness.


% DBW
0.4
0.3
0.2
0.1
0
-0.1
-0.2
21.00
1.00
5.00
9.00
13.00
17.00
21.00
TIME OF DAY
Foregut Fullness
Hindgut Fullness

this sample. However, the mean samples at 1700h and 2100h and 1700h and 0100h were not significantly different. Hence the increase in foregut fullness, first observed at 1700h, was sustained until after 0100h when a decrease is observed (Fig. 4.3). The mean hindgut fullness values show that the level of fullness at 0100h is significantly different from the samples taken at 1300h and 2100h (Table 4.1, Fig. 4.4).

4.3.1 Gut Fullness In Relation To Environmental Parameters

The cyclic environmental parameters of tide, light and temperature could have influenced gut fullness. As temperature, measured continuously every hour for a week, showed little change and will therefore be considered to be relatively constant, it was discounted as influencing the cyclicity of gut fullness. High and low tide times were calculated for the Lismore area where the cages were located. The pattern of foregut fullness did not mimic the rise and fall of tide levels (Figs. 4.3 and 4.5), since the period of the filling cycle, for the foregut, was 24 hours while the tide cycle period was approximately 12 hours. However, peaks in foregut fullness always occurred just after light levels had peaked, as is clear from the deviation diagram (Fig. 4.6). The peak of foregut fullness occurred between 1700h and 2100h when light levels were decreasing.

Figure 4.3: The average cycle of foregut fullness expressed as % DBW for a 24 hour period is shown. The level of gut fullness at 1700h is significantly different from that at 0500h ($p<0.001$, Table 4.1), 0900h ($p<0.01$, Table 4.1) and 1300h ($p<0.01$, Table 4.1). The vertical bars indicate standard error and the horizontal line below the axis shows the period of darkness.

PAGE(S) MISSING
NOT AVAILABLE

Fig. 4-3.

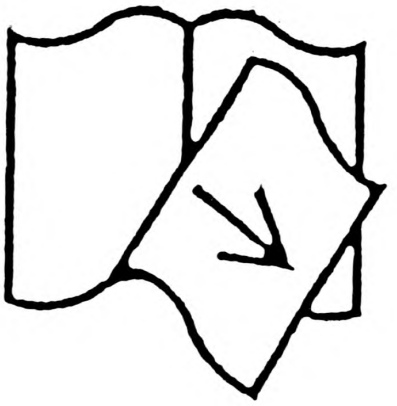
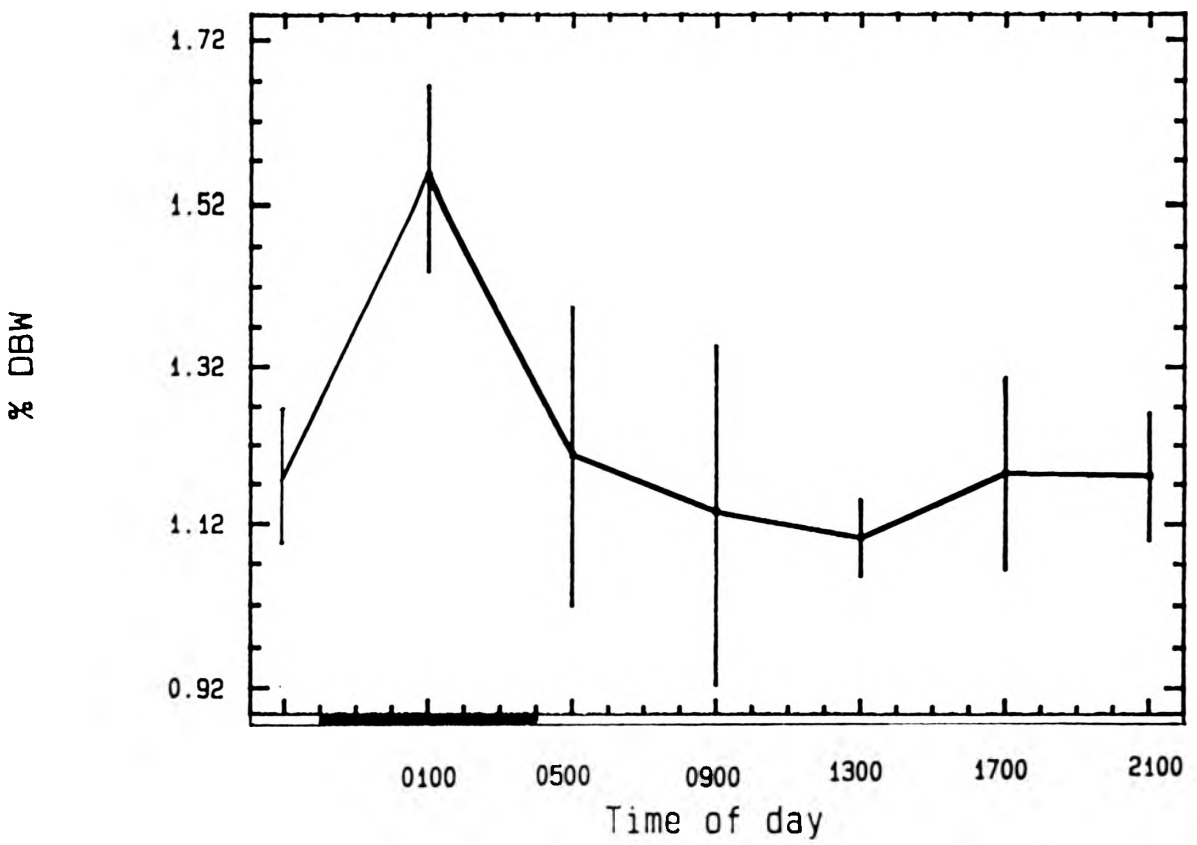
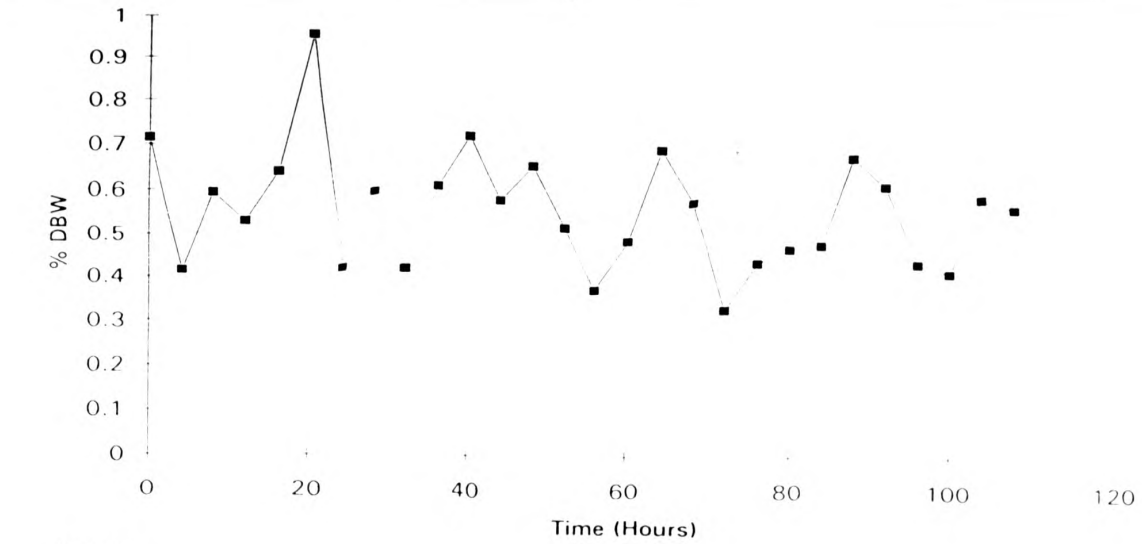


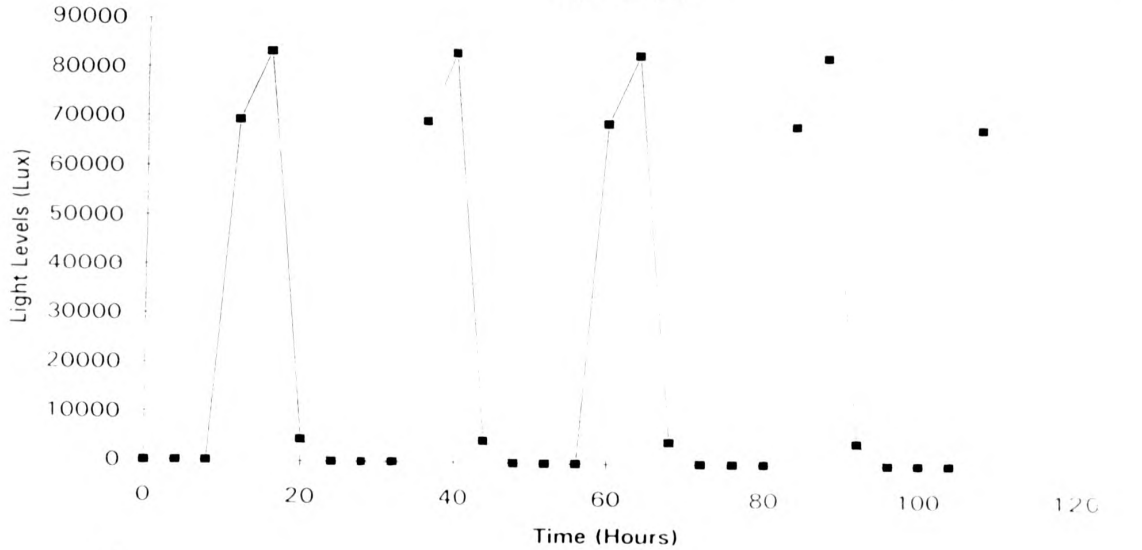
Figure 4.4: The average cycle of hindgut fullness expressed as %DBW for a 24 hr period is shown. The gut fullness level at 0100h is significantly different compared to both the levels at 1300h and 2100h ($p<0.05$, Table 4.1). The vertical bars show standard error and the horizontal bar below the axis shows the period of darkness.



Hindgut fullness in seacages

Figure 4.5: The environmental parameters of light (middle graph) and tide (bottom graph) are shown to fluctuate over the sampling period as foregut fullness (top graph) is monitored.


% DBW
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
120
Time (Hours)


Light Levels (Lux)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
20
40
60
80
100
120
Time (Hours)

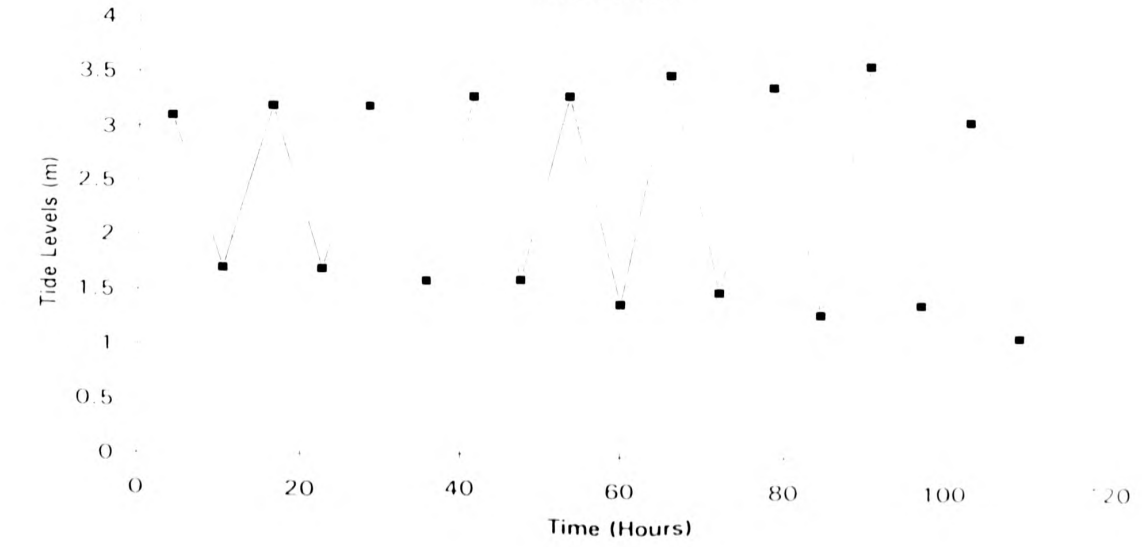
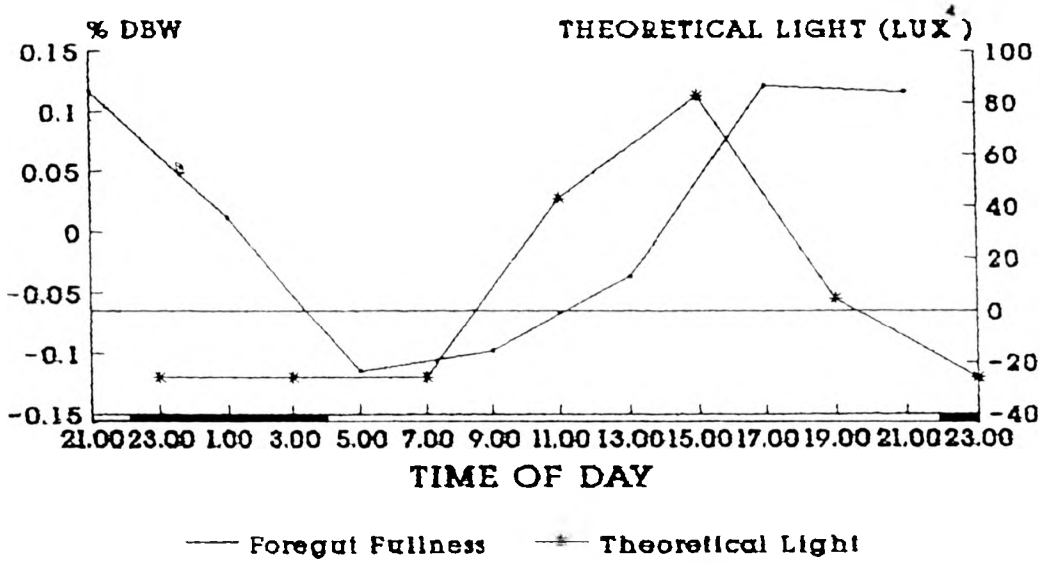

Tide Levels (m)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
Time (Hours)

Figure 4.6: The data calculated to show the deviation from the mean indicates an average cycle of foregut fullness and theoretical light levels for a 24 hr period. The horizontal bar below the axis shows the period of darkness.


% DBW
THEORETICAL LIGHT (LUX)
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
100
80
60
40
20
0
-20
-40
21.00 23.00 1.00 3.00 5.00 7.00 9.00 11.00 13.00 15.00 17.00 19.00 21.00 23.00
TIME OF DAY
Foregut Fullness
Theoretical Light

4.4 DISCUSSION

Atlantic salmon smolts held in sea cages during the summer and allowed to feed continuously throughout the day and night showed a diel fluctuation in the amount of food in the fore- and hindgut similar to that of Atlantic salmon in freshwater tanks in the summer (Chapter 3). Foregut fullness was significantly different between times and peaked between 1700h and 2100h. Kadri *et al* (1991) found a peak in both swimming speed and feeding response of Atlantic salmon, *S. salar* L., in sea cages during late afternoon. They also recorded a peak of both of these measurements in the early morning and concluded appetite showed 2 peaks during the day. As this work was carried out simultaneously with the present study there was clearly a difference in feeding pattern between the sites. A study investigating the growth of chinook salmon, *Oncorhynchus tshawytscha*, in British Columbia found site difference had the greatest effect on mean growth differences (Kreiberg *et al*, 1988). This may have been due to the different feeding patterns of the fish at the different sites. However, in the Kadri *et al* (1991) study the fish were fed only during daylight hours. The morning peak may therefore have been due to a period of food deprivation and a hunger response at daylight. Brett (1971) found on refeeding sockeye salmon, *O. nerka*, after an 11 hour period of food deprivation, the fish took a relatively large meal followed by a relatively small meal. Talbot *et al* (1984) also found that starved Atlantic salmon took a larger

meal than normal on refeeding.

Blyth et al (1993) tested an adaptive feeding system on Atlantic salmon in sea cages in Tasmania. The system recorded the levels of waste food beneath a feeding zone and so established the pattern of feeding by difference. An initial feeding peak was detected just after dawn and a significant feeding bout was observed two and half hours before total darkness. Once again, however, no food was offered during darkness.

Light levels showed rhythmic fluctuation every 24 hours. Foregut fullness also showed a rhythmic fluctuation over the same period. Foregut fullness was observed to increase as light decreased. This again implies Atlantic salmon may use light levels to synchronise their peaks of feeding. This has been reported in other fish species. Hoplosternum littorale started to increase their feeding at dusk with a peak between 0200h-0500h (Boujard et al, 1990) during which time 40% of their daily ration was consumed. When the light-dark cycle was advanced by 9 hours the feeding rhythm was advanced similarly. The light-dark cycle was then increased to cover 36 hours (i.e. 13.5L/22.5D and 25.5L/10.5D), the fish still responded to the light-dark change to synchronise their feeding rhythms rather than maintaining a 24 hour feeding rhythm (Boujard et al, 1991).

Atlantic salmon have been shown to use the light-dark

alteration to synchronize their locomotor activity rhythms (Richardson & McCleave, 1974). Some authors have suggested, tentatively, that activity may be indicative of feeding peaks. However, locomotor activity records a complex mixture of different behaviours. Swift (1962, 1964) noted that 'a trout feeds because it is active and is not active because it is feeding'. Even if feeding does take place during active phases, fish do not feed during the entire period of activity. It is therefore unwise (however tempting) to infer too close a relationship between activity and feeding (Boujard & Leatherland, 1992).

4.5 SUMMARY

1. The gut contents of juvenile Atlantic salmon in sea cages in the summer fluctuated rhythmically, in a similar pattern to that observed in freshwater tanks.
2. Foregut fullness was significantly different between times but not between days, peaking between 1700h and 2100h.
3. Tide and temperature fluctuations showed little influence on gut fullness.
4. Light was the only environmental parameter that showed a similar rhythmic pattern to that of foregut fullness. Further investigation is necessary to establish whether light may be influential in synchronizing feeding rhythms, and to determine which property of light is most important.

CHAPTER 5

EFFECTS OF CONTROLLING ENVIRONMENTAL PARAMETERS ON RHYTHMIC STOMACH FULLNESS

5.1 INTRODUCTION

The amount of food present in the gut of several fish species, fluctuates within a 24 hour period, for example in Atlantic salmon, Salmo salar L., (Hoar, 1941; Karpenko, 1982); perch, Perca fluviatilis L., (Thorpe, 1977); brook trout, Salvelinus fontinalis Mitchill, (Allan, 1978; 1981); pink salmon, Oncorhynchus gorbuscha Walbaum, (Godin, 1981); coho salmon, O. kisutch Walbaum, (Brodeur & Pearcy, 1987) and chinook salmon, O. tshawytscha Walbaum, (Sagar & Glova, 1988). Factors which influence both the timing and amplitudes of these fluctuations are important for understanding basic fish physiology. Information on feeding fluctuations is necessary both to interpret gut fullness data collected from wild populations and to manipulate feeding regimes of fish held under culture conditions.

Atlantic salmon is an important commercial species. By investigating the influences of environmental parameters on their feeding rhythms it is hoped to design more efficient feeding regimes. This would be advantageous commercially and may help to reduce food wastage, thereby cutting food costs.

Two of the most important environmental variables which may effect feeding rhythms are light and temperature. Jobling (1987) noted 'the effects of individual variables (such as light and temperature) have rarely been studied'. He was investigating their effects on growth of Arctic charr

and the identification of an endogenous rhythm. A feeding rhythm may be an expression of an endogenous or exogenous rhythm. If it is the expression of an endogenous rhythm then it will run free under constant conditions, i.e. a rhythm will still be apparent but not necessarily with a 24 hour periodicity (Minors & Waterhouse, 1986). Palsson *et al* (1992) investigated food intake of Arctic charr under continuous light and constant temperature. However, they were interested in long term variation and concluded that seasonal changes in food intake under constant conditions were apparent. Although an endogenous rhythm was identified the factors responsible for the variation were not.

Variations in the light-dark cycle have been indicated as important synchronisers of circadian rhythms (Muller, 1978a, 1978b; Moore-Ede, 1981). Sub-arctic areas provide excellent natural conditions for investigating biorhythms. The water temperature in the river Kaltisjokk, Northern Sweden, is a constant 0.4 ^{o}C from November to May and most chemical factors are stable with oxygen concentration at nearly 100%. Both the brown trout, *Salmo trutta*, and the minnow, *Phoxinus phoxinus*, showed a distinct change between activity and rest, while only one environmental factor showed a consistent variation, the daily light-dark changes (Muller, 1978a). However, as already discussed (Chapter 4) activity does not always imply feeding, although in the case of the sub-arctic river the

drift patterns are also dependent on light-dark changes (Muller, 1978a). Temperature is thought to be a secondary indicator of time (Nelson et al, 1975), but its importance may be in influencing the quantity of food consumed (Kaushik, 1986), as opposed to when it is taken. Clarke et al (1978) investigated the growth and adaptation to seawater in 'underyearling' sockeye (O. nerka) and coho (O. kisutch) salmon and reported that temperature controlled the rate of response to photoperiod changes. For example, changes in growth rate caused by manipulating photoperiod were apparent sooner at higher temperatures. Again in the sub-arctic river, Kaltisjokk, water temperature was concluded to be of secondary importance. Temperature determined the amount of activity but not the phase position of that activity (Muller, 1978a).

The present study was designed to test the following null hypotheses:

1. Gut contents fluctuate under all environmental conditions.
2. Peaks in gut contents cannot be attributed to either temperature or simulated photoperiod influence.
3. Temperature and foregut fullness, and simulated photoperiod and foregut fullness are not correlated.

5.2 MATERIALS AND METHODS

5.2.1 Fish Stocks

In May 1991, four groups of 100 1+ hatchery reared Atlantic salmon average weight 7.1g ($^{+}/_{-}0.2$) and average fork length 84.8mm ($^{+}/_{-}0.07$) were held in $1m^2$ radial flow tanks at S.O.A.F.D. Almondbank. Each tank was held under one of four controlled environmental regimes as follows (and 2 months prior to the start of the sampling):

i, Simulated ambient photoperiod, ambient temperature - SAPAT

ii, Simulated ambient photperiod, controlled temperature - SAPCT

iii, Constant light, ambient temperature - CLAT

iv, Constant light, controlled temperature - CLCT

All the tanks were held under fluorescent light. To simulate ambient photoperiod, the light levels were increased and decreased during the periods of dawn and dusk respectively, over a total range of c.140 lx. Between the periods of twilight light intensity remained constant. The fish therefore experienced ambient photoperiod (i.e. the correct number of hours of daylight for the respective time of the year). Simulated sunrise was 0335 and simulated sunset was 2020. The fish held under constant light experienced continuous light at an unfluctuating level throughout the study. Light meter readings were taken every 4 hours for each of the experimental regimes (Table 5.1).

Table 5.1: Environmental parameters for the four regimes tested

Regime	Parameters	Range	Mean
SAPAT	Simulated ambient photoperiod	0-136 Lx	--
	Ambient temperature	11.8-17.5^{o}C	14.5
SAPCT	Simulated ambient photoperiod	0-104 Lx	--
	Controlled temperature	13.2-16.7^{o}C	14.7
CLAT	Constant light	139-157 Lx	150.8
	Ambient temperature	11.8-17.5^{o}C	14.5
CLCT	Constant light	82-98 Lx	89.2
	Controlled temperature	13.2-16.7^{o}C	14.7

All the tanks used the same water source, the River Almond. For the controlled temperature regimes, a heating unit (Generator Systems) raised the ambient water temperature. The heater however, cut out if the ambient temperature rose above a pre-set limit of 15 ^{o}C. This resulted in reducing the amplitude of the fluctuations observed in the ambient water temperature without making the water temperature constant. The tanks which were held under ambient water temperature received water direct from the river. Both ambient and controlled temperature levels were monitored automatically using temperature probes (Squirrel logger, Grant Instruments, Cambridge (Table 5.1)).

5.2.2 Feeding Regime

The fish were fed 3g of a commercial salmon food every 20 minutes throughout the day and night. A caddymatic feeder was used to deliver the food along with the inflow of water. Before the onset of the sampling period (48 hours) the standard food was substituted for a diet labelled with an inert radio-opaque marker of iron powder (180-250 um) included at a rate of 5% of the diet. Weighed samples of labelled diet were x-rayed to determine the amount of marker present per unit weight of food.

5.2.3 Sampling

A sample of 20 fish was removed from each tank, anaesthetised, x-rayed and then returned to their respective

tanks. Subsequent samples were treated identically every 4 hours i.e. at 1000h, 1400h, 1800h, 2200h, 0200h, 0600h, for 112 hours. The initial sample was taken at 1000h on 20.05.91 and the last one at 0200h on 25.05.91.

From the developed plates the amount of food present in the fore-, hind- and wholegut for each fish at each time of sampling was assessed. Gut fullness was expressed as 100 times the ratio of dry weight of fish/ dry weight of food (Percentage Dry Body Weight, %DBW).

5.2.4 Calculations

The developed plates were analysed to calculate the amount of food present in the fore-, hind- and wholegut of each fish at each sampling time, using a relationship of marker count per unit weight of food. Gut fullness was expressed as percent dry body weight (% DBW), an index of gut fullness(as described above), for each fish sampled. The average gut fullness, for the sample of 20 fish sampled at each time calculated, with the corresponding standard error of the mean.

Data were also calculated as 'deviations from the mean' to obtain an average cycle for a 24 hour period. The following calculation was carried out for foregut fullness, light and temperature levels. The data were arranged in table form so each column represented a sampling time (i.e. 0200h, 0600h) and each row represented a day or cycle. The mean of each

sampling time (i.e. column) where n=5, also the mean of all values in the tables i.e. n=30, the grand mean, was calculated. The mean for each time (i.e. column) was taken from the grand mean to give 'the deviation from the mean'.

5.2.5 Statistical Analysis

Two way analysis of variance (ANOVA) was carried out on gut fullness data for the fore-, hind- and wholegut under the four environmental regimes tested. This allowed the differences betwen times-of-day and between different 24 hour periods to be calculated (Table 5.2).

Values for both fore- and wholegut fullness were consistently higher during the first 24 hours than during the next 4 cycles (Fig. 5.1). It is suspected that these high values resulted from a change in husbandry practice on the first day of the experimental sampling. After statistical analysis it was decided to omit these observations for the purposes of further statistical testing.

The missing observation of gut fullness at 116 hours was estimated by calculating the weighted average (x_{ij}), taking into account other observations made at the same time of day (i.e. 0600h), but during different cycles, and observations during the same cycle (i.e. cycle 5), but at different times of the day as follows:

Table 5.2: Summary of significance of difference in gut fullness between cycles and times of day

Regimes	Gut Portion	Between Cycles (3 d.f.)	Between times (5 d.f.)
SAPAT	Foregut	N.S.	***
	Hindgut	*	*
	Wholegut	N.S.	**
SAPCT	Foregut	N.S.	*
	Hindgut	*	N.S.
	Wholegut	*	*
CLAT	Foregut	N.S.	***
	Hindgut	*	N.S.
	Wholegut	*	*
CLCT	Foregut	N.S.	N.S.
	Hindgut	N.S.	N.S.
	Wholegut	N.S.	N.S.

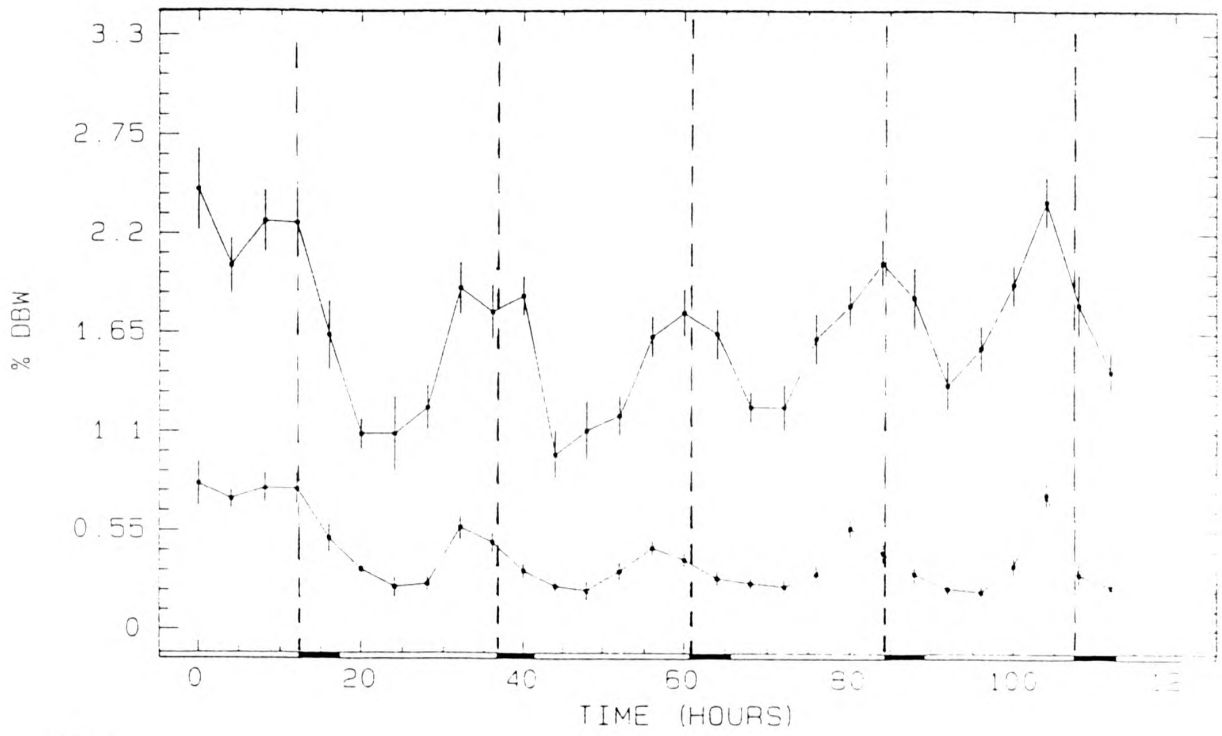
N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

*** - $p<0.001$

Figure 5.1: The foregut fullness levels (the lower recording) and whole gut fullness levels for simulated ambient photoperiod and ambient temperature (SAPAT). Each point represents the average gut fullness for the fish sampled at that time, time 0 is 1000h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling.



SAPAT

$$x_{ij} = \frac{rx_{j.} + cx_{.j} - x_{..}}{(r-1)(c-1)}$$

where x_{ij} is the required estimate

r and c are rows and columns respectively

$x_{j.}$ is the sum of the observations in the same row as the missing value

$x_{.j}$ is the sum of the observations in the same column as the missing value

$x_{..}$ is the sum of all recorded observations

Correlations between gut fullness and temperature and gut fullness and light were calculated for the 23 sets of observations made for cycles 2-5. Correlations between light levels and gut fullness, for regimes where the light levels were simulated ambient, were inappropriate, because the light levels were either zero or constant and so the variable was not linear. However, correlations between daylight levels (i.e. levels recorded from 0600h to 1800h) and the respective levels of fullness were caried out (Table 5.3).

Table 5.3: Summary of significance of correlations between gut fullness and light, and gut fullness and temperature

Regimes	Gut Portion	Correlation with light levels	Correlation with temperature
SAPAT	Foregut	N.S.	***
	Hindgut	N.S.	*
	Wholegut	N.S.	**
SAPCT	Foregut	N.S.	N.S.
	Hindgut	N.S.	N.S.
	Wholegut	N.S.	N.S.
CLAT	Foregut	N.S.	**
	Hindgut	N.S.	N.S.
	Wholegut	N.S.	*
CLCT	Foregut	N.S.	N.S.
	Hindgut	N.S.	N.S.
	Wholegut	N.S.	N.S.

N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

*** - $p<0.001$

5.3 RESULTS

5.3.1 Gut fullness under simulated ambient photoperiod, ambient temperature (SAPAT).

Both hind- and wholegut fullness fluctuated rhythmically with a period of 24 hours. Foregut contents showed a distinct peak at 1800h (Fig. 5.1). The mean foregut fullness value at 1800h was significantly different from all the mean samples taken at other sampling times (Table 5.4, Fig. 5.2). Hence more food was present between 1400h-1800h than at other times of the day. The hindgut was maintained at a relatively high level of fullness throughout the experiment (Fig 5.3). Two way analysis of variance showed no significant differences in fore- and wholegut fullness between cycles, but significant differences in both fore ($p<0.001$) and wholegut ($p<0.01$) fullness between sampling times (Table 5.2). The hindgut showed significant differences in fullness at the 5% level both between cycles and times (Table 5.2).

5.3.2 Gut fullness under simulated ambient photoperiod, controlled temperature (SAPCT)

Gut contents fluctuated with time (Fig. 5.4), but no consistent peak in foregut fullness was observed at any time of the day. The mean foregut fullness was only significantly different from the mean sample at 0600h ($p<0.05$) indicating a difference in gut fullness between late evening (1800h -

Table 5.4: Summary of signifcance levels between gut fullness at 1800h and at other times

Regimes	1800h & 1000h	1800h & 1400h	1800h & 2200h	1800h & 0200h	1800h & 0600h
SAPAT	**	**	*	**	**
SAPCT	N.S.	N.S.	N.S.	N.S.	*
CLAT	***	*	*	**	**
CLCT	N.S.	N.S.	N.S.	N.S.	N.S.

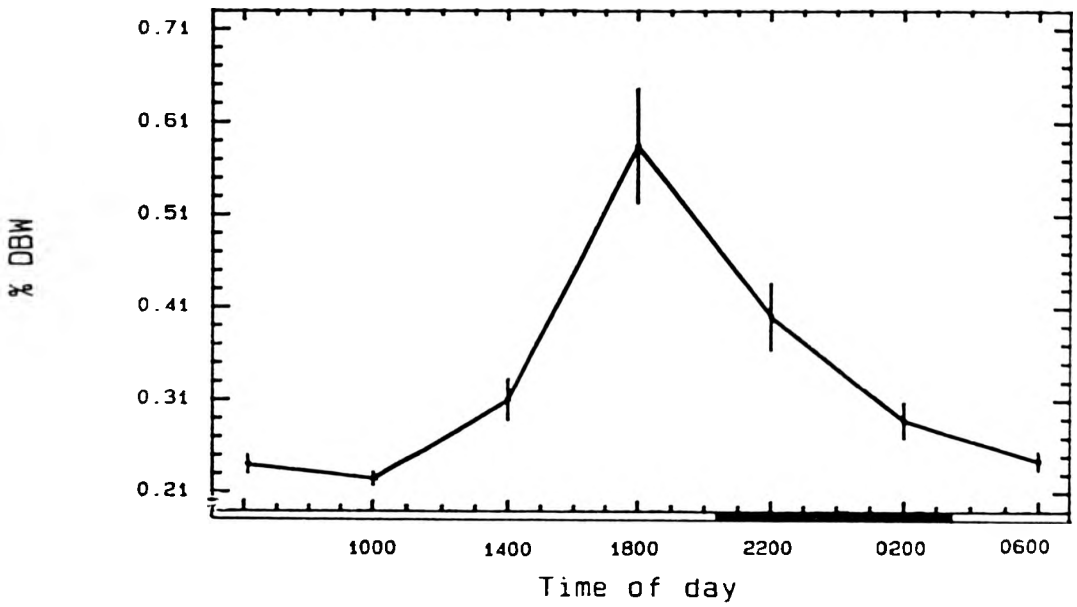
N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

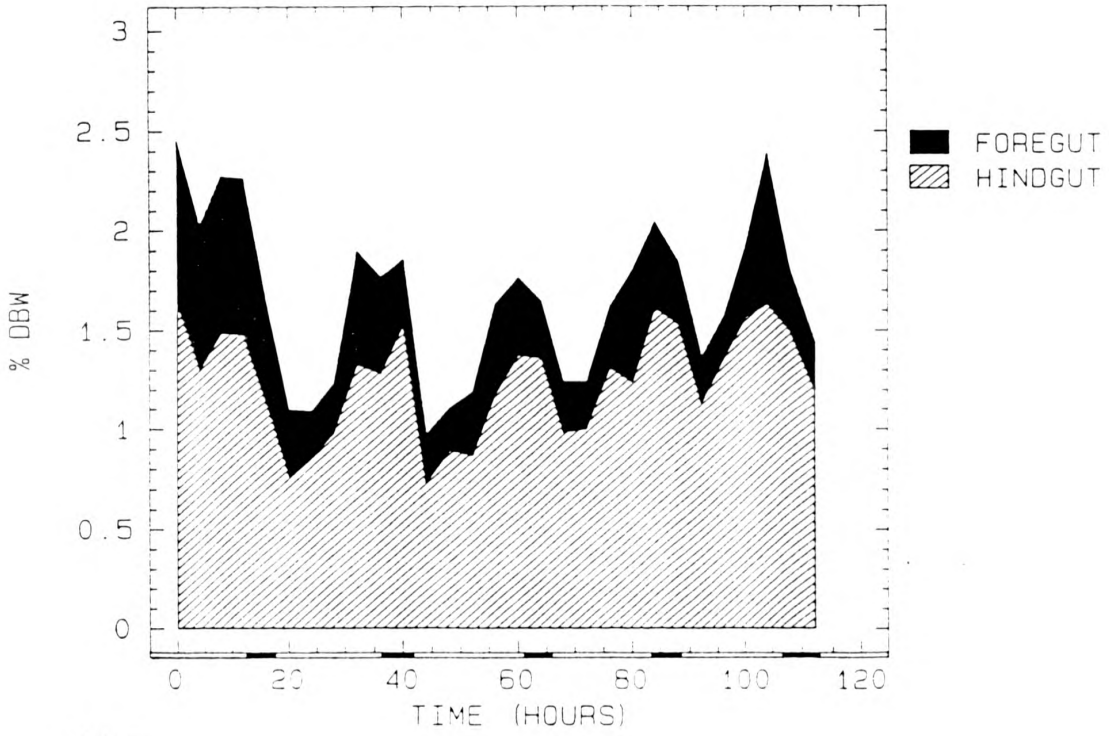
*** - $p<0.001$

Figure 5.2: The average cycle of foregut fullness for a 24 hr period, expressed as %DBW, shows a peak at 1800h. Fullness levels at 1800h are significantly different from the average level at all other times (Table 5.4). The vertical bars indicate standard error and the horizontal bar, below the axis, shows the period of darkness.


0.71
0.61
0.51
0.41
0.31
0.21
% DBW
1000
1400
1800
2200
0200
0600
Time of day

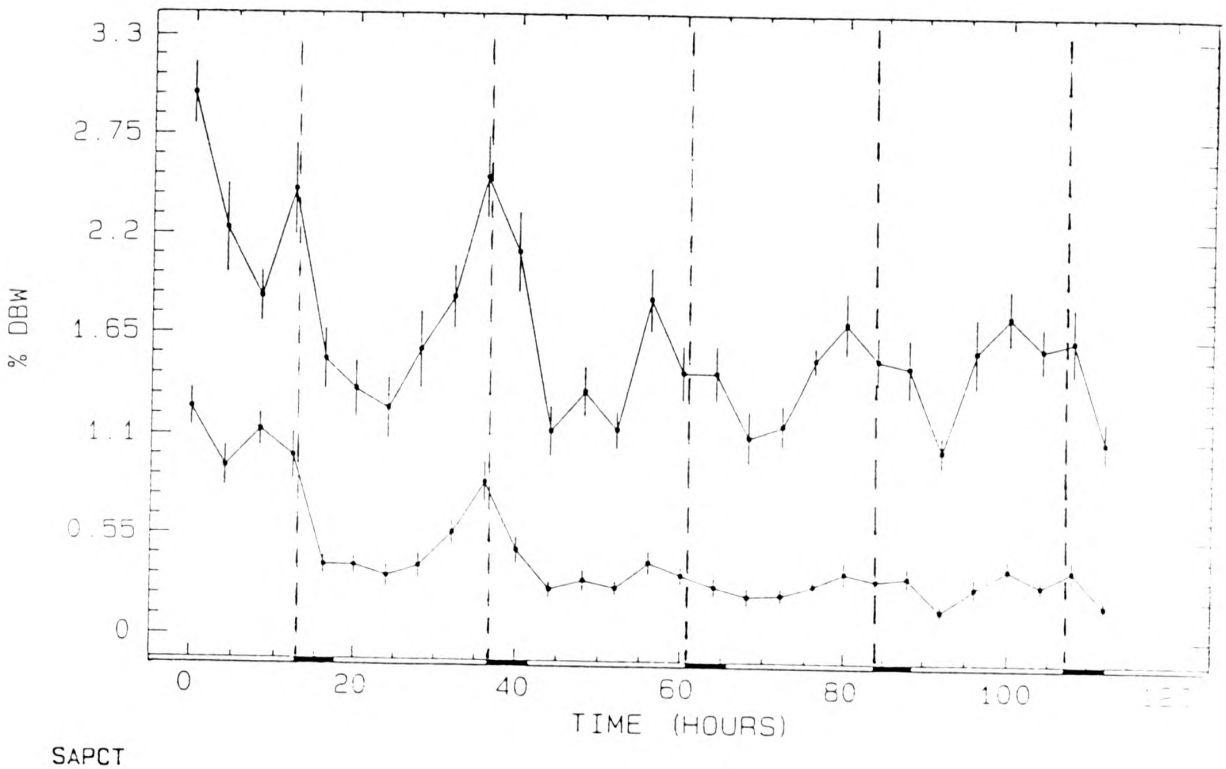
SAPAT

Figure 5.3: Hindgut (hatched area) and foregut (shaded area) fullness levels for fish held under SAPAT, time 0 is 1000h. The horizontal bar, below the axis, shows periods of darkness.


3
2.5
2
1.5
1
0.5
0
% DBW
0
20
40
60
80
100
120
TIME (HOURS)
FOREGUT
HINDGUT

SAPAT

Figure 5.4: Foregut (the lower recording) and wholegut (the upper recording) fullness levels for fish held under the environmental regime, simulated ambient photoperiod and controlled temperature (SAPCT). Each point represents the average gut fullness for the fish sampled at that time, time 0 is 1000h. The vertical bars show standard error and the horizontal bar, below the axis, indicates the periods of darkness during sampling.



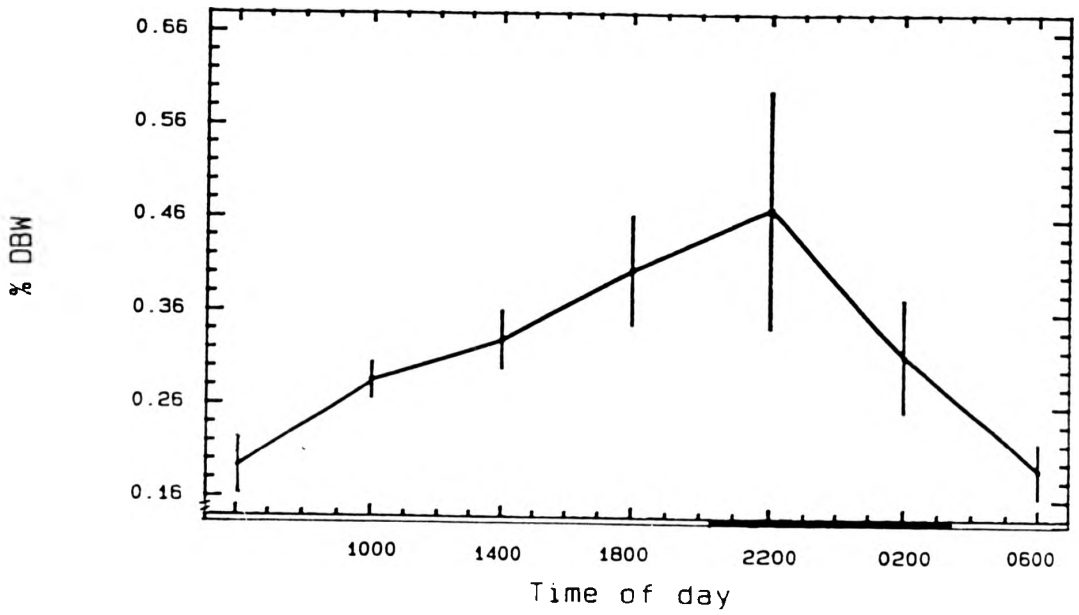
SAPCT

2200h) and early morning (0600h (Table 5.4, Fig. 5.5)). Although an overall increase is seen the maximum point at 2200h it is not significantly different from the mean samples prior to or after it. This was probably due to the un-characteristic increase of food present at 36 hours. The hindgut was again maintained at a relatively high level of fullness, while the foregut showed more erratic increases in fullness than under SAPAT (Fig 5.6). Two way ANOVA showed that foregut fullness differed significantly both between cycles and between times. Wholegut fullness did not differ significantly between cycles but was significantly different between sampling times ($p<0.05$). Lastly hindgut fullness did not differ significantly either between cycles or times (Table 5.2).

5.3.3 Gut fullness under constant light, ambient temperature (CLAT)

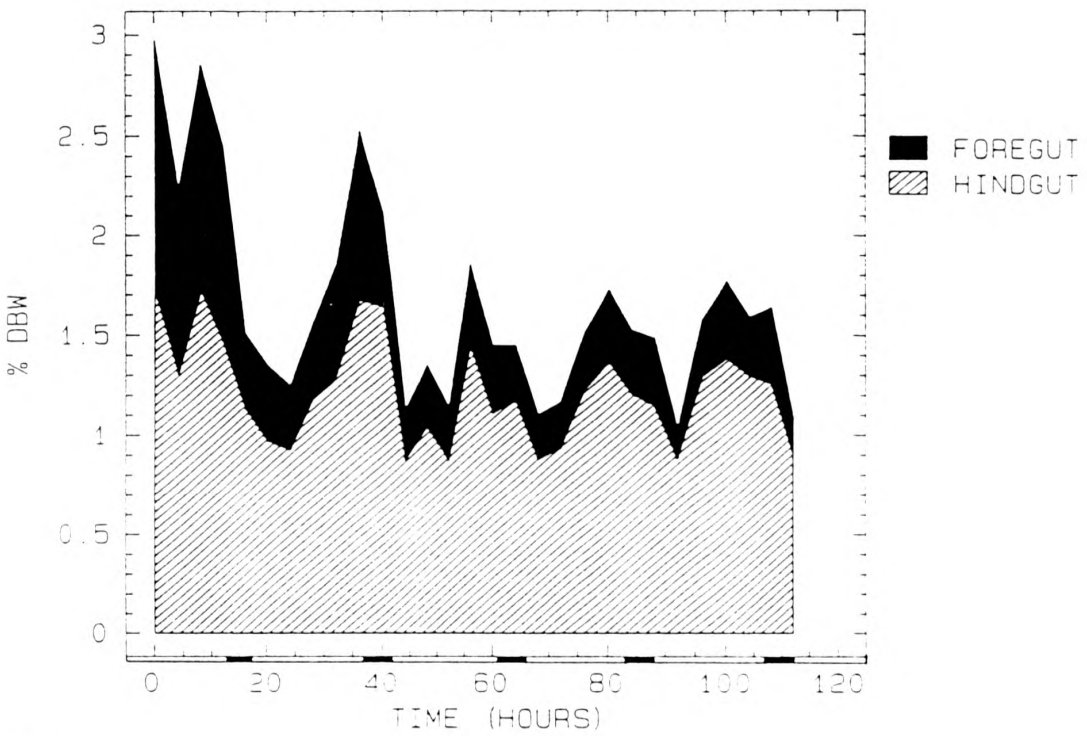
With light intensity maintained around 150 lux, and ambient water temperature the gut contents fluctuated with time (Fig 5.7). Patterns of hind- and wholegut fullness were less rhythmic, compared to SAPAT, but the peaks of foregut fullness observed at 1800h were similar in amplitude to those under SAPAT. The mean of the samples for 1800h was significantly different from the mean of the samples at all other times (Fig. 5.8, Table 5.4). Hindgut fullness was maintained at a relatively high level compared to foregut fullness, with the exception of the last 2 cycles (Fig.

Figure 5.5: The average foregut fullness cycle for a 24 hr period for fish held under SAPCT. Only the fullness level at 0600h is significantly different from the sample at 1800h ($p<0.05$, Table 5.4). The vertical bars indicate the standard error and the horizontal bar, below the axis, shows the period of darkness.


0.66
0.56
0.46
0.36
0.26
0.16
% DBW
1000
1400
1800
2200
0200
0600
Time of day

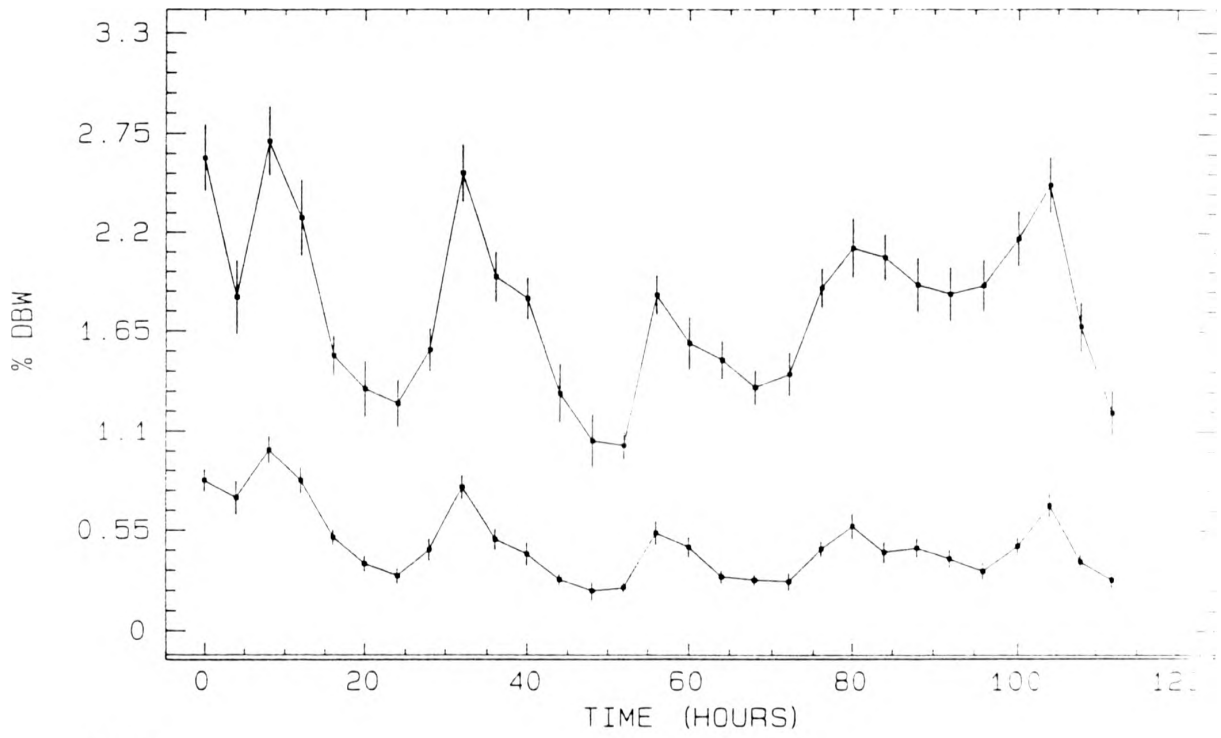
SAPCT

Figure 5.6: The fullness levels for the hindgut (hatched area) and foregut (shaded area) for fish held under the SAPCT regime. The average gut fullness for the fish sampled at each sampling time was calculated, time 0 was 1000h. The horizontal bar, below the axis, shows periods of darkness during the sampling.


3
2.5
2
1.5
1
0.5
0
% DBW
0
20
40
60
80
100
120
TIME (HOURS)
FOREGUT
HINDGUT

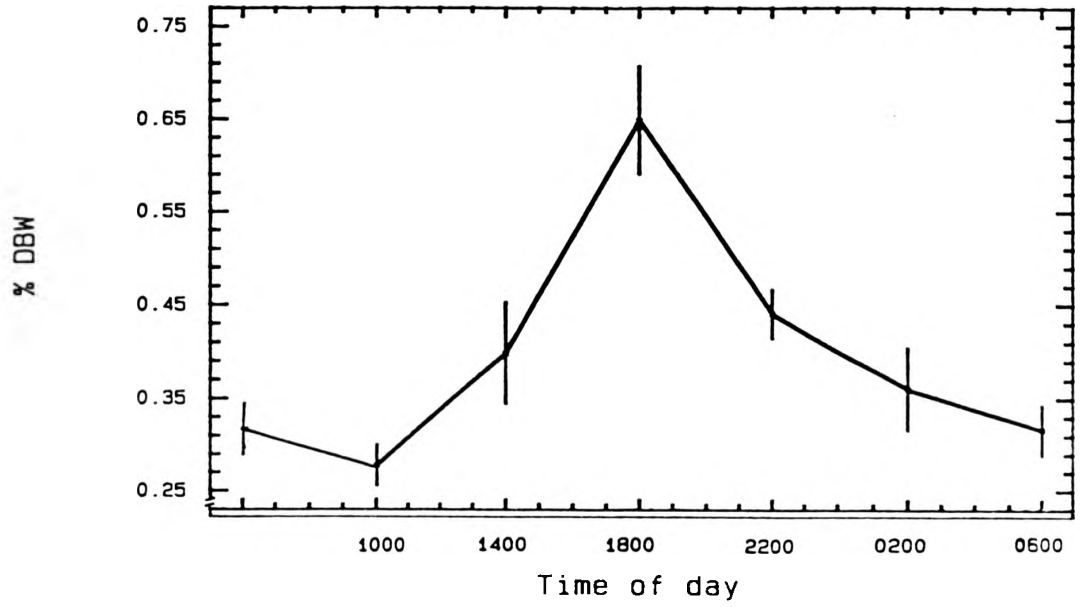
SAPCT

Figure 5.7: Wholegut fullness (the higher recording) and foregut fullness (the lower recording) for fish held under the environmental regime, constant light ambient temperature (CLAT). Each point shows the average gut fullness for the fish sampled at each sampling time, time 0 was 1000h. The vertical bars indicate standard error and the horizontal bar, below the axis, shows that there were no periods of darkness during the sampling.



CLAT

Figure 5.8: The average foregut fullness cycle for fish held under the environmental regime CLAT. The foregut fullness level at 1800h is significantly different from the levels at all other times (Table 5.4). The vertical bars show standard error and the horizontal bar, below the axis, indicates there were no period of darkness.


0.75
0.65
0.55
0.45
0.35
0.25
% DBW
1000
1400
1800
2200
0200
0600
Time of day

CLAT

5.9). There was no significant difference in foregut fullness between cycles, but a significant difference between sampling times ($p<0.001$). The hindgut differed significantly between cycles ($p<0.05$) but not between times, and wholegut contents were significantly different both between cycles and times ($p<0.05$, Table 5.2).

5.3.4 Gut fullness under constant light, controlled temperature (CLCT)

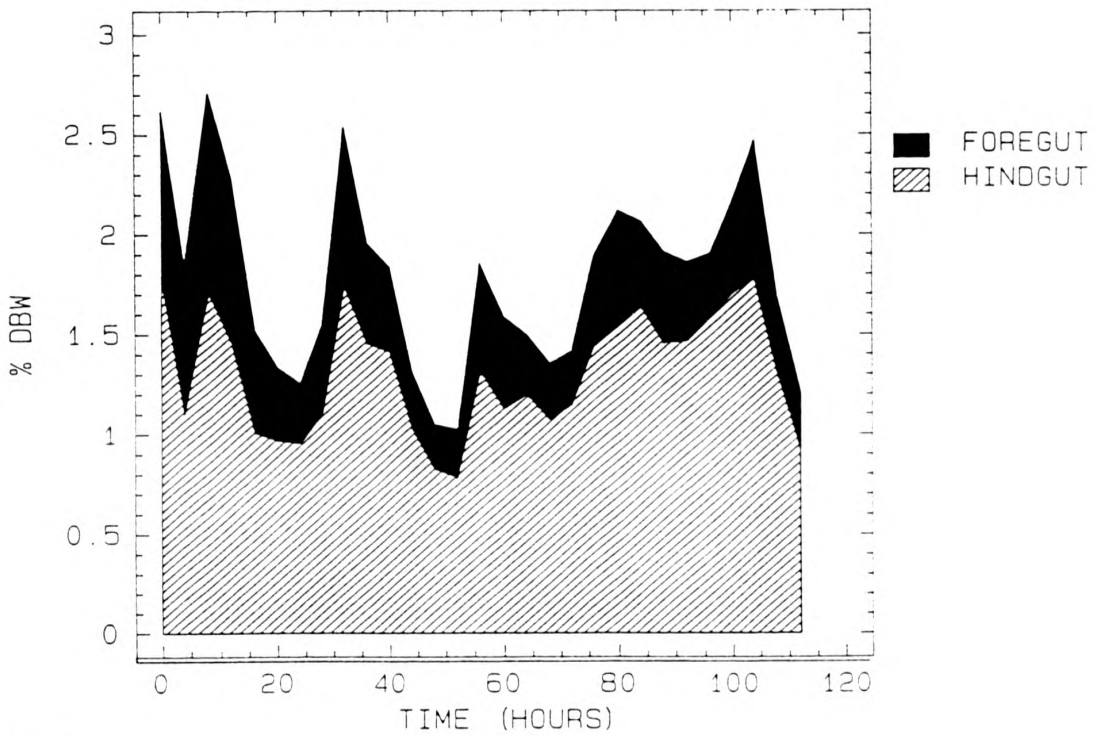
With the light maintained at 90 lux and a controlled water temperature of 15 ^{o}C rhythmic gut fullness fluctuations were not apparent (Fig. 5.10). The mean of the samples taken at 1800h was not significantly different from those at any other time. However, as under SAPCT the mean sample at 2200h was higher than that at 1800h, although not significantly different (Fig. 5.11, Table 5.4). There were no significant differences either between cycles or times for fore-, hind- or wholegut contents. The hindgut was maintained at a lower level of fullness than under any of the other sets of environmental conditions (Fig 5.12).

5.3.5 Relationship between environmental variables and gut fullness

- under simulated ambient photoperiod and ambient temperature.

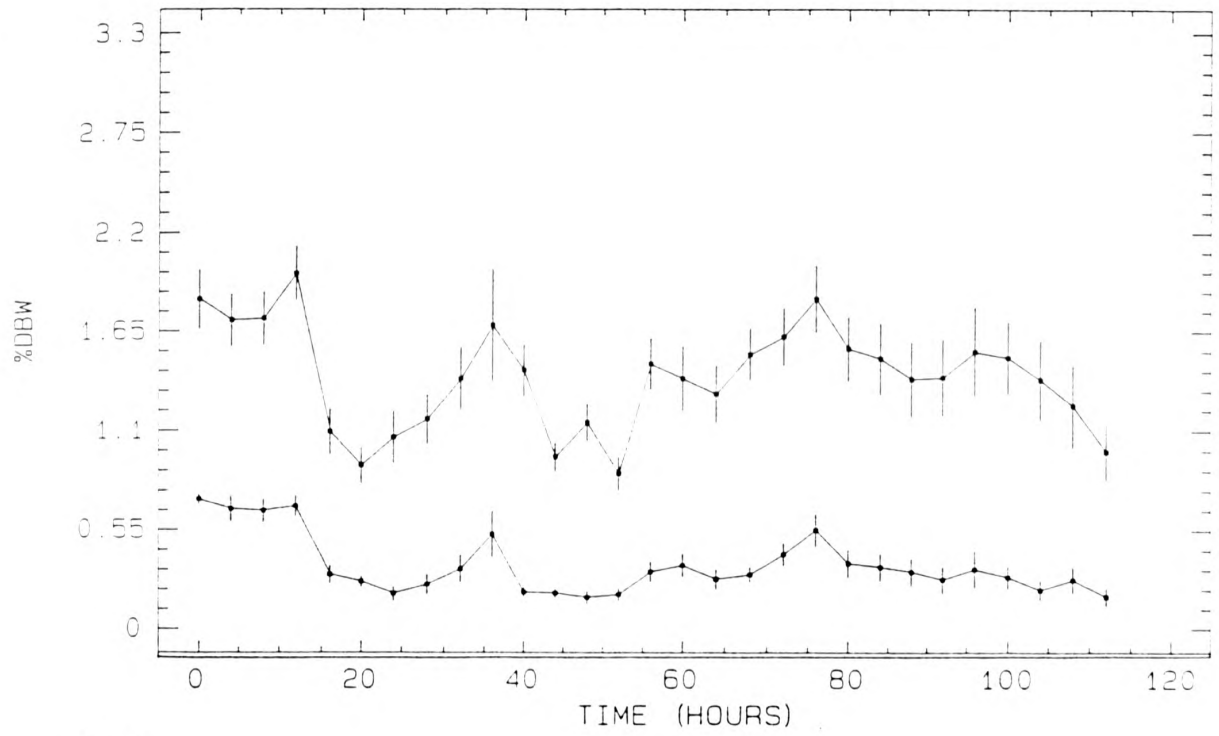
Foregut fullness peaked towards the end of the simulated daylight period when temperature levels were highest (Fig. 5.13).

Figure 5.9: The hindgut fullness (hatched area) and foregut fullness (shaded area) levels for fish held under the CLAT regime. Average fullness levels are calculated for the fish sampled at each time, time 0 is 1000h. The vertical bars indicate standard error and the horizontal bar, below the axis, shows that there were no periods of darkness during sampling.


3
2.5
2
1.5
1
0.5
0
% DBW
0
20
40
60
80
100
120
TIME (HOURS)
FOREGUT
HINDGUT

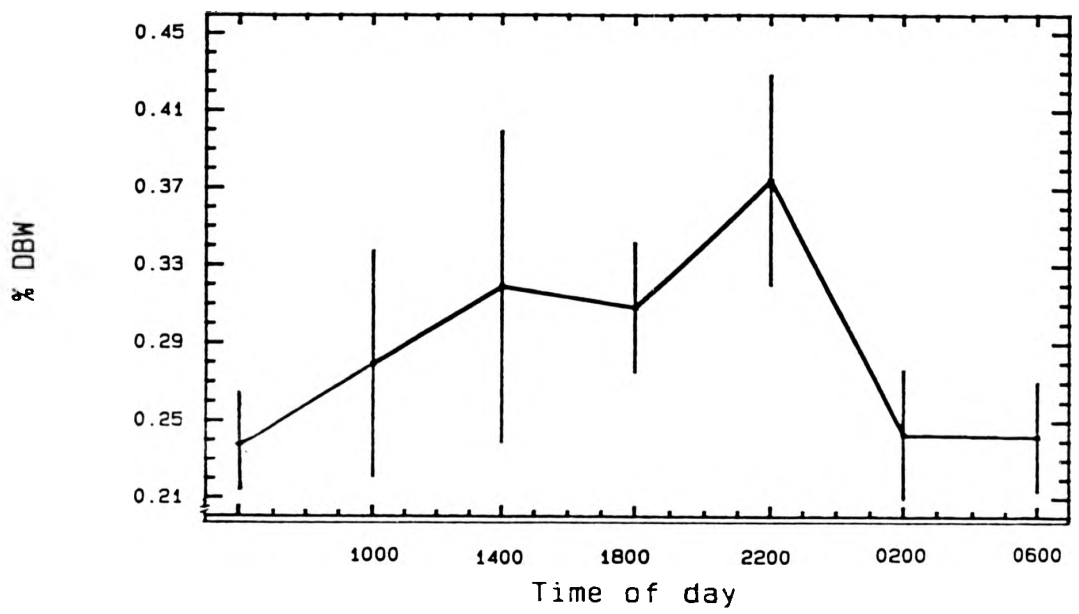
CLAT

Figure 5.10: The wholegut (the upper recording) and the foregut (the lower recording) fullness levels for fish held under the environmental regime, constant light, controlled temperature. Each point represents the average gut fullness level for the sampled at each time, time 0 being 1000h. The vertical bars show standard error and the horizontal bar, below the axis, indicates that there were no periods of darkness during sampling.



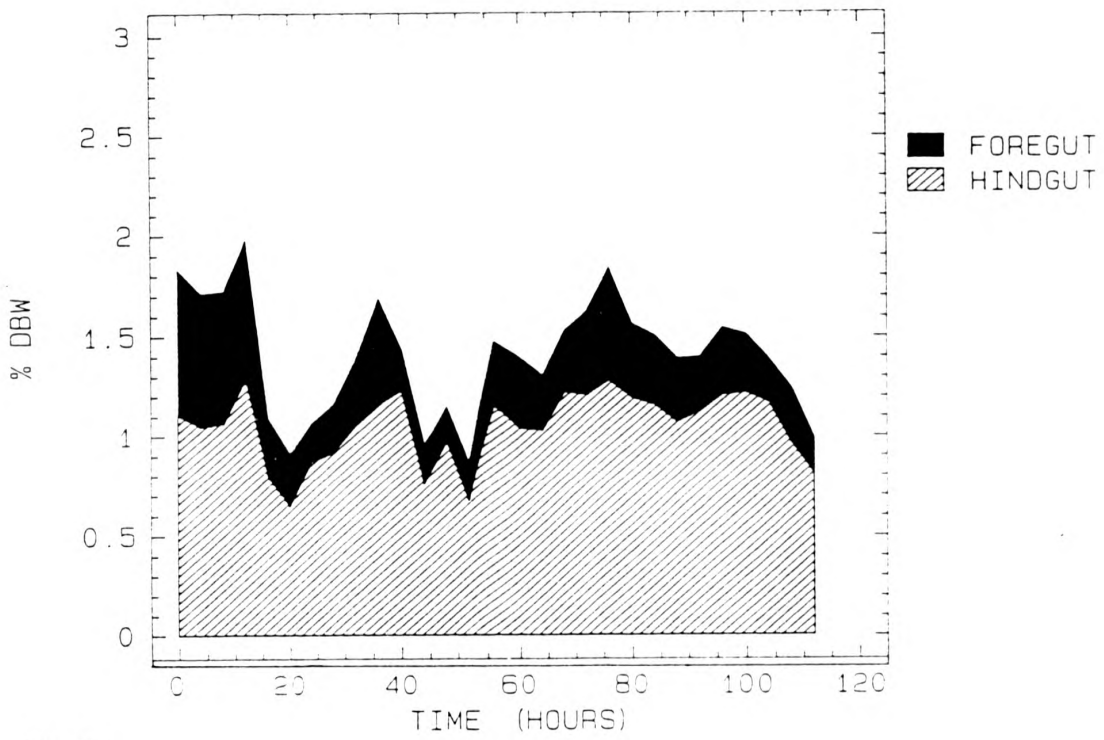
CLCT

Figure 5.11: The average foregut fullness cycle for a 24 hr period for fish held under CLCT. The level of foregut fullness at 1800h is not significantly different from the average level at any of the other sampling times (Table 5.4). The vertical bars show standard error and the horizontal bar, below the axis, indicates there were no period of darkness during sampling.


0.45
0.41
0.37
0.33
0.29
0.25
0.21
% DBW
1000
1400
1800
2200
0200
0600
Time of day

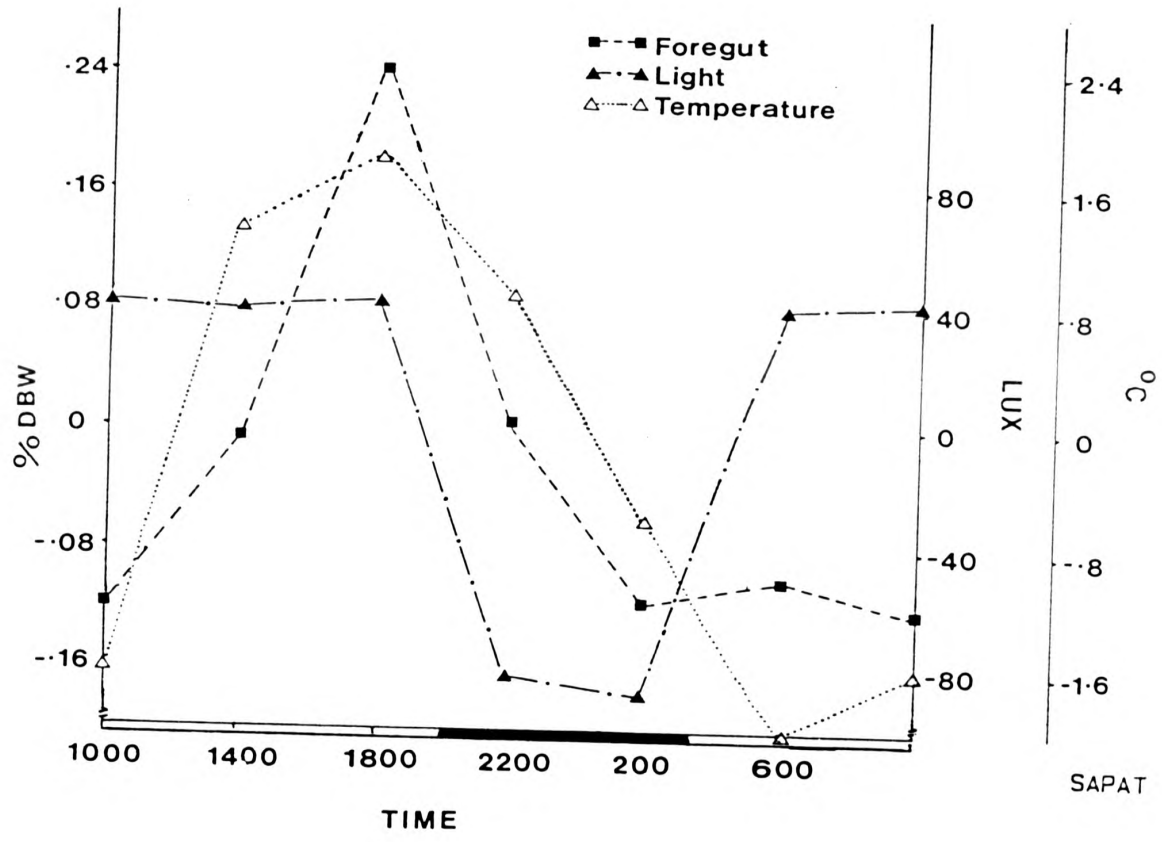
CLCT

Figure 5.12: The hindgut fullness (hatched area) and foregut fullness (shaded area) levels for fish held under CLCT. The horizontal bar, below the axis, shows no periods of darkness during the sampling.


3
2.5
2
1.5
1
0.5
0
% DBW
0
20
40
60
80
100
120
TIME (HOURS)
FOREGUT
HINDGUT

CLCT

Figure 5.13: The data calculated as the deviation from the mean for foregut fullness, light and temperature levels for fish held under SAPAT.


Foregut
Light
Temperature
·24
·16
·08
0
-·08
-·16
% DBW
80
40
0
-40
-80
LUX
2·4
1·6
·8
0
-·8
-1·6
°C
1000
1400
1800
2200
200
600
TIME
SAPAT

There was no significant correlation between light levels and fullness of any part of the gut. Temperature levels were significantly correlated with foregut fullness at the 0.1% level, with hindgut fullness at the 5% level and wholegut fullness at the 1% level (Table 5.3).

- simulated ambient photoperiod and controlled temperature

Foregut fullness continued to increase between 1800h-2200h while both light and temperature levels were continually decreasing (Fig. 5.14). Although controlled temperature was on a downward trend during this period (1800h-2200h), light was only decreasing during the latter part of this 4 hour period. Neither light nor temperature levels showed a significant correlation with the level of fullness of any part of the gut (Table 5.3).

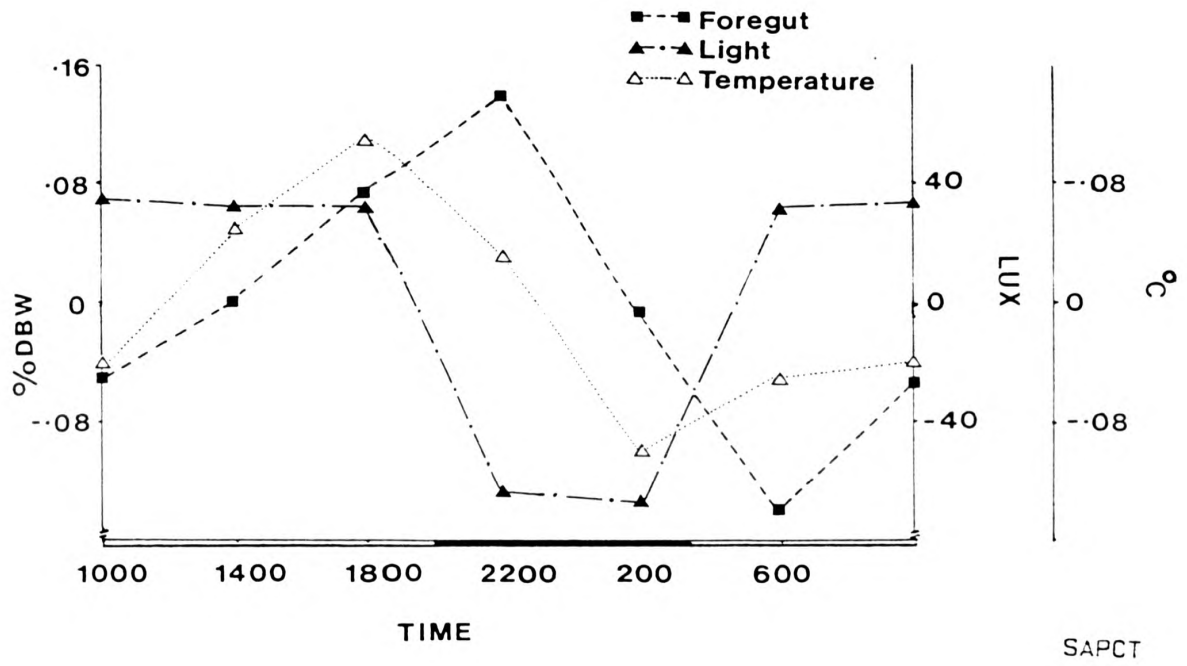
- constant light and ambient temperature

The fluctuations in light levels were statistically not significant, and therefore light can be termed constant. Foregut fullness peaked at 1800h as did ambient temperature (Fig 5.15). Both fore- and wholegut fullness were significantly correlated with temperature levels (foregut $p<0.01$, wholegut $p<0.05$), but not hindgut fullness. No significant correlation was observed between light levels and fullness and any part of the gut (Table 5.3).

- constant light and controlled temperature

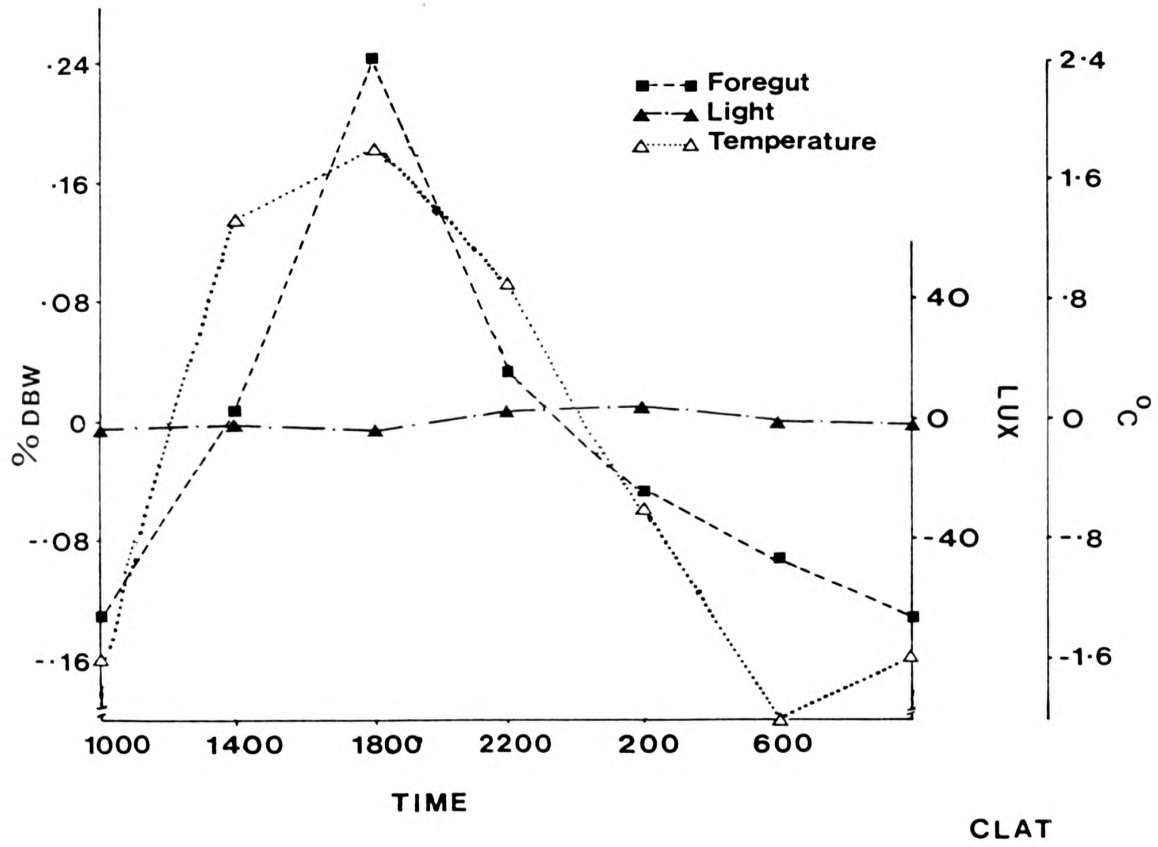
The fluctuations in foregut fullness were greatly reduced

Figure 5.14: The data calculated as deviation from the mean for foregut fullness, light and temperature levels for fish held under SAPCT.


Foregut
Light
Temperature
·16
·08
0
-·08
%DBW
40
0
-40
LUX
-·08
0
-·08
°C
1000
1400
1800
2200
200
600
TIME

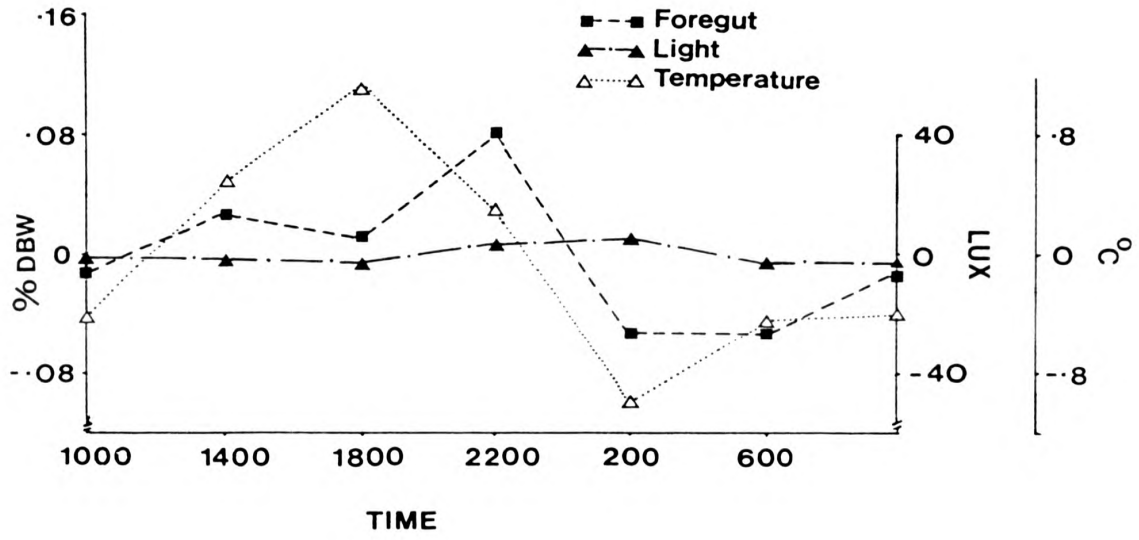
SAPCT

Figure 5.15: The data calculated as deviation from the mean for foregut fullness, light and temperature levels for fish held under CLAT.


Foregut
Light
Temperature
·24
·16
·08
0
-·08
-·16
% DBW
40
0
-40
LUX
2·4
1·6
·8
0
-·8
-1·6
°C
1000
1400
1800
2200
200
600
TIME
CLAT

with a peak at 2200h (as under SAPCT), while controlled temperature was decreasing (Fig.5.16). No significant correlations were observed between either light or temperature levels and fullness of any part of the gut (Table 5.3).

Figure 5.16: The data calculated as deviation from the mean for foregut fullness, light and temperature levels for fish held under CLCT.


Foregut
Light
Temperature
·16
·08
0
-·08
% DBW
40
0
-40
LUX
·8
0
-·8
°C
1000
1400
1800
2200
200
600
TIME

CLCT

5.4 DISCUSSION

The gut contents of Atlantic salmon parr were seen to fluctuate rhythmically under all conditions except under the combination of constant light and controlled temperature. The ceasing or dampening out of the feeding periodicity is not convincing proof that the feeding rhythm is either endogenous or exogenous in nature. It could be that the periodicity is un-observed due to too unfavourable an environment (Aschoff, 1960). However, this is unlikely in these circumstances since feeding is still observed, only the predictable feeding peak in late afternoon has been lost. Eriksson & Veen (1980) put forward the theory that in the brown bullhead, Ictalurus nebulosus (Teleostei), a multi-oscillatory circadian system operates. This system needs the presence of the light-to-dark transitions to synchronize the loosely coupled circadian oscillators. Otherwise arrhythmicity is observed in fish held under constant conditions. Although this may be the situation in this study a third possiblity should be considered, namely that the feeding rhythm has no endogenous component and is entirely exogenous. Further investigation is required before any firm conclusions can be drawn concerning the nature of the feeding rhythm in Atlantic salmon.

Previous experiments investigating gut fullness of Atlantic salmon have indicated that gut fullness was related to changes in light levels whilst temperature levels

affected the amount of food present only when the light levels remained constant (Rawlings & Talbot unpublished). The present results appear to contradict such findings. In previous studies however, the fish were held under natural ambient light not under simulated ambient photoperiod. They were therefore exposed to long periods of increasing and decreasing natural light levels/intensities. In the present study all fish were held under artificial illumination, and fish held under simulated ambient photoperiod only experienced changing light levels during dawn and dusk periods. It may have been the difference either in light intensities or spectral composition between natural and artificial light that caused the discrepancy. However, it is unlikely to be due to differences in spectral composition as Atlantic salmon show no difference in growth, regardless of the spectral composition of the light, under which they are held (Stefansson & Hansen, 1989). Further investigations on the effect of different light intensities on feeding and growth are necessary, since work already carried out has concentrated largely on the effects of several constant light intensities on growth (Wallace et al, 1988; Stefansson et al, 1990). Jacob & Nair (1983) noted that feeding was rhythmic in two species of larvivorous fishes. They concluded that feeding in these species was related to environmental fluctuations in light, since their study was carried out in the tropics, where light intensity rather

than season or photoperiod length was thought to be of paramount importance. Due to the relatively short periods of increasing and decreasing light levels in the present study, the fish may have been unable to utilize these changes in light levels as environmental cues. Hence, under these conditions no correlation was observed between feeding and light levels.

It is necessary to determine which property of light is essential to elicit a feeding response from the fish as it enables us to understand why light is important to them. It has been hypothesised that light may be used to indicate 'biological time' (Schwassmann, 1971), although there is indecision about how the light is actually utilised to indicate time. Two different time measuring mechanisms have been identified in a wide variety of insects and birds. One is known as the hour glass mechanism and has been well demonstrated in some insects (Lees, 1966). The other is based on a daily rhythm in sensitivity to light, which has been demonstrated in birds (e.g. Farner, 1970) and 2 species of fish in relation to reproductive cycles: sticklebacks, Gasterosteus aculeatus L., (Baggerman, 1980) and the Asian catfish, Heteropneustes fossilis, (Sundararaj and Vasal, 1973, 1976). The hour glass mechanism measures the length of the light or dark period in the 24 hour cycle. This mechanism is set in motion by an external signal (e.g. dawn and dusk, the natural light/dark cycle) this timer is set

into motion every day (Baggerman, 1980). The second mechanism, based on a daily rhythm of sensitivity to light, starts at the beginning of each day when it becomes light e.g. zero hour. The system underlying the photoperiodic response is not sensitive or is very insensitive, to stimulation by light and remains so for the first 6-8 hours. However its sensitivity begins to increase until a maximum is reached after 16 hours, after which it declines to zero again at the end of the 24 hour period. This mechanism has been investigated in fish in relation to the reproductive cycle and the neuro-endocrine gonadal system has been reported to be sensitive to light (Baggerman, 1980). It is thought that the light is the actual stimulator of the neuro-endocrine reaction, when the photoperiod overcomes the photoreactivity threshold, but that the daily light-dark cycle serves to synchronise the circadian light sensitivity rhythm so that it remains in phase to a 24 hour rhythm (Baggerman 1980). More detailed investigation into feeding under controlled light regimes is necessary to further our understanding of the relationship between feeding and light levels.

In the present study, gut contents were observed to be significantly correlated with ambient water temperature regardless of whether light was simulated ambient photoperiod or constant. Under controlled water temperature conditions however, there was no significant correlation

between temperature and gut fullness. Very little work has been carried out to investigate the effect of fluctuations of temperature within a 24 hour period. Our study suggests that although temperature may be linked to the quantity of food taken, when fluctuations in temperature were reduced either the fish could no longer detect the change in temperature or the changes were too slight to elicit a response. Berg *et al* (1990) reported that Atlantic salmon parr grew better under rhythmically alternating temperatures than under constant temperature conditions. This may be due to the experimental fish responding to the changes in water temperature or due to them experiencing a longer period of higher temperature compared to the controls.

5.5 SUMMARY

1. Gut contents fluctuated rhythmically under all environment regimes, except the combination of constant light and controlled temperature.
2. The nature of the feeding rhythm (whether it is an expression of an exogenous or endogenous component or both) has not been conclusively demonstrated.
3. A peak in foregut fullness was observed only when the fish were held under ambient temperature. Although with simulated ambient photoperiod and controlled temperature a difference was observed between late afternoon (1800h) and early morning (0600h).
4. When the fish were held under ambient temperature

a correlation between temperature and foregut fullness was observed.

5. No significant correlation was observed between light levels during the daylight period of simulated ambient photoperiod and foregut fullness, the null hypothesis is not rejected.

6. The effect of light on gut fullness needs to be further investigated since results gained with the simulated ambient photoperiod were contradictory to all other data so far collected.

CHAPTER 6

THE EFFECT OF THREE DIFFERENT LIGHT REGIMES ON RHYTHMIC GUT FULLNESS

6.1 INTRODUCTION

Food intake by fish, both the quantity taken during an individual feeding bout and when the feeding bouts occur, is influenced by complex interactions of factors both physiological and environmental. One of the physiological factors is stomach fullness. Fange & Grove (1979) state that the return of appetite is related to the time taken to reduce the stomach contents. Holmgren *et al* (1983) however, go further, suggesting: "fish will eat varying amounts depending on stomach fullness and at intervals determined by the rate of stomach emptying". Observations by Tugendhat (1960) and Beukema (1968) indicate that stomach emptiness/fullness alone does not control the amount of food ingested and therefore, by inference, when it is ingested.

Survival is the highest priority of an organism. To achieve this an organism must become hungry before the tissues are depleted of their metabolic requirements. The nutritional system has evolved in such a way as to anticipate possible future depletion and act acordingly (Armstrong, 1980). In mammals, other physiological factors have been identified in the control of appetite: changes in circulating levels of glucose, fatty acids, glycerol and essential amino acids, are all monitored by the liver and brain, and the hormones, insulin and cholecystokinin, may be effective on key centres of the brain (Holmgren *et al*, 1983). Comparatively little is known about similar

physiological mechanisms in fish. However, several hormones in fish vary significantly in circulating titre within a 24 hour period (Spieler, 1979). Thus it may be advantageous for fish to feed at specific times coinciding with favourable rhythms and therefore ensuring maximum utilization of the available nutrients consumed. For example channel catfish, Ictalurus punctatus, fed at 2 different times of the day showed significant differences in body fat (Noeske-Hallin et al, 1985). Hormone changes have been directly related to feeding times (Boujard & Leatherland, 1992), as in Dicentrarchus labrax where plasma insulin levels peaked after feeding on natural prey items. However, plasma thyroxine (T_4) concentrations peaked in Carassius auratus at 1600h regardless of when the fish were fed (Spieler & Noeske, 1981). Later, Spieler & Noeske (1984) found that, after shifting the light/dark phase, the T_4 peak always appeared during the late photophase regardless of feeding time, indicating synchronicity between the peak in hormone levels and the light cycle.

When food was available ad libitum, Boujard & Leatherland (1992) found that the feeding rhythm was also synchronized by the light/dark cycle. Fish fed on restricted diets, with specific scheduled meals show a preference to feed at specific times (indicated by increased growth) in relation to the light/dark cycle. For example the nocturnal catfish, Heteropneustes fossilis, gained more weight when fed during

the mid to late portion of the dark phase than at other times (Sundararaj et al, 1982).

Although these reports indicate both the hormone and feeding rhythms are linked to the light/dark cycle, it does not clearly indicate which component of the light/dark cycle is important in relation to feeding. As feeding peaks for some fish species (e.g. rainbow trout, O. mykiss) occur either at dawn or dusk or at both times, it was thought that the rapid change in light intensity during these periods was important (Landless, 1976). In early experiments the change in light intensity at the beginning and end of the light period was large and abrupt (often lights-on lights-off). In nature the change in light intensity is not abrupt and light levels change constantly throughout the day, it is therefore unlikely that rhythms are synchronized by the abrupt changes mentioned above.

The present study tested the following null hypotheses:
1. The feeding pattern does not differ under the three light regimes; simulated ambient photoperiod; simulated ambient light and photoperiod; ambient light and photoperiod.
2. Feeding rhythms of Atlantic salmon in freshwater tanks are unrelated to photoperiod.
3. Changes in light intensities are important in synchronizing feeding rhythms.
4. Temperature is of primary importance in establishing feeding rhythms.

6.2 MATERIALS AND METHODS

6.2.1 Fish Stocks

On 05.11.91 at Almondbank hatchery six groups of 100 Atlantic salmon, of average weight 24.5g ($^{+}$/_0.6) and average length 12.7cm ($^{+}$/_0.1), were placed in radial flow tanks under one of the following three light regimes:

i, Ambient light and photoperiod (control) - ALP

ii, Simulated ambient photoperiod - SAP

iii, Simulated ambient light and photoperiod - SALP

Each treatment was replicated.

On 24th March 1992 the same six groups of fish were transported from Almondbank to Pitlochry in aerated water contained in plastic lined bins. This move was to increase acessibility to the fish and so the fish could be held in a purpose designed aquarium (i.e. better facilities, for this experiment). The fish were again placed in radial flow tanks and held under identical light regimes.

The control tanks were in a room exposed to natural light (through skylights), and no artificial lighting was used. All the tanks for the simulated regimes were contained in black plastic tents and a centrally controlled computer was used to alter the light intensity illuminating each tank.

The SAP tanks were exposed to the same number of hours of

daylight as the time of the year, but light levels were increased and decreased to simulate dawn and dusk respectively, and between these twilight periods the light levels remained at a pre-set maximum constant of 220 lux.

The SALP tanks were also exposed to the same number of hours of daylight as the time of the year, but the light levels were increased continuously to a pre-set maximum, of 220 lux, at the mid-point of the period of simulated daylight and then decreased. For all three regimes, sunrise was 0338h and sunset was 2016h (25.05.92, Whittaker's Almanack). Light meter readings were taken every 4 hours for each experimental regime tested.

All tanks used the same water source from Loch Faskally. Water temperature readings were taken every 4 hours throughout the sampling period. However, due to the nature of the loch, water temperature showed very little fluctuation between readings ($^{+}/_{-}$ 0.5^{o}C).

The average weight and fork length of the fish one week after sampling was, 42.1g ($^{+}/_{-}$ 7.7) and 15.5cm ($^{+}/_{-}$ 0.9) respectively.

6.2.2 Feeding Regime

The fish were fed a commercial diet delivered from bucket feeders (Ewos, Bathgate, Lothian, Scotland) onto a spinning disc, the food entered the tank with the inflow of water (as

previously described). All feeders were triggered by a central computer to deliver food every 10 minutes day and night. Before the onset of the sampling period (48 hours) the standard food was substituted for a diet labelled with 3% ballotini (Jencons Ltd, Leighton Buzzard, UK: 300-355 um).

Weighed quantites of labelled food were x-rayed and the amount of marker per unit weight of food was calculated.

6.2.3 Sampling

The six tanks were sampled every 4 hours for 116 hours at 0200h, 0600h, 1000h, 1400h, 1800h, 2200h, from 0200h on 25.05.92 up to and including 2200h on 29.05.92, providing 6 samples for each of 5 days for each tank. For each sample 15 fish were anaesthetised, x-rayed and returned to their respective tanks. The x-ray plates were developed, and the amount of marker and therefore the weight of food present in the fore-, hind- and wholegut, for each fish, at each sampling time, was calculated.

6.2.4 Calculations

The amount of food present in the fore-, hind- and wholegut was expressed as an index of gut fullness calculated as percent of the dry body weight of the fish (% DBW):

$$\% \text{ DBW} = 100 \text{ (Dry weight of food/ Dry weight of fish)}$$

The mean gut fullness of the 15 fish sampled was calculated for each sampling time to show the pattern of gut fullness over the 5 consecutive days.

To give an indication of the average pattern of fullness and light levels for one 24 hour period for each light regime, the average deviation from the overall mean (n=30) was calculated for each sample time (n=5). These values for mean foregut fullness allowed assessment of significance of differences between times of the day.

6.2.5 Statistical Analysis

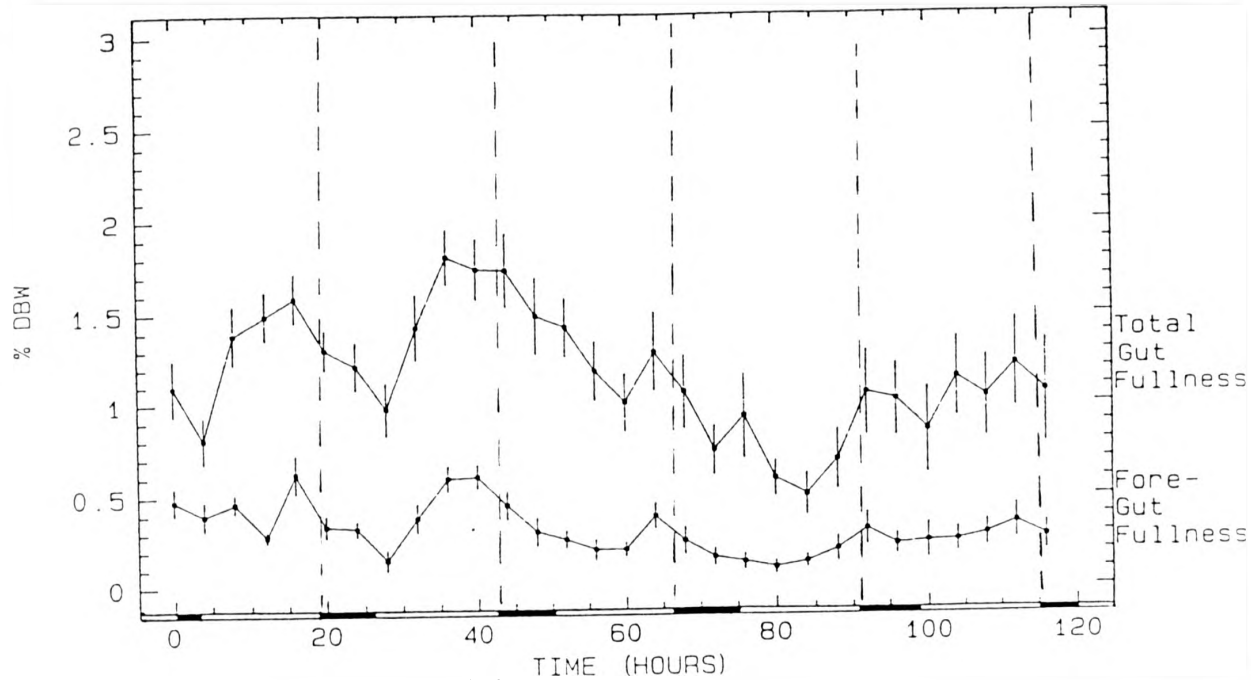
Two way analysis of variance (ANOVA, Campbell 1989) was carried out on gut fullness data for the fore-, hind- and wholegut under the three light regimes. The differences between time of day sampled and between different 24 hour periods was calculated. The two F-ratios were tested for significance at the 5% ($p<0.05$), 1% ($p<0.01$) and 0.01% ($p<0.01$) levels.

A one sample paired t-test was used to assess whether the mean foregut fullness taken at one specific time (1800h) compared separately to all other sampling times during the same 24 hour period was significantly different.

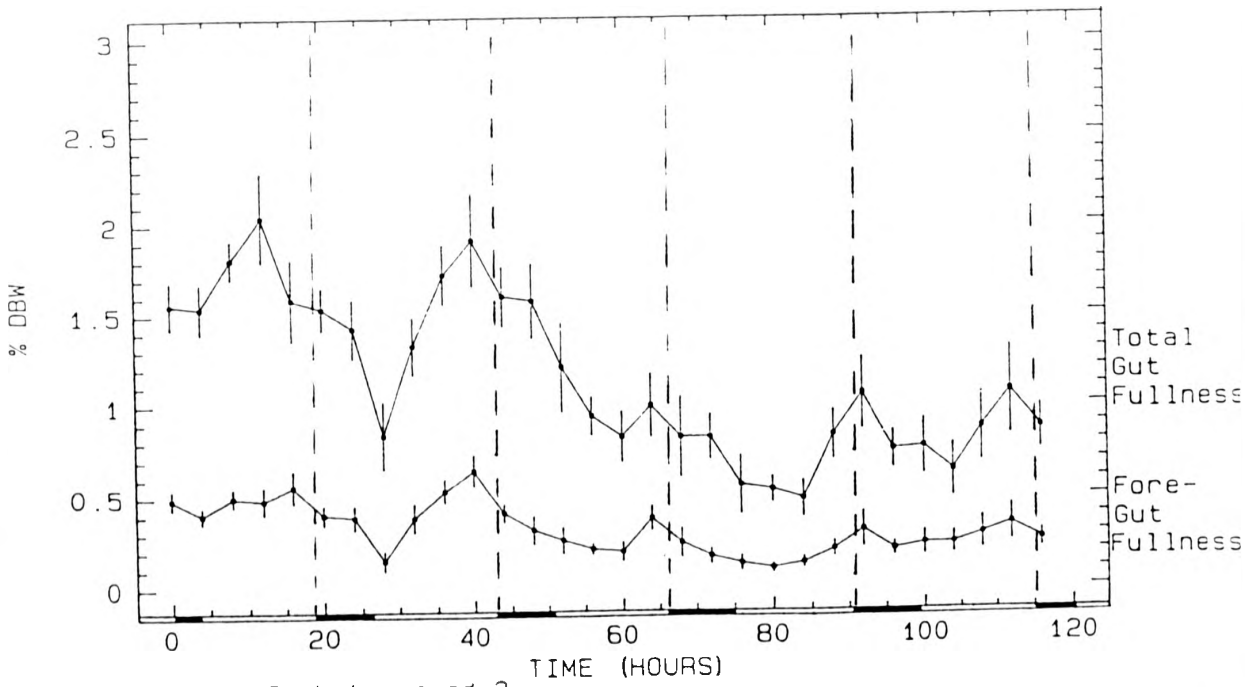
Table 6.1: Summary of minimum and maximum gut fullness levels for all light regimes tested

Regimes		Minimum			Maximum		
		Mean	S.E.	Time	Mean	S.E.	Time
ALP 1	Foregut	0.101	.036	1000	0.619	.100	1800
	Hindgut	0.367	.081	1400	1.281	.133	2200
	Wholegut	0.501	.112	1400	1.803	.140	1400
ALP 2	Foregut	0.100	.025	1000	0.647	.085	1800
	Hindgut	0.351	.078	1400	1.559	.190	1400
	Wholegut	0.483	.101	1400	2.042	.245	1400
SAP 1	Foregut	0.137	.031	0200	0.775	.137	2200
	Hindgut	0.279	.049	0600	1.247	.154	2200
	Wholegut	0.410	.085	0600	1.996	.231	2200
SAP 2	Foregut	0.132	.040	0200	0.718	.186	2200
	Hindgut	0.303	.109	1400	1.457	.268	0200
	Wholegut	0.601	.170	0600	1.964	.367	0200
SALP 1	Foregut	0.065	.028	1000	0.752	.121	1800
	Hindgut	0.155	.044	1400	1.352	.209	2200
	Wholegut	0.224	.068	1400	2.282	.274	1800
SALP 2	Foregut	0.067	.023	1000	0.754	.097	1800
	Hindgut	0.307	.034	1400	1.469	.095	1400
	Wholegut	0.377	.048	1000	2.027	.149	1400

Figure 6.1: Fore- and total- (or whole-) gut fullness levels for fish held under ambient light and photoperiod (ALP) for tanks 1 and 2. Each point represents the mean gut fullness of the 15 fish sampled at each sampling time, time 0 was 0200h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling.



Ambient light & photoperiod 1



Ambient light & photoperiod 2

Fore- and wholegut fullness fluctuated throughout the day with a repeatable increase just before the onset of darkness each day (Fig. 6.1). Two way analysis of variance showed that in tank 1 all portions of the gut were significantly different between days, ($p<0.01$), but that only the foregut showed significant differences between times of sampling ($p<0.05$, Table 6.2). Similarily in tank 2 the fore-, hind- and wholegut were all significantly different between days ($p<0.001$) and again only the foregut was significantly different between times of sampling ($p<0.05$, Table 6.2).

In both tanks after the first 4 samples fore- and hindgut fullnesses fluctuated together (Fig. 6.2). An increase in foregut fullness was usually accompanied by a corresponding increase in hindgut fullness. However, during the first 4 samples the hindgut fullness increased although the foregut only increased slightly. This may have been a stress reaction as this was the only period in which this was evident.

In both tanks foregut fullness peaked in late afternoon at 1800h. In tank 1 the mean foregut fullness at 1800h was significantly different from values at 0200h, 0600h and 1000h (Table 6.3), indicating an increase after 1000h and a decrease after 2200h (Fig. 6.3). In tank 2 the mean value of foregut fullness at 1800h was significantly different from those at 0200h, 1000h and 1400h (Table 6.3).

PAGINATION ERROR

P. 215.

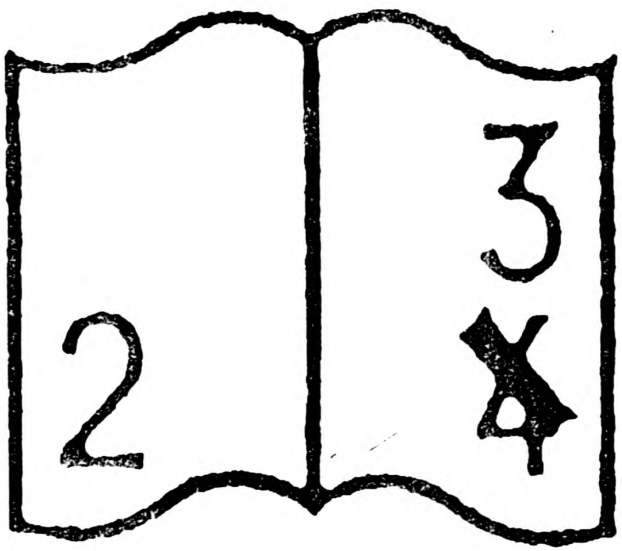
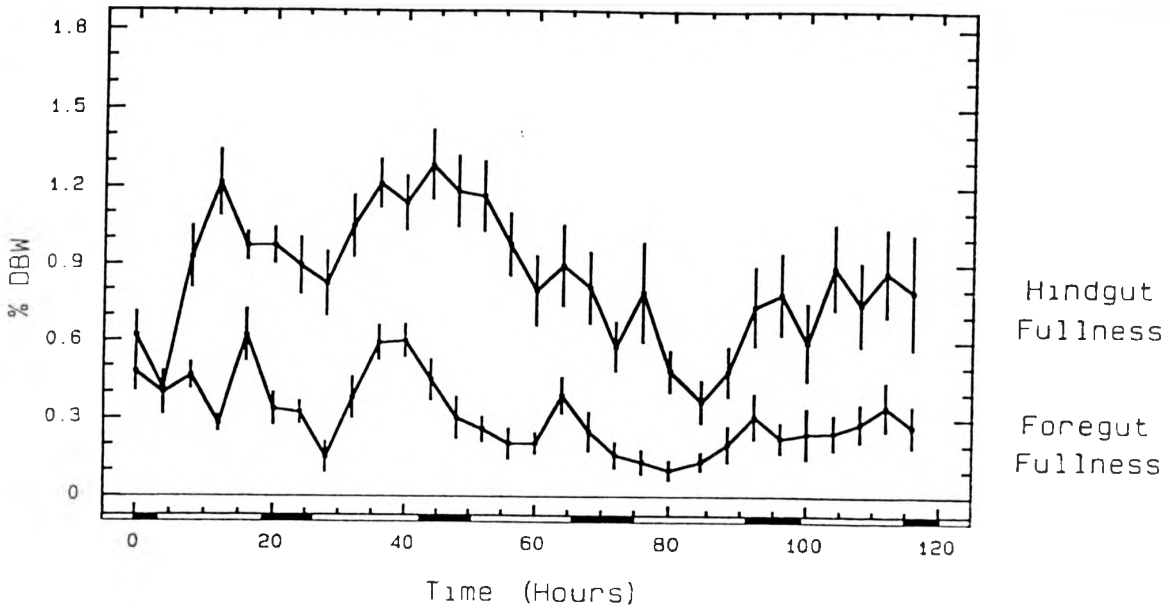
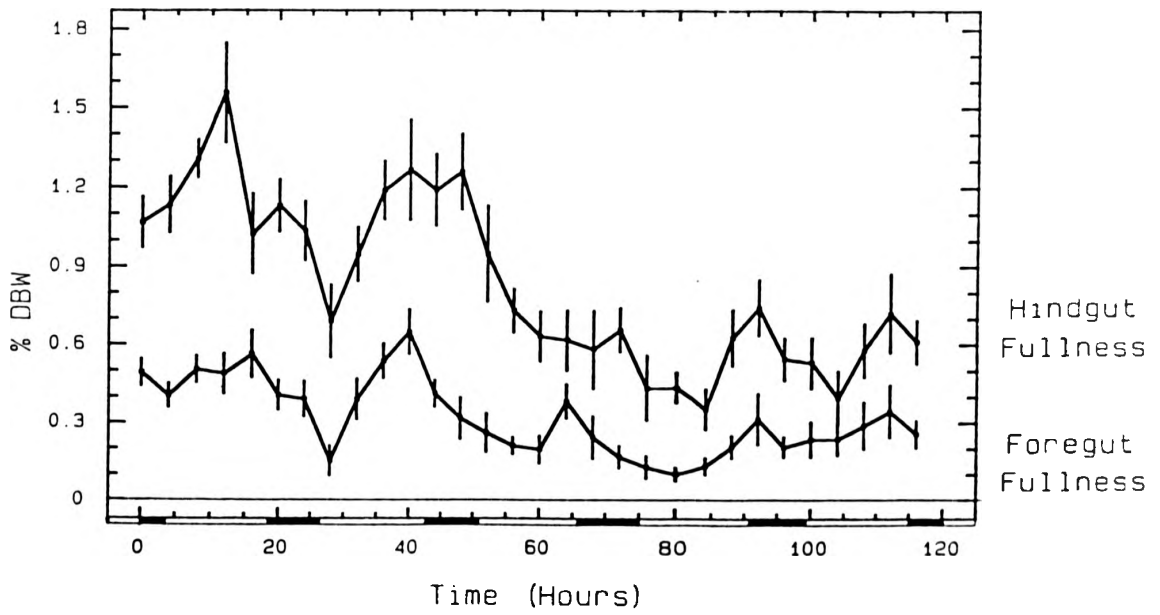


Figure 6.2: Hind- and foregut fullness levels for fish held under ALP for both tanks 1 and 2. Again each point represents the mean gut fullness level for all fish sampled at each sampling time, time 0 was 0200h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling.



Ambient light & photoperiod 1



Ambient light & photoperiod 2

Table 6.3: Summary of significance levels between foregut fullness levels at 1800h and at all other times

Regimes	Tank	1800 & 0200	1800 & 0600	1800 & 1000	1800 & 1400	1800 & 2200
ALP	1	*	*	**	N.S.	N.S.
	2	*	N.S.	*	*	N.S.
SAP	1	N.S.	N.S.	N.S.	N.S.	N.S.
	2	N.S.	N.S.	N.S.	N.S.	N.S.
SALP	1	**	**	*	*	N.S.
	2	***	**	**	*	N.S.

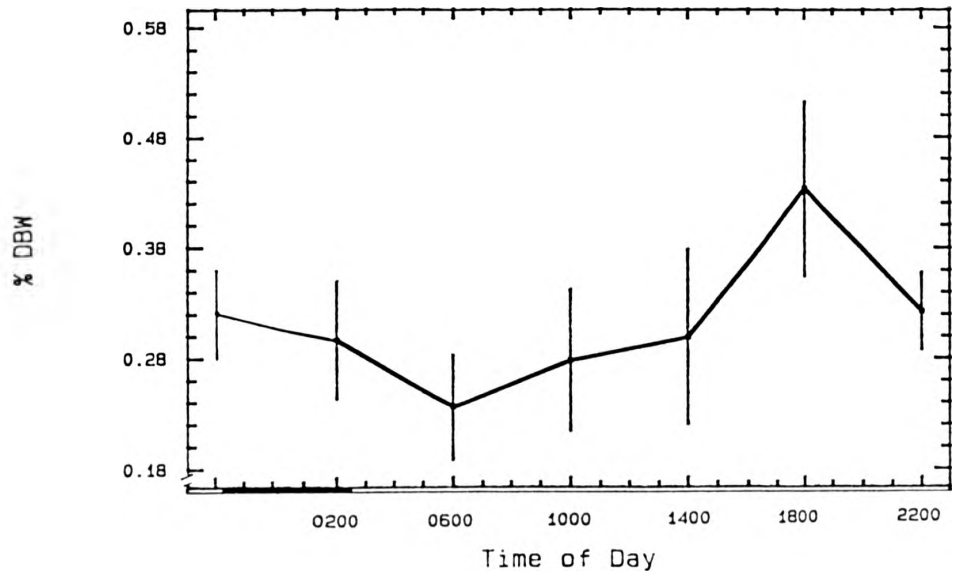
N.S. - Not Significant

* - $p<0.05$

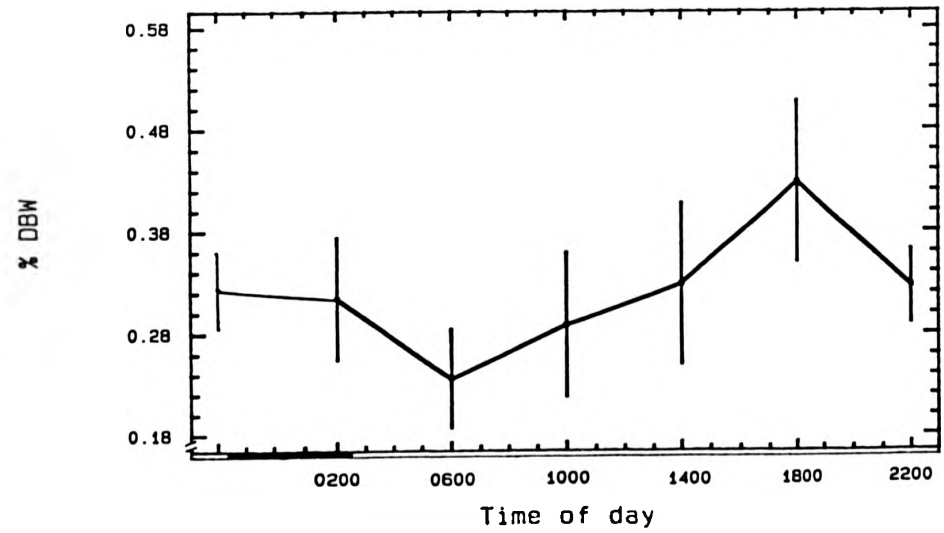
** - $p<0.01$

*** - $p<0.001$

Figure 6.3: The average foregut fullness cycle for a 24 hr period for fish held under ALP for both tanks 1 and 2.In tank 1 the level of fullness at 1800h was significantly different from those at 0200h, 0600h and 1000h (Table 6.3). In tank 2 the level of fullness at 1800h was significantly different from those at 0200h, 1000h and 1400h (Table 6.3). The vertical bars show standard error and the horizontal bar, below the axis, indicate the period of darkness.



Ambient light and photoperiod 1



Ambient light and photoperiod 2

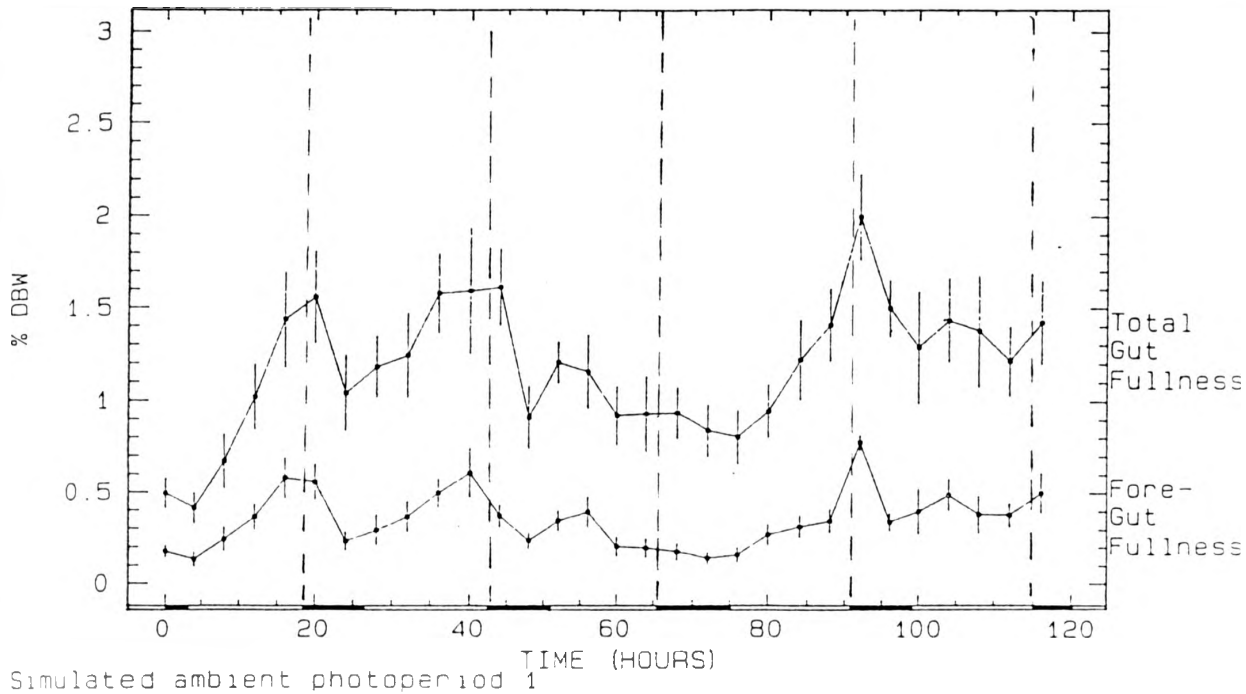
6.3.2 Gut fullness under simulated ambient photoperiod (SAP)

Under SAP 24 hour periodicity of gut fullness was less noticeable (Fig. 6.4) and the levels of wholegut fullness were more erratic, than under ALP. The highest foregut fullness level over a 24 hour period was recorded at 1800h only during cycle 2 for both tanks. In tank 1 minimum foregut fullness was 0.137 % DBW ($^{+}$/_ 0.031) at 0200h and maximum 0.775 % DBW ($^{+}$/_ 0.137) at 2200h. The minimum wholegut fullness was 0.410 % DBW ($^{+}$/_ 0.085) at 0600h and maximum was 1.996 % DBW ($^{+}$/_ 0.231) at 2200h. In tank 2 minimum foregut fullness was 0.132 % DBW ($^{+}$/_ 0.040) at 0200h and maximum was 0.718 % DBW ($^{+}$/_ 0.186) at 2200h. The minimum wholegut fullness was 0.601 % DBW ($^{+}$/_ 0.170) at 0600h and maximum 1.964 % DBW ($^{+}$/_ 0.367) at 0200h.

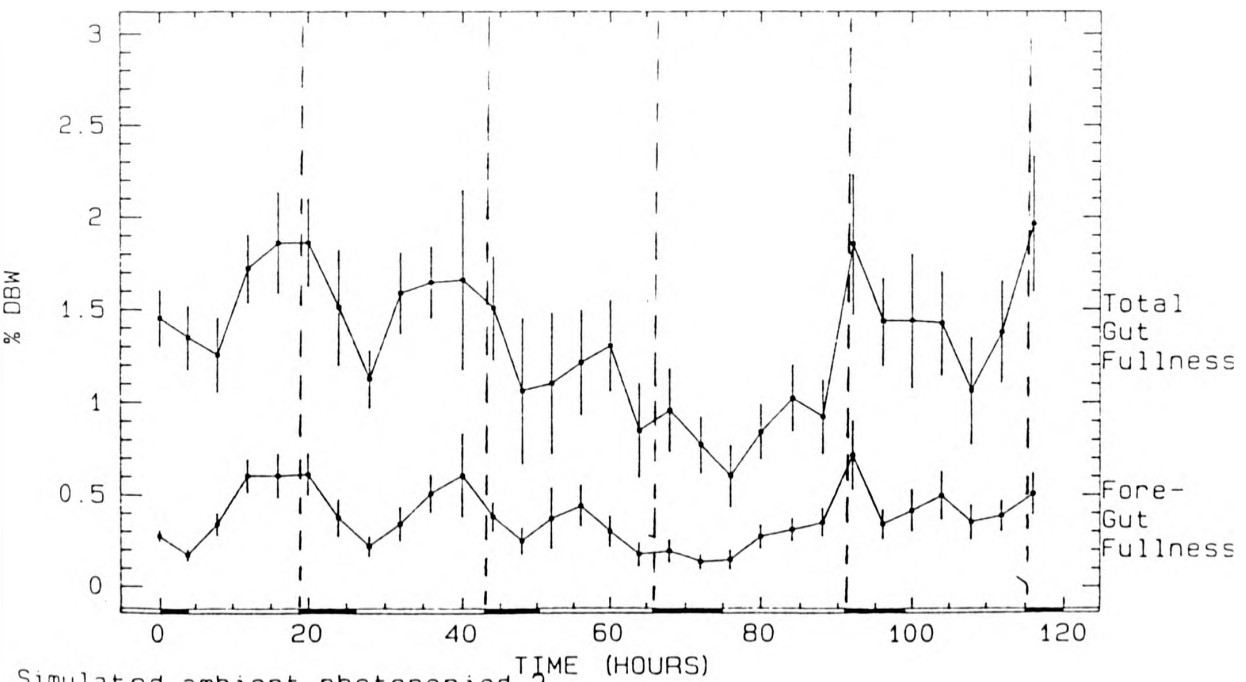
Two way ANOVA showed that in tank 1 the foregut was not significantly different either between days or times sampled; the hindgut was significantly different both between days ($p<0.01$) and times ($p<0.05$); and the wholegut was also significantly different both between days and between times ($p<0.05$, Table 6.2). In tank 2 foregut fullness was again not significantly different between days or times; the hindgut was significantly different only between days ($p<0.001$) as was the wholegut ($p<0.01$, Table 6.2).

In tank 1 fore- and hindgut fullnesses were closely

Figure 6.4: The fore- and total- (or whole-) gut fullness levels of fish held under simulated ambient photoperiod (SAP) for both tanks 1 and 2. Each point represents the mean level of gut fullness for the fish sampled at each sampling time, time 0 was 0200h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during the sampling.



Simulated ambient photoperiod 1



Simulated ambient photoperiod 2

related from the beginning of the sampling period. In tank 2 after an initial period the fullness levels of the 2 portions of the gut mirrored each other relatively closely. In both tanks the fore- and hindgut fullness levels were relatively erratic and a periodicity was not evident (Fig.6.5).

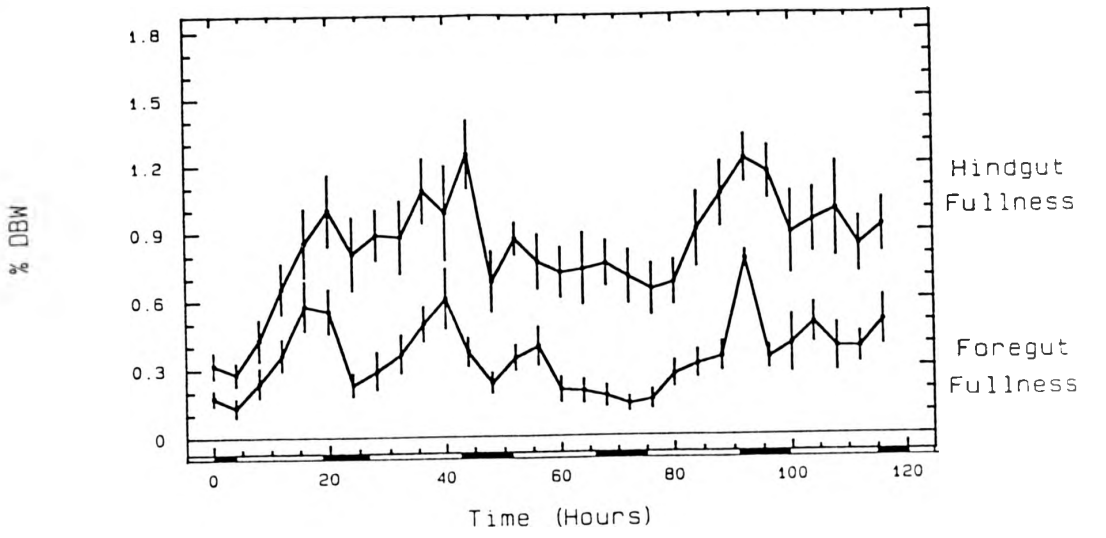
There was no late afternoon peak in foregut fullness (Fig. 6.6). The mean foregut fullness at 1800h, in both tanks was not significantly different from any of the values at other sampling times (Table 6.3). Mean foregut fullness increased steadily throughout the 24 hour period reaching a maximum at 2200h, unlike under ALP, where a relatively sudden increase was observed at one point each day.

6.3.3 Gut fullness under simulated ambient light and photoperiod (SALP)

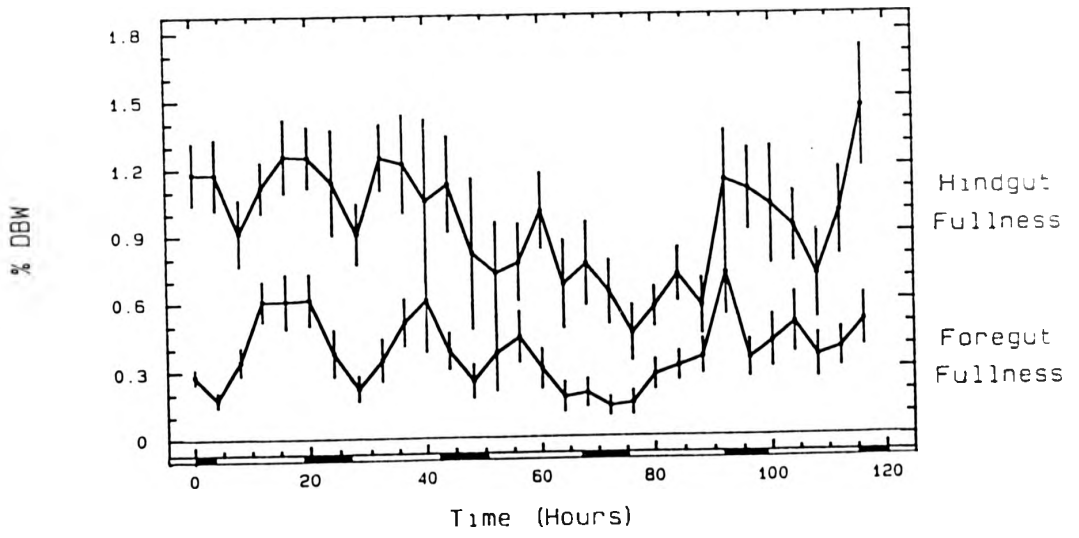
Gut fullness fluctuated with time (Fig 6.7) and a 24 hour periodicity was observed in both the fore- and wholegut fullness levels, similar to that under ALP. However, both these tanks were badly affected by the pump failure and the resulting large amounts of suspended solids in the water column. After 64 hours of sampling all levels of gut fullness fell rapidly and did not start to increase again until 88 hours, when the water cleared.

In tank 1 minimum foregut fullness was 0.065 % DBW ($^{+}/_{-}$ 0.028) at 1000h and maximum 0.752 % DBW ($^{+}/_{-}$ 0.121) at

Figure 6.5: The hind- and foregut fullness levels for fish held under SAP for both tanks 1 and 2. Each point represents the mean gut fullness level of all fish sampled at each sapling time, time 0 was 0200h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling

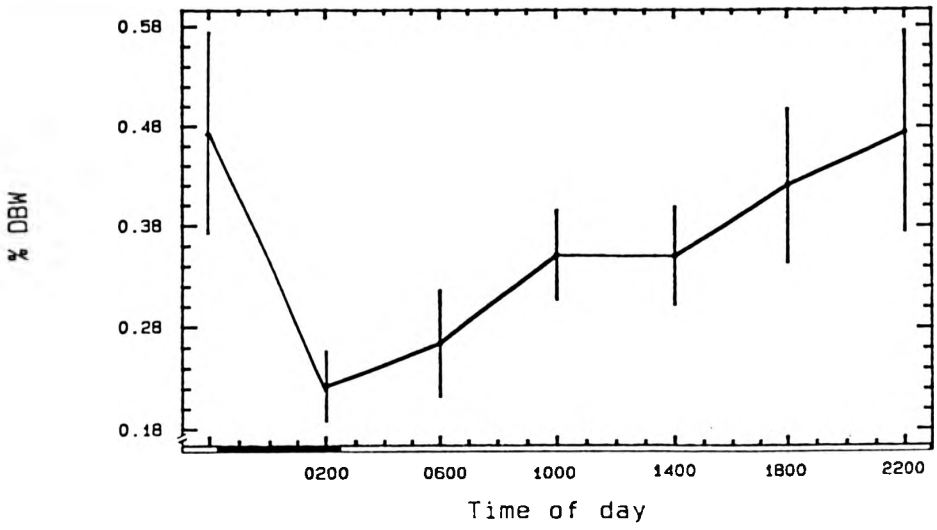


Simulated ambient phototperiod 1

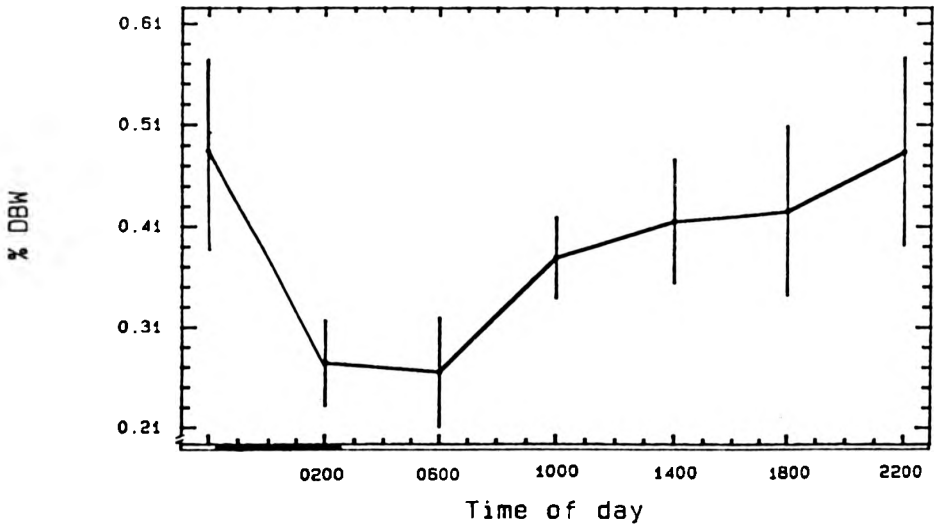


Simulated ambient photoperiod 2

Figure 6.6: The average foregut fullness cycle for a 24 hr period for fish held under SAP for both tanks 1 and 2. The mean gut fullness level at 1800h was not significantly different from the level at any other time for fish in both tanks (Table 6.3). The vertical bars show standard error and the horizontal bar, below the axis, indicates the period of darkness.

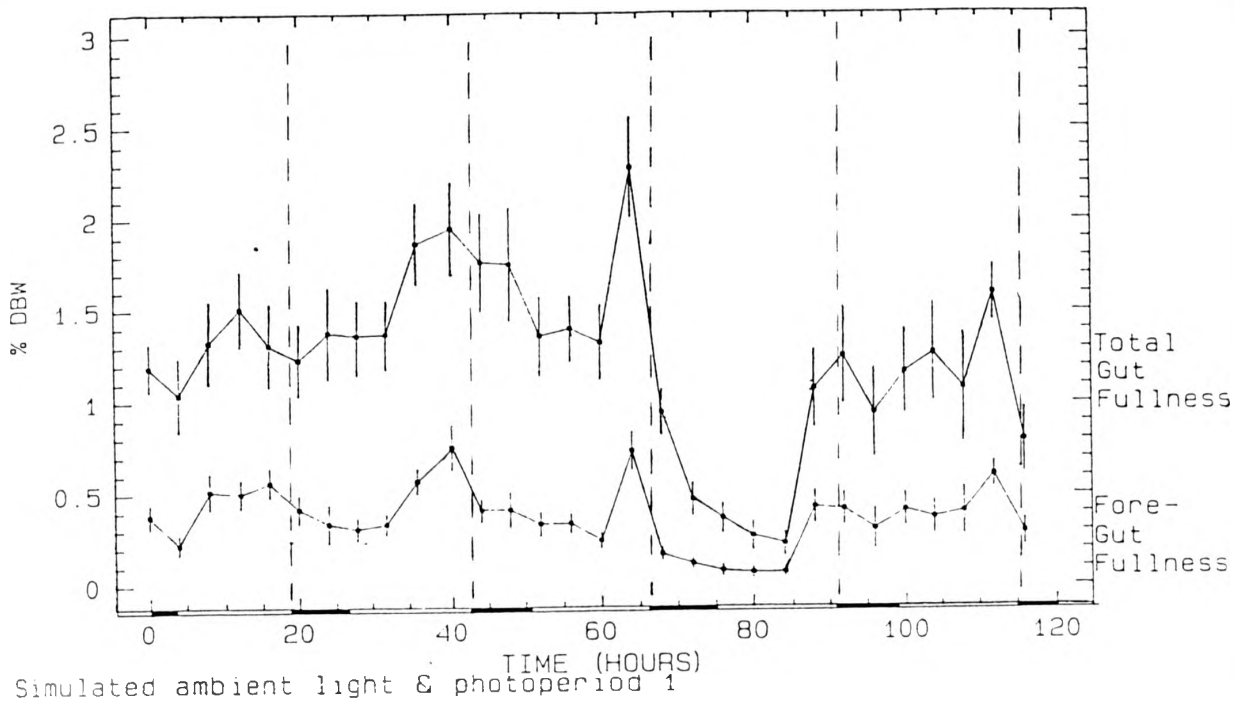


Simulated ambient photoperiod 1

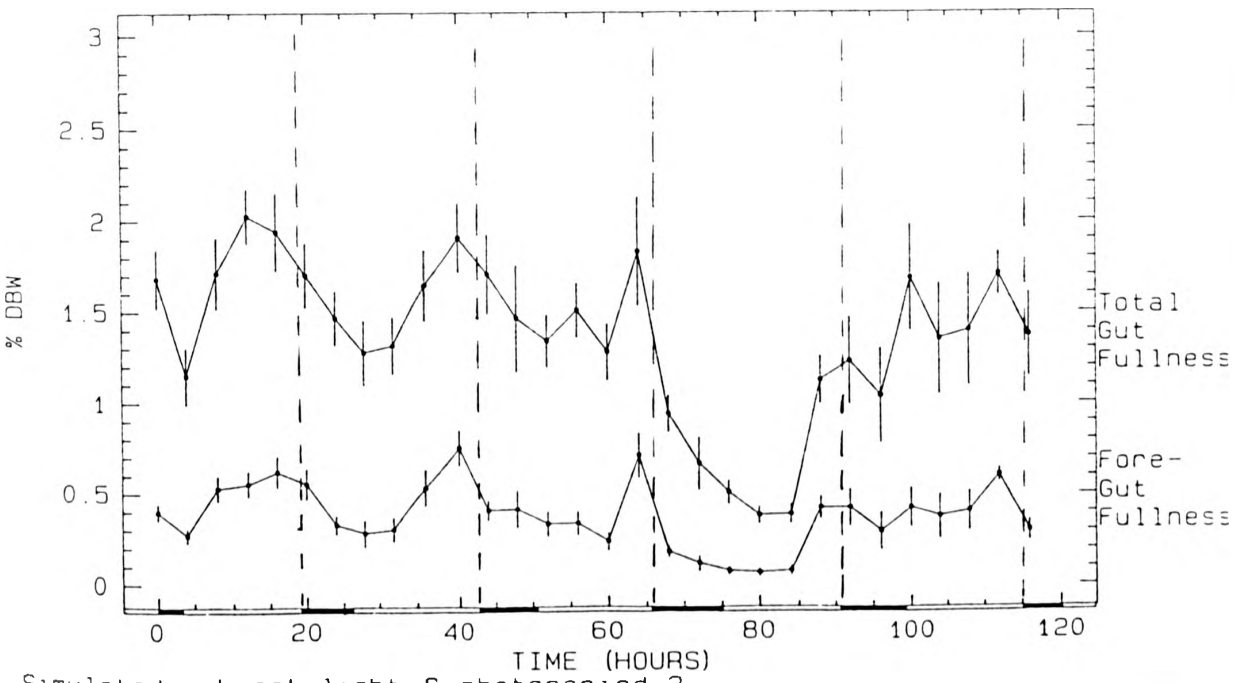


Simulated ambient photoperiod 2

Figure 6.7: The fore- and total- (or whole-) gut fullness levels for fish held under simulated ambient light and photoperiod (SALP) for both tanks 1 and 2. Each point represents the mean level of fullness for the fish sampled at each time, time 0 was 0200h. The vertical bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling.



Simulated ambient light & photoperiod 1



Simulated ambient light & photoperiod 2

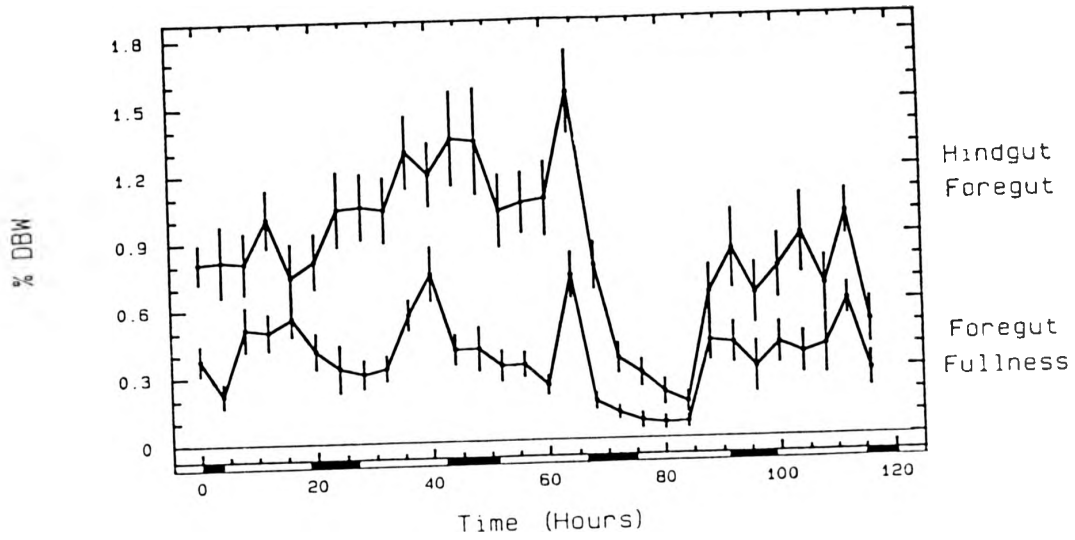
1800h. The minimum wholegut fullness was 0.224 % DBW ($^{+}/_{-}$ 0.068) at 1400h and maximum 2.282 % DBW ($^{+}/_{-}$ 0.274) at 1800h. In tank 2 minimum foregut fullness was 0.067 % DBW ($^{+}/_{-}$ 0.023) at 1000h and maximum 0.754 % DBW ($^{+}/_{-}$ 0.097) at 1800h. The minimum wholegut fullness was 0.377 % DBW ($^{+}/_{-}$ 0.048) at 1000h and maximum 2.027 % DBW ($^{+}/_{-}$ 0.149) at 1400h.

Two way ANOVA showed that in tank 1 foregut fullness was significantly different both between days and times ($p<0.01$). Hindgut fullness was significantly different between days ($p<0.001$) as was wholegut fullness ($p<0.01$), but both these portions of the gut were not significantly different between times. In tank 2 foregut fullness levels were significantly different between days ($p<0.01$), as was the hindgut ($p<0.001$) and wholegut ($p<0.001$, Table 6.2). However, no portions of the gut were significantly different between times.

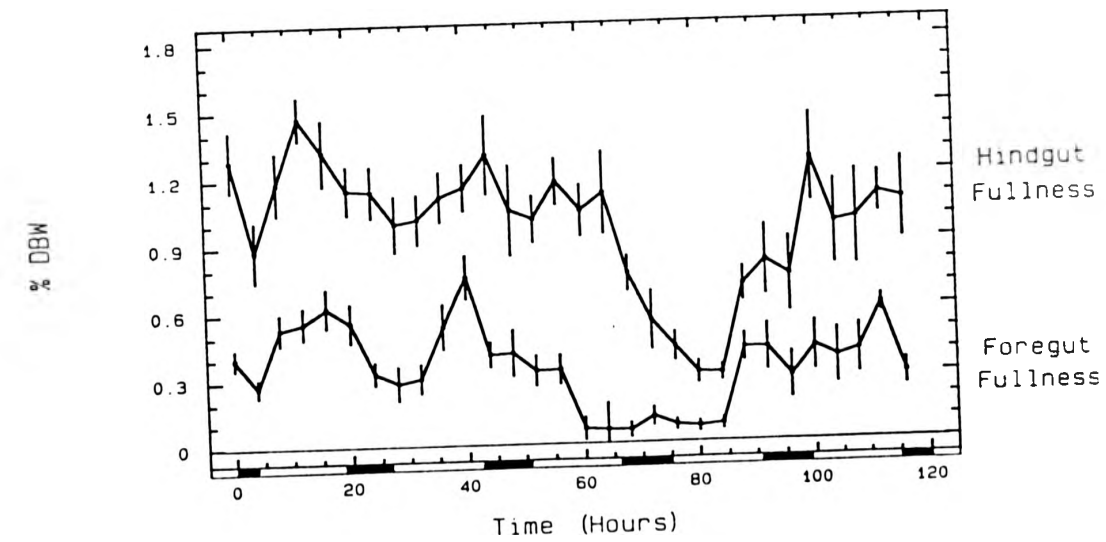
In both tanks fore- and hindgut fullness levels mirrored each other relatively closely. An increase in foregut fullness was accompanied by an increase in hindgut fullness. This is particularly well illustrated during the pump failure after 64 hours of sampling until the increase in gut fullnesses at 88 hours (Fig. 6.8).

In both tanks a peak in foregut fullness was observed at 1800h as under ALP but contradictory to the fish held

Figure 6.8: The hind- and foregut fullness levels for fish held under SALP for both tanks 1 and 2. Each point represents the mean level of gut fullness for fish sampled at each sampling time, time 0 was 0200h. The vertival bars show standard error and the horizontal bar, below the axis, indicates periods of darkness during sampling.



Simulated ambient light & photoperiod 1



Simulated ambient light & photoperiod 2

under SAP (Fig 6.9). The mean level of foregut fullness at 1800h was significantly different from all values from 0200h-1400h. In both tanks only values at 1800h and 2200h were not significantly different (Table 6.3).

6.3.4 Relationship between the different light regimes and gut fullness

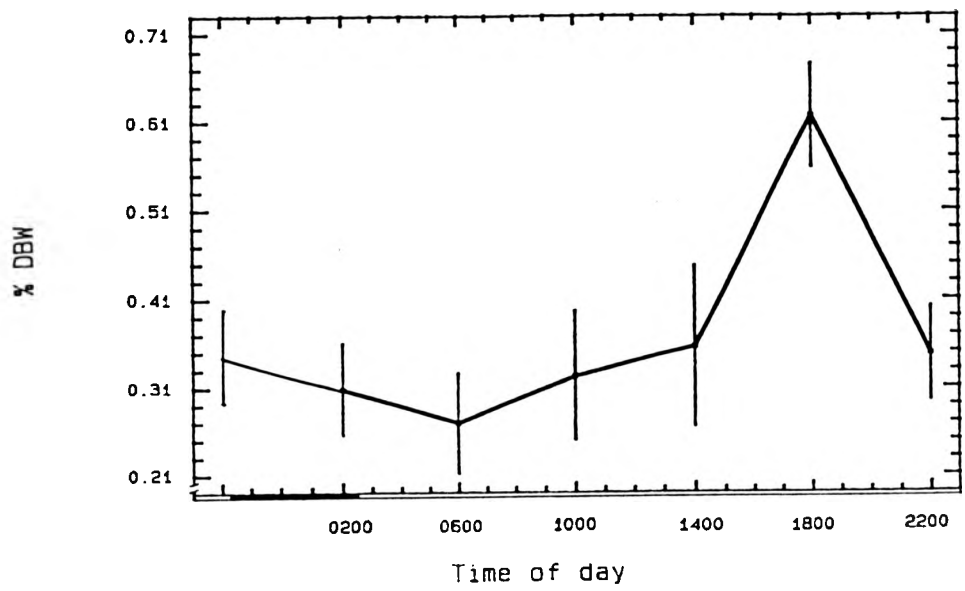
- ambient light and photoperiod

Under ALP, the light levels, within a 24 hour period, increased for the period of dawn. They continued to rise after this period and started to decrease before dusk occurred (Fig. 6.10). From the data calculated to show deviation from the mean foregut fullness levels peaked at 1800h when ambient light levels were on a downwards trend (Fig. 6.11). Fullness levels did not again start to increase until well after dawn.

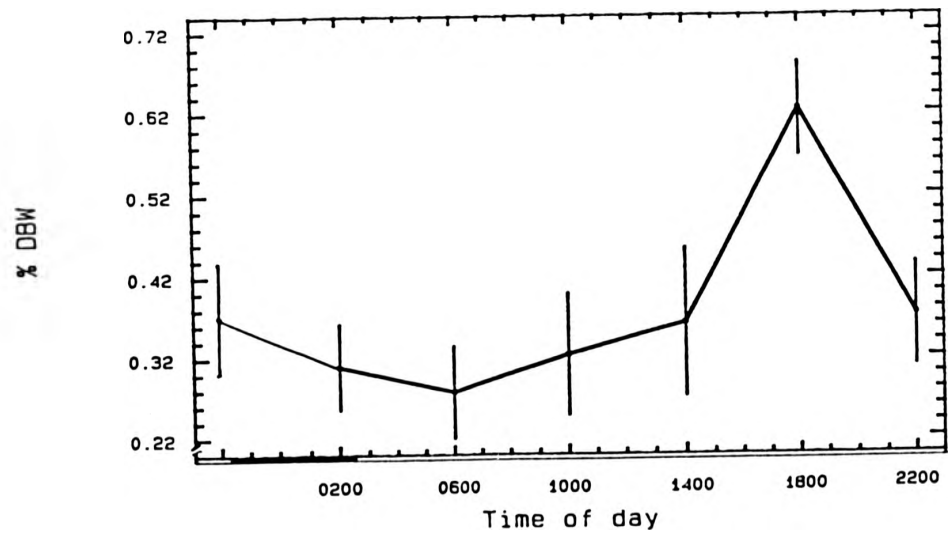
- simulated ambient photoperiod

Under SAP, the only period of increasing light was during simulated dawn, once this period had occured the light levels remained constant (Fig. 6.10). Likewise the only period of decreasing light was during simulated dusk. From the data calculated to show deviation from the mean it canbe senn that foregut fullness levels were highest (for a 24 hour period) at 2200h, the sample taken after simulated dusk. Fullness levels did not begin to fall until the period of darkness (Fig 6.12) and did not start to increase again until after simulated dawn.

Figure 6.9: The average foregut fullness cycle for a 24 hr period for fish held under SALP for both tanks 1 and 2. In both tanks the level of mean fullness at 1800h was significantly different from the samples at 0200h, 0600h, 1000h and 1400h (Table 6.3). The vertical bars show standard error and the horizontal bar, below the axis, indicates the period of darkness.

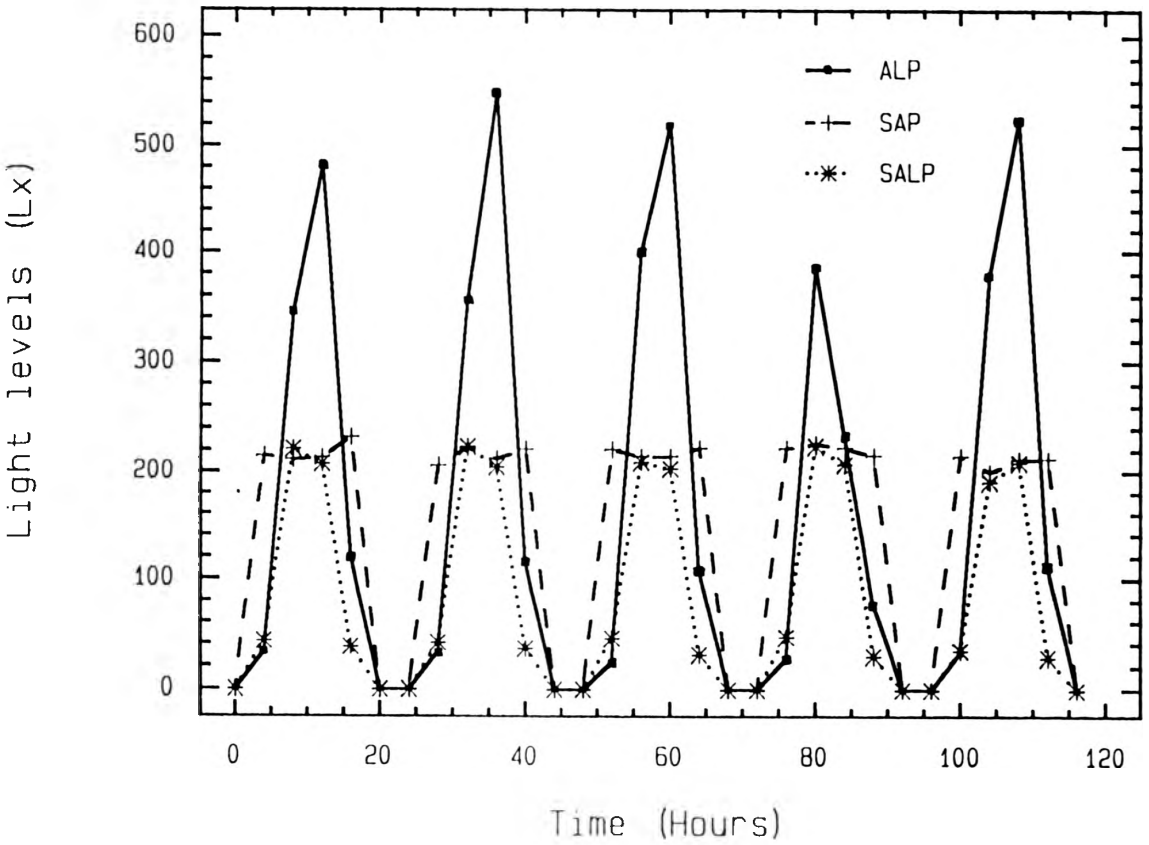


Simulated ambient light & photoperiod 1



Simulated ambient light & photoperiod 2

Figure 6.10: The pattern of light levels for the regimes- ambient light and photoperiod (ALP, dot), simulated ambient photoperiod (SAP, cross) and simulated ambient light and photoperiod (SALP, star). Light levels were expressed in lux and time 0 was 0200h.



Light levels

Figure 6.11: The data calculated as deviation from the mean for light (dot) and foregut fullness levels for fish held under ALP for both tanks 1 (cross) and 2 (star). The horizontal bar, below the axis, shows the period of darkness.

PAGE(S) MISSING
NOT AVAILABLE

Fig. 6.11

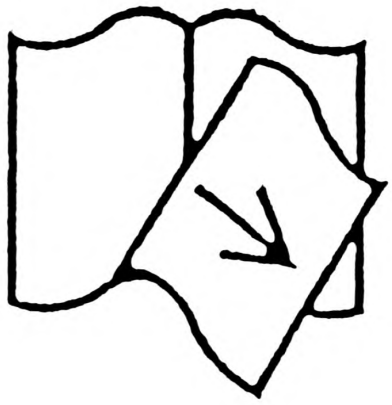
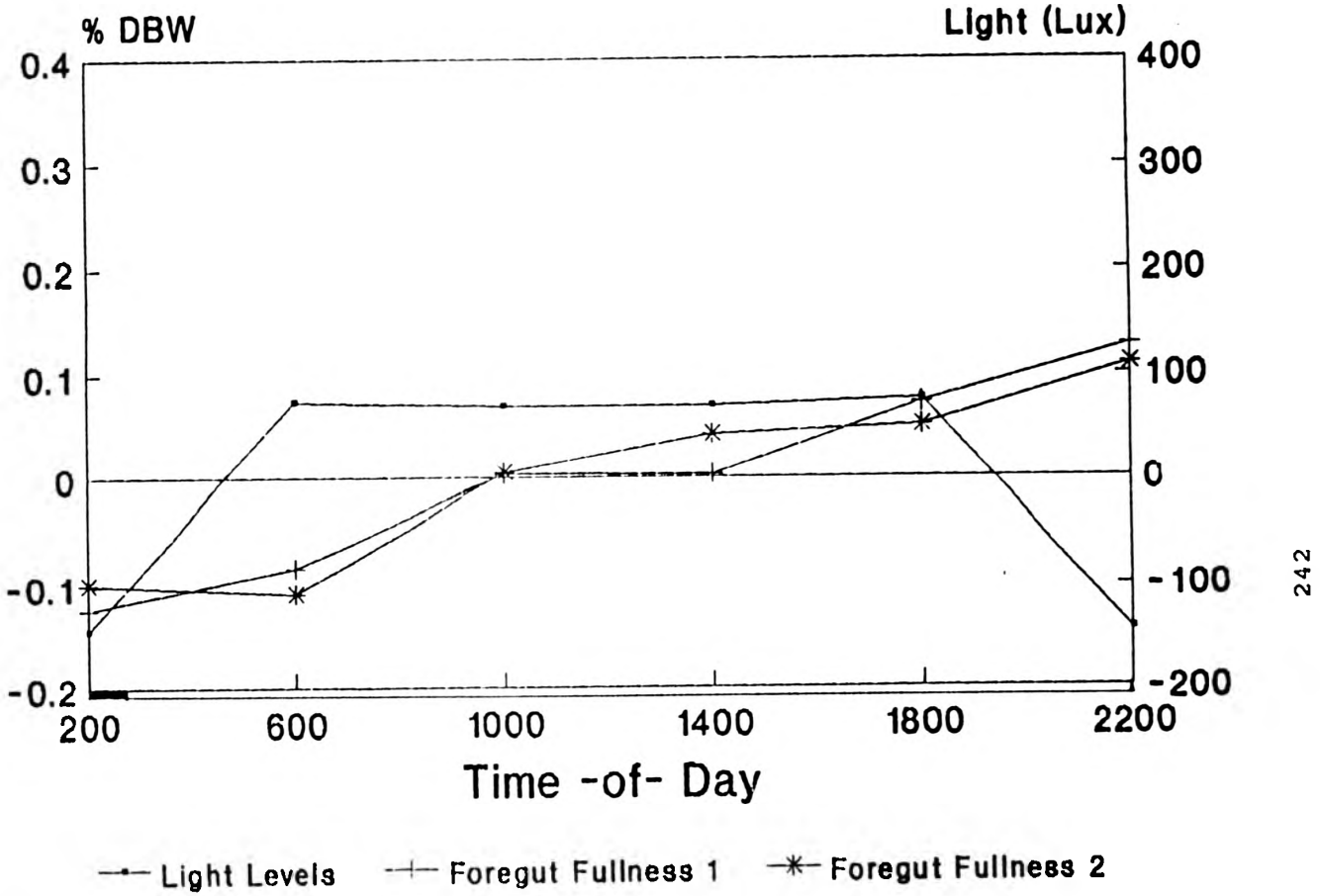


Figure 6.12: The data calculated as deviation from the mean for light (dot) and foregut fullness levels for fish held under SAP for both tanks 1 (cross) and 2 (star). The horizontal bar, below the axis, shows the period of darkness.

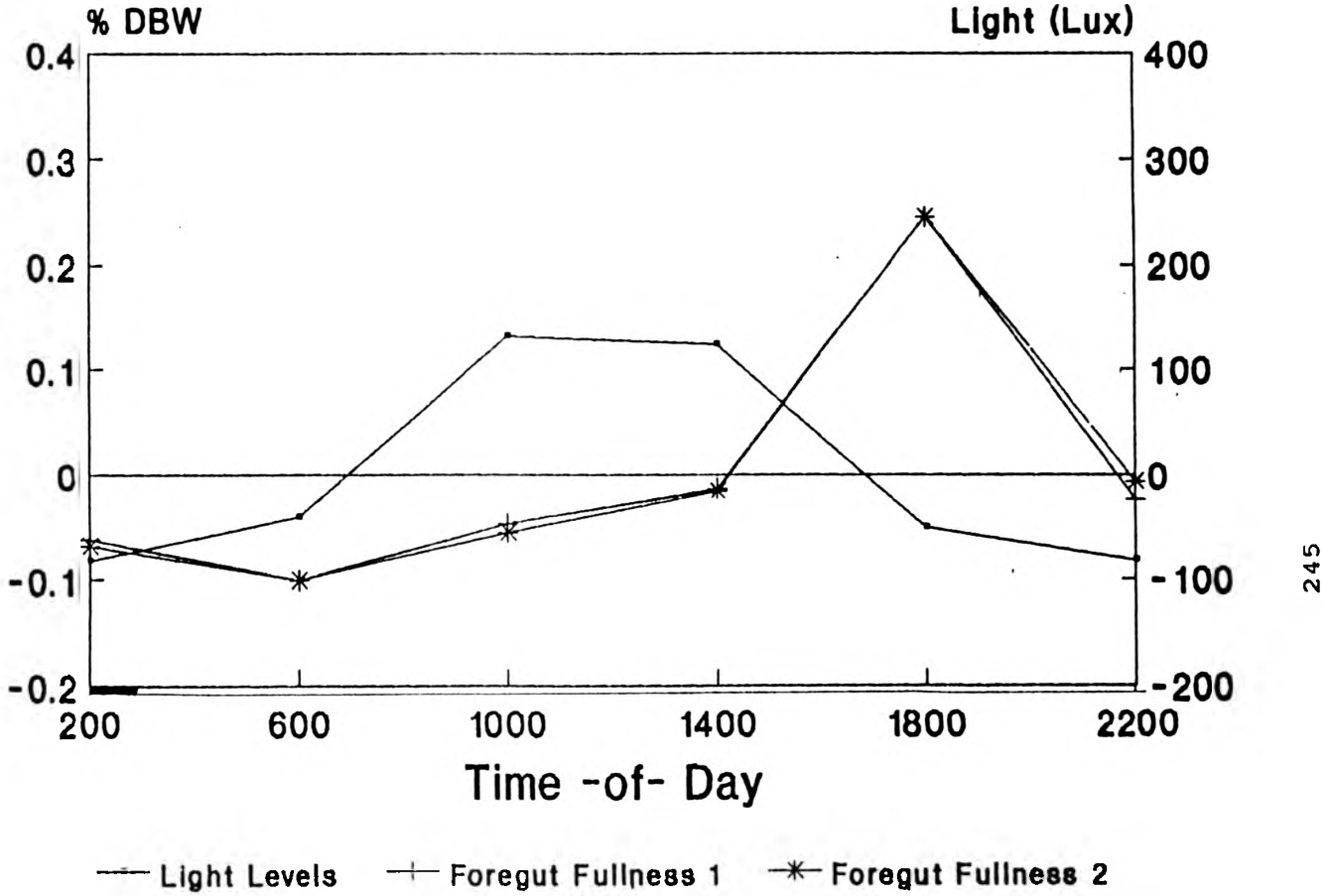


Simulated Ambient Photoperiod

- simulated light and photoperiod

Under SALP, light levels initially increased during a simulated dawn, but again as under ALP continued to increase after this period. Similarly light levels started to decrease before simulated dusk occurred (Fig 6.10). Although the high midday natural light levels of ambient light were never achieved, the fish still responded comparably. From the data calculated to show the deviation from the mean the level of foregut fullness started to increase while light levels were decreasing, but fell during the period of darkness. Foregut fullness levels began to increase well after simulated dawn had occurred (Fig.6.13).

Figure 6.13: The data calculated as deviation from the mean for light (dot) and foregut fullness levels for fish held under SALP for both tanks 1 (cross) and 2 (star). The horizontal bar, below the axis, shows the period of darkness.



Simulated Ambient Light and Photoperiod

6.4 DISCUSSION

Feeding was rhythmic under both ALP and SALP but not under SAP. The only changes the fish could respond to were the dawn and dusk periods experienced in all tanks, or the daytime light intensity changes in ALP and SALP.

In most organisms, living in cyclical environments, physiological timing devices have evolved to match the temporal structure of the environment. The diel light/dark cycle forms the most reliable basis for information about time of day. Eriksson & Alanara (1992) suggesed that, in principle, salmonids could respond in a purely mechanistic manner to light changes, becoming active in response to lights-on or lights-off. However, they added "it is probably not that simple since activity is far from constant during periods of light or dark". Also the relative importance of dawn and dusk (nature's lights on/off) as a regulator of activity or of feeding behaviour remains unclear (Boujard & Leatherland, 1992). This view was also held by Harker (1964), who stated "the setting of the phases of a rhythm is by no means dependent upon a change from absolute darkness to light, or vice versa". In a previous experiment (Chapter 5) only fish held under simulated ambient photoperiod and controlled temperature (SAPCT) showed no peak in foregut fullness, but a difference in gut fullness between late evening (1800h - 2200h) and early morning (0600h). These results are similar to those of the current experiment where

fish were held under almost identical regimes (SAP), hence indicating further evidence that simulated photoperiod alone is insufficent to synchronize feeding peaks.

In the experiment of Chapter 5, under simulated ambient photoperiod and ambient temperature (SAPAT), and constant light and ambient temperature (CLAT), the temperature peaked regularly and so did foregut fullness, independent of light conditions. All tanks in the current experiment shared a common water source, with no daily fluctuation in temperature. The only comparable regimes between the two experiments were those of SAPCT and SAP, but the similarity of gut fullness patterns between them shows that, in the absence of light change between dawn and dusk, there is a response to temperature. This is also observed in mammals for example in mice and rats, when food is continuously available, the timing of circadian rhythms is determined primarily by the daily light/dark cycle, and temperature has only a secondary effect (Nelson et al, 1975). Also the most important factor influencing fish activity is the alternation between light and darkness, while temperature is only of secondary importance (Muller, 1978a).

If photoperiod alone is insufficent to elicit a feeding response, the change in light experienced during the day must be critical. It is not however the absolute intensity of light that is the influencing variable but

the change in intensity (Chaston, 1968; Gibson & Keenleyside, 1966; Molina Borja et al, 1990). This is well illustrated in this experiment: while fish held under SALP experienced the same temporal pattern of intensity change, but not the same absolute light intensities as those under ALP, the results were comparable. These results support the hypothesis described in Chapter 5, that a time measuring mechanism may be present in fish based on a daily rhythm of sensitivity to light (Baggerman, 1980).

Most information on light intensity influencing feeding has come from studies in the wild because in most experimental studies the intensities used have not varied realistically (Harker, 1964). This may have lead to the conclusion that intensity had little or no influence, since only photoperiod change was being studied.

6.5 SUMMARY

1. Gut fullness in Atlantic salmon fluctuated and foregut fullness peaked under ambient light and photoperiod and simulated ambient light and photoperiod.
2. Simulated ambient photoperiod was insufficient to synchronize a feeding rhythm in Atlantic salmon similar to that seen under natural conditions.
3. Temperature is of secondary importance and only influences feeding rhythms when light levels remain constant during the period of daylight.

4. Changing light levels, as opposed to specific light intensites, are important in synchronizing feeding rhythms and peaks.

CHAPTER 7

THE EFFECTS OF TEMPORAL DIFFERENCES IN FEEDING ON MEAL SIZE

7.1 INTRODUCTION

Atlantic salmon, in culture both in fresh water (Rawlings, 1989) and in sea water (Rawlings *et al*, 1991; Chapter 4) feed rhythmically. When offered a constant supply of food throughout the day, fish showed a pronounced peak of gut fullness in the late afternoon (Chapter 3, 4 and 6). Several species of fish when fed during specific times of the day take larger meals and show differing physiological responses (Sundararaj *et al*, 1982; Noeske & Spieler, 1984). The effect of timing a daily meal to coincide with a peak of preferential feeding in salmon may have similar affects, the mechanisms of which are still to be defined (Sundararaj *et al*, 1982). In mammals the time of day of feeding a single daily meal can affect weight gain and possibly food conversion efficiency (Nelson *et al*, 1973, 1975; Philippens *et al*, 1977).

The timing of salmonids feeding in the wild has been considered as an evolutionary compromise between metabolic demand, vision capacity, predation risk and food availability (Eriksson & Alanara, 1992). Salmonids have survived under these conditions for millions of years but have only recently (in evolutionary terms) been subjected to intense farming conditions. Although farmed salmonids may have adapted their feeding behaviour to fish farm feeding regimes it is unlikely that their physiological mechanisms have become equally well adapted. Therefore, although the

fish may take food when fed, intake may not be maximised and the nutrients may not utilized to their best advantage.

The present experiments tested the null hypotheses that the size of a single meal for salmon in sea cages in the summer is unaffected by the time it is offerred, and that when multiple meals are offerred the amount taken does not alter with the time of day.

7.2 MATERIALS AND METHODS

7.2.1 Fish Stocks

The following study was carried out at Lerang Research Station (59° 5′N 6° 2′E) near Stavanger, Norway. Hatchery reared Atlantic salmon post-smolts, were held in sea water tanks at the land based site from November 1991 to June 1992. On the 1st July 1992 350 salmon (mean weight 177.9g, mean fork length 26.3cm) were moved to each of six 5mx5mx5m sea cages, for 60 days.

7.2.2 Feeding Regime

The fish were fed on a commercial diet of expanded Royale feed (BP Nutrition, Wincham, UK) at a level of 2.5% of their body weight per day throughout the trial. Two replicate cages were fed morning only between 0500h-0900h, two replicate cages were fed afternoon only between 1500h-1900h and two replicate cages were fed both morning and afternoon. Both the morning and afternoon periods of feeding were during daylight hours. The food was delivered by bucket feeders with a vibrating delivery disc (Skretting, Norway 50L), in 5g bursts, throughout the 4-hour feeding periods. The feeding was controlled centrally by computer. The computer was also used to record daily input of food as well as temperature, salinity and oxygen levels at 2 metres depth, where the fish tended to aggregate.

7.2.3 Sampling

On the 25 and 26 August 1992 the diet was replaced by one labelled with a radio-opaque marker of ballotini (Jencons, Leighton Buzzard, UK) at 3 % (w/w), which had been prepared commercially. The food fed during the morning was labelled with ballotini size 8.5 and that in the afternoon with ballotini size 6.

At 0900h (25.08.92) 32 fish were removed from each of the 2 cages fed morning only and from each of the 2 cages fed both morning and afternoon. The fish were held in bins, anaesthetised, weighed then placed on cassettes loaded with prepacked Industrex Kodak film and x-rayed using a Triton portable x-ray machine. The x-ray plates were repacked ready for transport back to SOAFD Almondbank, Perthshire, Scotland for developing. The fish were allowed to recover before returning them to their original cages.

At 1900h on 25.08.92 32 fish were removed from each of the 2 cages fed afternoon only and from each of the 2 cages fed both morning and afternoon. The fish were treated as previously described. The same procedure was repeated on the 26.08.92 at both 0900h and 1900h.

7.2.4 Calculations

Samples of food were collected and a calibration relationship between the food and amount of marker was

determined. Known amounts of food were x-rayed and the count of marker present per unit of food was calculated. The plates were analysed to assess the amount of food present in the intestine of the fish at each sampling time, and expressed as percent dry body weight (% DBW) as before.

Average values of gut fullness were calculated for fish for each of the cages. For the cages fed both am and pm, on the evening of the 25.08.92 and both the samples on the 26.08.92, it was possible to calculate the residual food left in the gut from the previous meal as well as the food consumed in the immediate 4 hours prior to sampling and consequently the total amount of food present. Average evacuation rates were also calculated for these cages both during the day and overnight.

7.2.5 Statistical Analysis

A two-sample t-test was used to test that the means of the replicate samples were not significantly different. This t-test was also used to calculate whether the data sets from different treatments and different days, were significantly different.

PAGINATION ERROR

P. 256

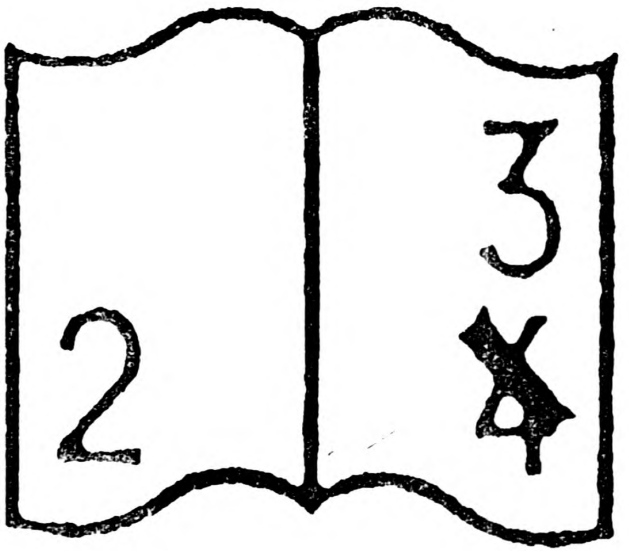


Table 7.1: Mean gut fullness values

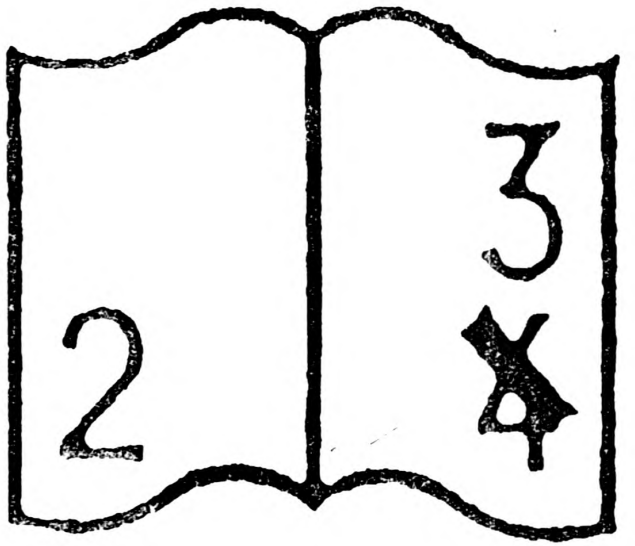
Time Fed	Cage No	Time of Sample				
			Morning		Afternoon	
		Meal Size	Am	Total	Pm	Total
25.08.93						
Morning	1	Mean	1.657	--	--	--
		S.E.	0.266	--	--	--
Morning	2	Mean	1.951	--	--	--
		S.E.	0.103	--	--	--
Morn/After	1	Mean	1.519	--	3.636	4.044
		S.E.	0.134	--	0.245	0.259
Morn/After	2	Mean	1.662	--	2.795	3.217
		S.E.	0.200	--	0.225	0.258
Afternoon	1	Mean	--	--	3.424	--
		S.E.	--	--	0.331	--
Afternoon	2	Mean	--	--	3.153	--
		S.E.	--	--	0.341	--

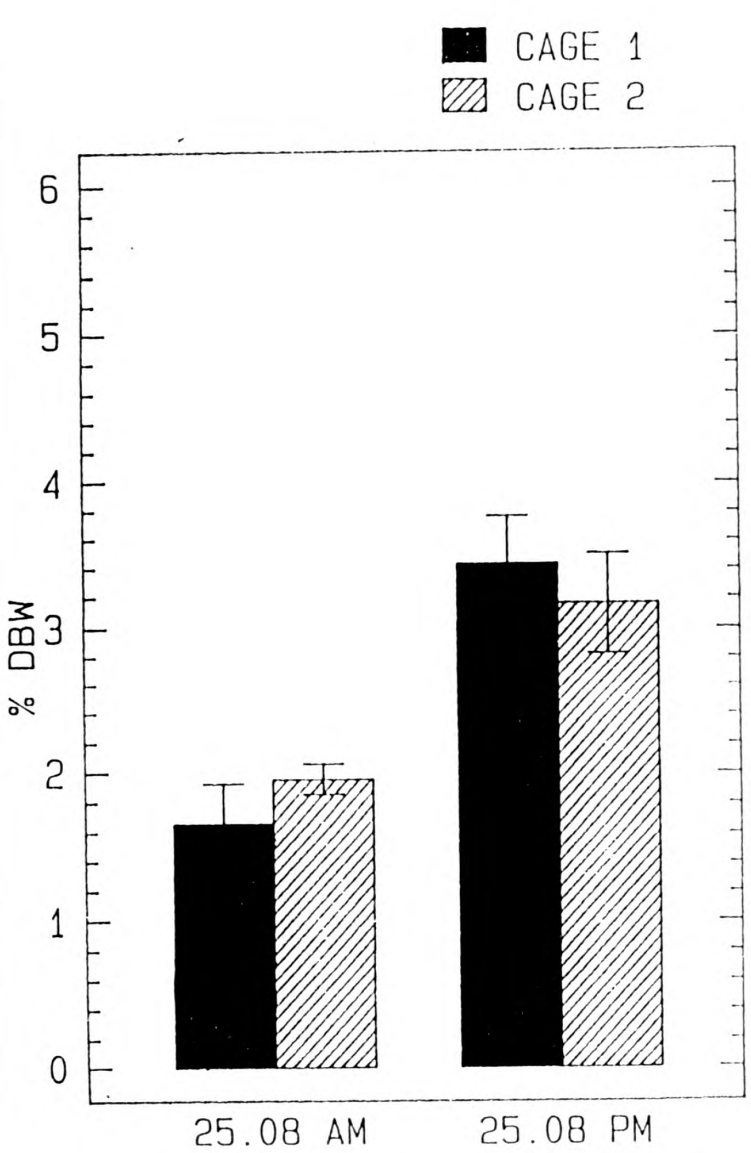
Table 7.1: Continued

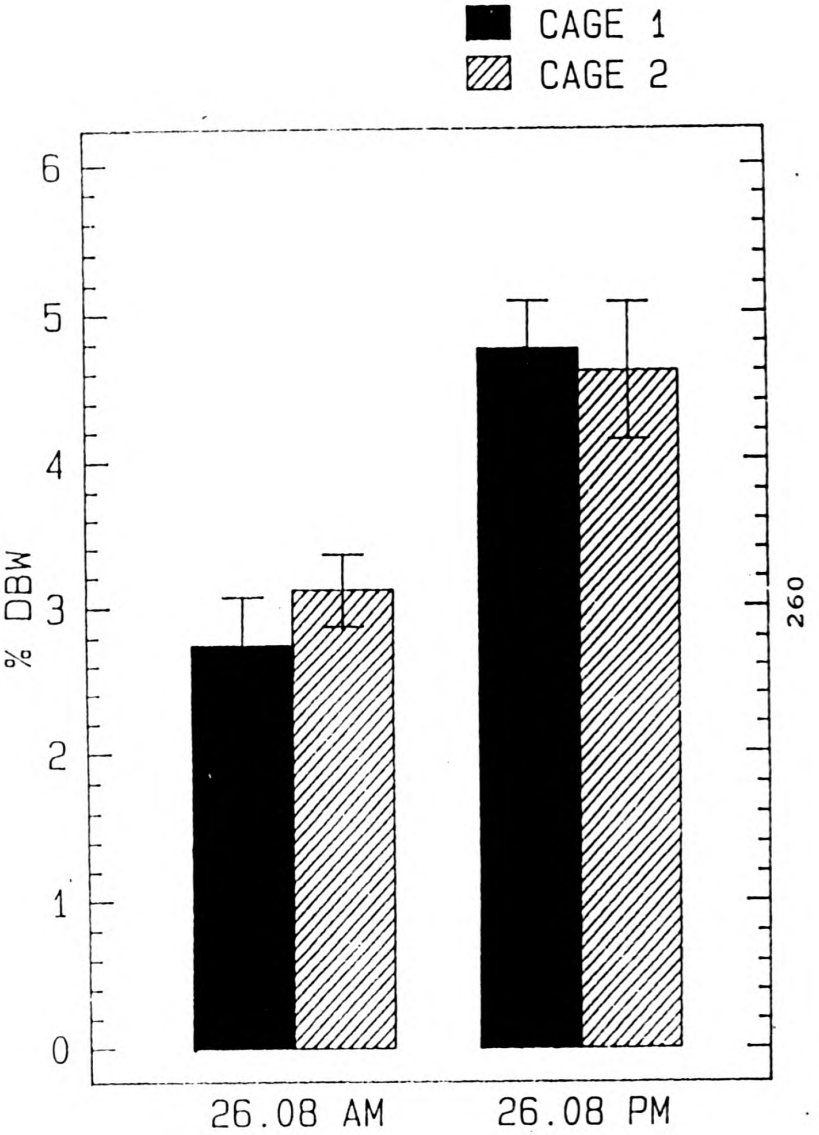
Time Fed	Cage No.	Time of Sample				
			Morning		Afternoon	
		Meal Size	Am	Total	Pm	Total
26.08.93						
Morning	1	Mean	2.745	--	--	--
		S.E.	0.320	--	--	--
Morning	2	Mean	3.110	--	--	--
		S.E.	0.246	--	--	--
Morn/After	1	Mean	1.675	2.712	3.972	4.500
		S.E.	0.210	0.281	0.375	0.406
Morn/After	2	Mean	1.512	1.918	3.376	3.801
		S.E.	0.188	0.209	0.351	0.392
Afternoon	1	Mean	--	--	4.750	--
		S.E.	--	--	0.332	--
Afternoon	2	Mean	--	--	4.602	--
		S.E.	--	--	0.472	--

PAGINATION ERROR

P. 259




CAGE 1
CAGE 2
% DBW
0
1
2
3
4
5
6
25.08 AM
25.08 PM


CAGE 1
CAGE 2
% DBW
0
1
2
3
4
5
6
26.08 AM
26.08 PM

260

Table 7.2 : Summary of significant differences of gut fullness for fish fed a single daily meal

Day	Time Sampled	Cage No.	Significant Difference
25.08	Morning	1	N.S.
	Morning	2	
	Afternoon	1	N.S.
	Afternoon	2	
	Morning	1 & 2	***
	Afternoon	1 & 2	
26.08	Morning	1	N.S.
	Morning	2	
	Afternoon	1	N.S.
	Afternoon	2	
	Morning	1 & 2	***
	Afternoon	1 & 2	
25.08	Morning	1 & 2	***
26.08	Morning	1 & 2	
25.08	Afternoon	1 & 2	***
26.08	Afternoon	1 & 2	

N.S. - Not Significant ** - p<0.01

* - p<0.05 *** - p<0.001

on the first morning, only shows the amount of food consumed that morning. It gives therefore no indication of the reisidue of food left from the meal the night before (24.08.92). In all other samples the amount taken at the previous meal was distinguishable, since the markers labelled each meal separately. The residue left from the previous meal was a relatively small proportion of the total amount in the gut at the time of sampling (Fig. 7.2, see later for actual percentages). Therefore, gut fullness due to the meal just consumed, not including the residue of the meal consumed previously, will be considered.

For the fish fed two meals a day, there were no significant differences in gut fullness in the mornings (Table 7.3). However, the replicate samples taken at 1900h were significantly different ($p<0.001$, Table 7.3) on the 25th, but not on the 26th. Therefore the data for replicate cages on 26.08.92 were pooled for further statistical analysis, but replicate cages were treated separately for 25.08.92.

Fish from cages 1 and 2 on both days had significantly more food ($p<0.001$, Table 7.3) in the gut in the afternoon samples (Fig 7.3).

Since the morning and afternoon feeds were labelled with different markers it was possible to distinguish food remaining from the previous meal and food that had been

Figure 7.2: The mean meal sizes for fish fed twice daily on the afternoon of 25 and the morning and afternoon of the 26 August 1992, for both cages. Meal 1 is shaded, meal 2 is heavily hatched and the total is lightly hatched.The vertical bars indicate standard error.

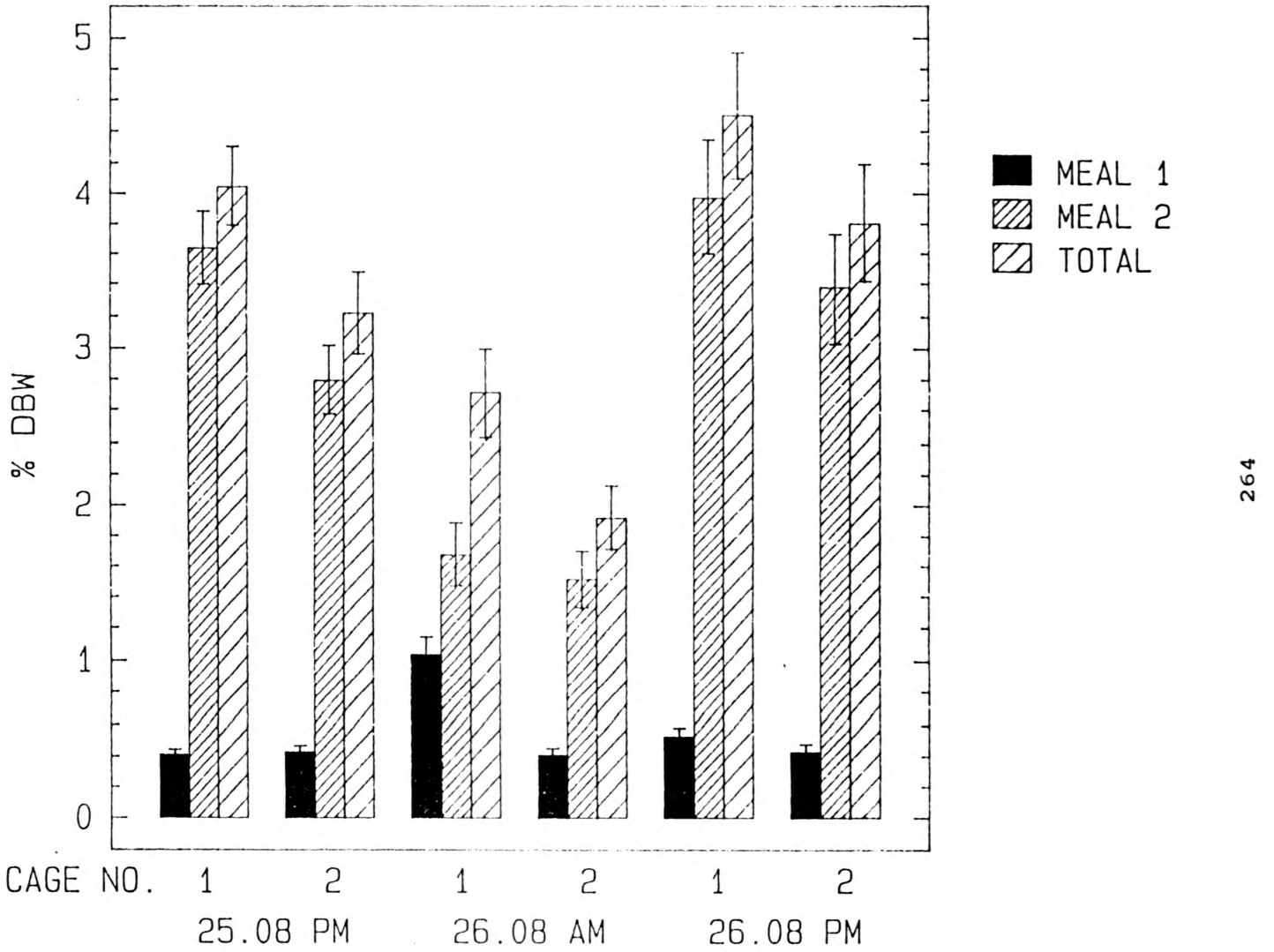

5
4
3
2
1
0
% DBW
MEAL 1
MEAL 2
TOTAL
CAGE NO. 1 2 1 2 1 2
25.08 PM
26.08 AM
26.08 PM

Table 7.3 : Summary of significant differences of gut fullness for fish fed two meals a day

Day	Time Fed	Time Sampled	Cage No.	Significant Difference
25.08	Morn/After	Morning	1	N.S.
		Morning	2	
		Afternoon	1	***
		Afternoon	2	
		Morning	1	***
		Afternoon	1	
		Morning	2	***
		Afternoon	2	
26.08	Morn/After	Morning	1	N.S.
		Morning	2	
		Afternoon	1	N.S.
		Afternoon	2	
		Morning	1 & 2	***
		Afternoon	1 & 2	

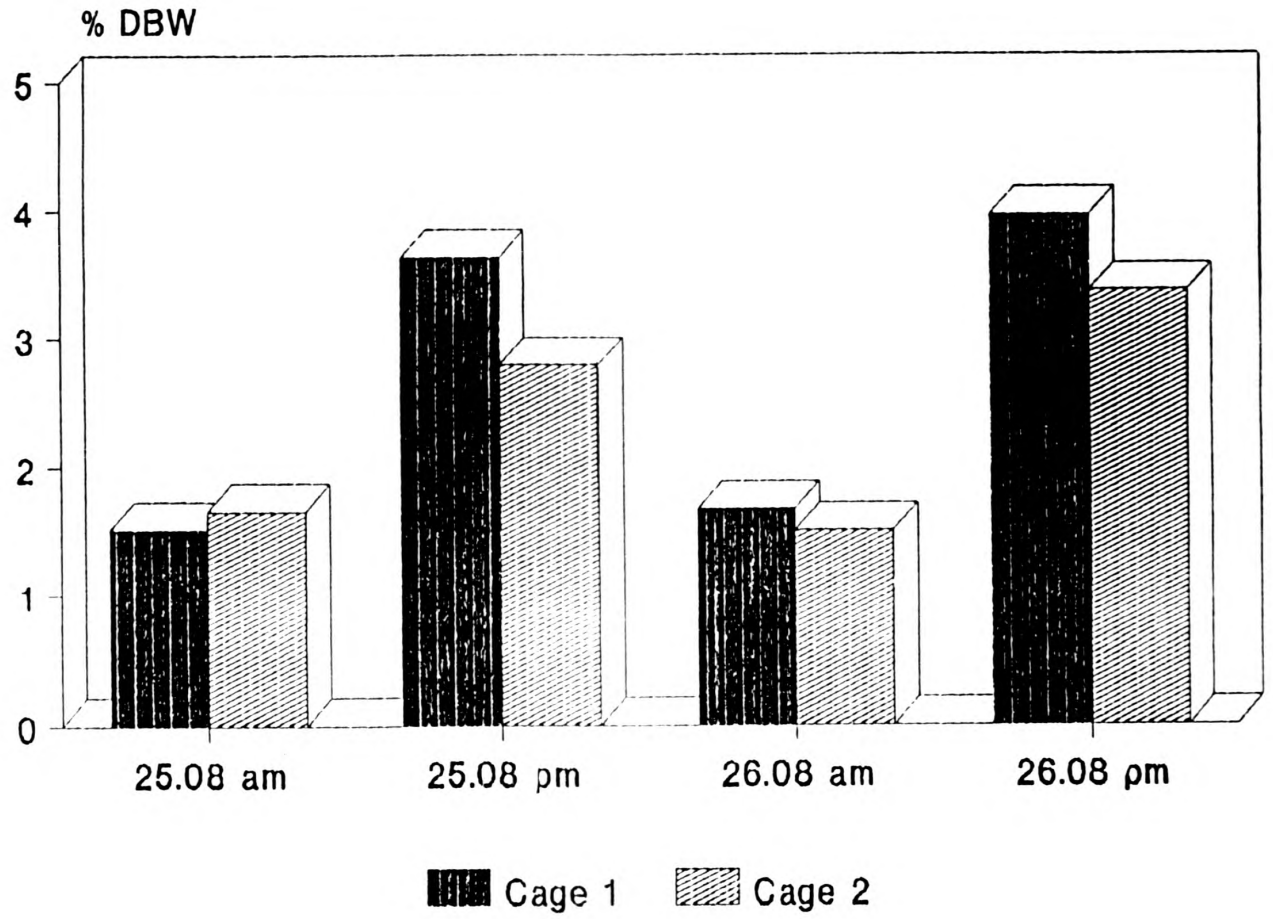
N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

*** - $p<0.001$

Figure 7.3: The mean meal size for fish fed both morning and afternoon, sampled after feeding, indicating the amount of food present. Cage 1 represented by shading and cage 2 represented by hatching.



Fish fed both morning and afternoon

consumed within the last 4 hours prior to sampling. Evacuation rates were higher, for both replicate cages, during the overnight (1900h-0900h) period than during both daytime periods (0900h-1900h; Table 7.4). However the amounts taken during the afternoon feeds were also larger than the amounts taken in the mornings. A clearer picture is given if the percentage of each meal in relation to the total amount of food present in the gut at any one time is considered.

On the afternoon of the 25 August the average fish in cage 1 had 27 % of the previous meal remaining in their gut. This constituted only 10 % of the gut contents after the second meal had been consumed, therefore the new meal contributed 90% of the average gut fullness. In cage 2 again the average fish had 27 % of the previous meal remaining in their gut. This made up 13 % of the gut contents after the afternoon feed, so the new meal resulted in 87 % of the gut fullness. On the morning of the 25 August the average fish in cage 1 had 28 % of the previous meal remaining and this constituted 38 % of the gut fullness after the first meal of the day had been fed. Therefore 62 % of the gut fullness resulted from the new meal. In cage 2 on average only 14 % of the meal consumed the night before remained, this contributed to 21 % of the gut fullness after the first meal of the day. Therefore 79 % of the average gut fullness resulted from the meal just consumed. On the afternoon of the 26 August the average fish in cage 1 had 31 % of the morning meal

Table 7.4 : Mean evacuation rates (g/hour) for fish fed both morning and afternoon

Cage No.	Day		
	25.08		26.08
	0900h-1900h	1900h-0900h	0900h-1900h
1	0.870	1.980	1.126
2	1.221	1.706	0.954

remaining in their gut, this contributed 12 % of the gut fullness after the second meal of the day was consumed. Therefore 88 % of the average gut fullness was due to the meal just consumed. Lastly in cage 2 the average fish had 28 % of the previous meal remaining in their gut, this contributed 11 % of the average gut fullness after the last meal of the day was consumed. Therefore 89 % of the gut fullness was due to the meal fed in the afternoon.

7.3.3 Comparison of single and multiple meal size fed on the same day and at the same time

For the morning of the 25th August two sample t-tests showed there were no significant differences (Table 7.5) between meal size irrespective of whether it was the only meal of the day or if it was one of two meals (Fig. 7.4). This was also observed for the afternoon samples, there were no significant differences (Table 7.5) between the size of the meal regardless of it being the only meal of the day or the second one (Fig. 7.4). On the 26th August however, the mean meal sizes in the morning were significantly greater ($p<0.001$, Table 7.5) for the single meal of the day than for the first of two meals (Fig. 7.4). Similarly the single afternoon meal was significantly greater ($p<0.01$, Table 7.5) than the size of the second meal of the day (fed in the afternoon).

Table 7.5: Summary of significant differences of gut fullness for fish fed single meals compared to multiple meals

Day	Time Fed	Time Sampled	Cage No.	Significant Differences
25.08	Morning	Morning	1	N.S.
	Morn/After	Morning	1	
	Morning	Morning	2	N.S.
	Morn/After	Morning	2	
	Afternoon	Afternoon	1	N.S.
	Morn/After	Afternoon	1	
	Afternoon	Afternoon	2	N.S.
	Morn/After	Afternoon	2	
26.08	Morning	Morning	1 & 2	***
	Morn/After	Morning	1 & 2	
	Afternoon	Afternoon	1 & 2	**
	Morn/After	Afternoon	1 & 2	

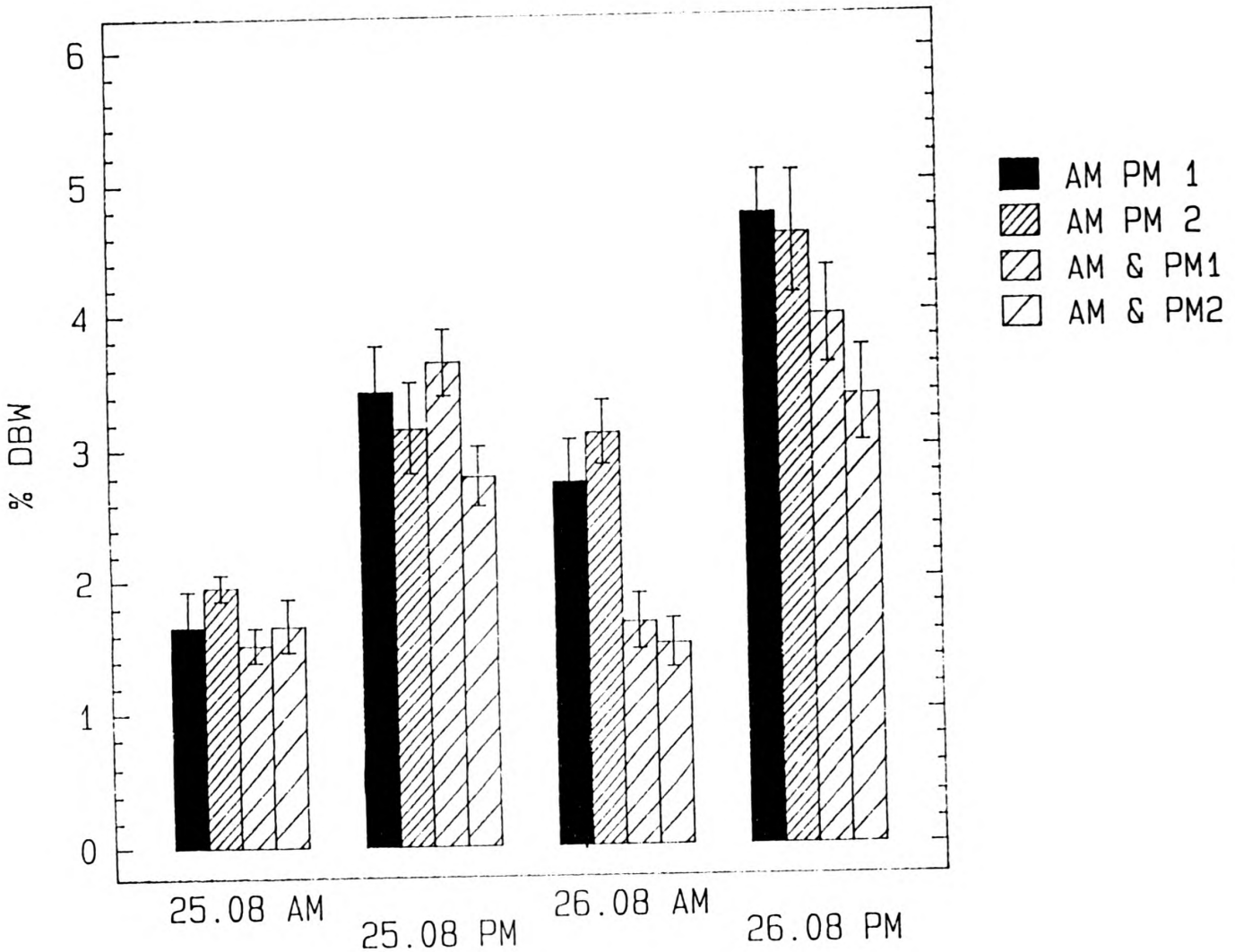
N.S. - Not Significant

* - $p<0.05$

** - $p<0.01$

*** - $p<0.001$

Figure 7.4: The mean meal size for fish fed both once or twice daily for days and for replicate cages. The shaded area indicates mean meal size for fish fed either morning or afteroon only for cage 1, the heavily shaded area shows mean meal size for fish fed either morning or afternoon for cage 2, the lightly shaded area shows mean meal size for fish fed twice daily for cage 1 and lastly the very lightly shaded area shows mean meal size for fish fed twice daily for cage 2.


6
5
4
3
2
1
0
% DBW
25.08 AM
25.08 PM
26.08 AM
26.08 PM
AM PM 1
AM PM 2
AM & PM1
AM & PM2

7.4 DISCUSSION

Of the fish fed only once per day, those fed in the afternoon took significantly larger meals. This was consistent with the previous findings that foregut fullness of Atlantic salmon peaked in late afternoon during the summer both in freshwater tanks (Chapter 3) and in sea water cages (Chapter 4). This peak has been linked to light cycles experienced under both natural ambient light and simulated ambient photoperiod and light regimes (constantly changing light levels) (Chapter 6).

Fish fed in the morning only consumed half that of fish fed in the afternoon only, although they were fed the same size ration. Since the food that is unaccounted for does not appear in the fish it must be lost from the cage. Therefore it appears that time of day of feeding is important in reducing feed loss. This need has been previously highlighted by both Eriksson & Alanara (1992) and Seymour & Bergheim (1991). Observations on marine cage farms which produce salmon commercially estimate feed loss to be as high as 19%. When figures were broken down, 1.4 % of the wastage was attributed to food delivered by hand-feeding and 40.5% was from food dispensed from an automatic feeder (Thorpe et al, 1990b).

The emptying of a meal from a fish stomach has been shown to be influenced by number of factors. In this study

evacuation rates differed between multiple meals consumed at different times of the day. One identified factor is temperature which shows a negative correlation with gastric emptying time (reviewed by Fange & Grove, 1978). In this study however temperature was reported as remaining relatively stable throughout sampling. Another factor that has been reported is the interaction between meals. This has been shown in several fish species, for example, in the, perch, Perca fluviatilis, roach, Rutilus rutilus, (Persson, 1984), Atlantic salmon (Talbot et al, 1984) and coho salmon, Oncorhynchus kisutch, (Ruggerone 1989). However, in these studies the time between meals was relatively long, Ruggerone (1989) fed meals two hours apart whilst Talbot et al (1984) fed continuously, only identifying a three hour period with labelled food. During our study, since so little of a residue meal was remaining when a new meal was fed, it is unlikely that interaction between meals could account for the differing evacuation rates. Another factor which affects evacuation rates is meal size, an increase in meal size prolonging the time required to empty the stomach, but increasing the actual evacuation rate (Flowerdew & Grove, 1979). In the current study, the afternoon meals were larger and the evacuation rates higher, indicating that meal size did affect evacuation rate. It would be interesting to observe the evacuation rate of the same meal size over differing times of the day to see if this is also an influence. Although we have data for similar meal sizes,

i.e. fish fed morning and afternoon for replicate 2 (2.795 %DBW) and fish fed morning only for replicate 1 (2.745 %DBW), we only have evacuation data for fish fed twice daily. Fish fed once daily were only sampled once and complete evacuation had taken place in that time.

Brett (1971) found that in sockeye salmon, O. nerka, the relationship between voluntary food intake and the length of time of food deprivation was sigmoidal, reaching a plateau after 25-30 hrs of deprivation.

For the single meals the period of deprivation was 20 hrs, for fish fed morning or afternoon only, therefore meal size should have been similar for these two periods. However, the morning meal size was consistently smaller. For the multiple meals the period between the finish of the morning feed and the onset of the afternoon feed was 6 hours, but the period between the finish of the afternoon feed and the onset of the morning feed was 10 hours. However the morning meal size was consistently smaller despite the longer period of food deprivation.

Lastly it would appear logical that meal size is in proportion to ration size. However the ration size for each single meal was double that of each of the multiple meals, but meals fed at the same time of day never reflected this difference. The termination of the feeding bouts may have been due to a full stomach (Toates, 1981; Holmgren et al,

1983; Knight, 1985). However, the stomachs of the fish sampled never appeared over-distended (on the x-ray plates) and they frequently appeared less than full. Atlantic salmon in a Newfoundland River were rarely found to have their stomachs above half full, although brook trout, Salvelinus fontinalis, from the same river frequently registered a full stomach (competition for food was reduced by spatial segregation) (Thonney & Gibson, 1989). It appears that in salmon under normal feeding conditions (i.e. no prolonged bouts of food deprivation), in the summer, feeding is not regulated by stomach fullness.

Since all cages were fed ultimately the same amount of food per day, the cages fed twice a day can account for more of the ration in terms of food consumption and must have experienced a reduced food loss. From these results therefore, it appears that if a single meal is to be fed this should be in the afternoon. However, if the resources are available two meals should be offered, the size of the afternoon one twice that of the morning. This feeding regime should increase food consumption and therefore reduce food deposition on the sea bed.

7.5 SUMMARY

1. When fed a single daily meal, Atlantic salmon held in sea cages ate more if fed in the afternoon rather than in the morning.

2. Fish fed twice a day took more food during the afternoon meal.

3. Meal size was influenced by time of day rather than consumption of previous meals.

4. Time of day of feeding also overrode the effect of length of food deprivation in influencing meal size.

5. Stomach fullness is not the sole regulatory factor of feeding in Atlantic salmon in the summer.

6. Multiple meals resulted in more food consumption and therefore reduced feed loss, although priority should be given to the second meal within a 24 hour period.

7. Evacuation rates differed with regard to meal size.

CHAPTER 8

GENERAL DISCUSSION

8.1 RHYTHMIC STOMACH FULLNESS 8.1.1 Fresh water

8.1.2 Sea water

8.2 SEASONAL EFFECTS

8.3 INFLUENCE OF TEMPERATURE

8.4 INFLUENCE OF LIGHT AND PHOTOPERIOD

8.5 TEMPORAL DIFFERENCES

8.6 IMPORTANCE FOR WILD FISH POPULATIONS

8.7 IMPORTANCE FOR COMMERCIAL PRODUCTION

8.1 RHYTHMIC STOMACH FULLNESS

Most organisms, including fish, do not feed constantly but exhibit a "circadian-like" prandial pattern (Boujard & Leatherland 1992). This feeding behaviour is not a random process but predictable and periodic (Armstrong, 1980).

Prandial periodicity can be detected using careful monitoring. Since feeding involves movement, whether to "snatch" at a passing prey item or to search actively for food, the recorded movements of a fish (i.e. the activity rhythms) are often taken as an indication of feeding activity (Boujard & Leatherland, 1992). These activity rhythms, although useful, often lead to misleading conclusions. For example, for Alosa pseudoharengus swimming activity is diurnal (Richkus & Winn, 1979), feeding however, is nocturnal (Kohler & Ney, 1980). Also, although Eriksson (1978) observed both diurnal and nocturnal activity in the same population of Ictalurus nebulosus, feeding took place mainly at dusk and dawn. A more direct monitoring approach, such as the one used in the present experiments - x-radiography, gives an indication of the feeding pattern by establishing the rhythmic stomach fullness of the fish.

Feeding patterns are subject to several variables the first of which is the type of water.

8.1.1 Fresh water

The variation of feeding patterns or patterns of stomach fullness, have been recorded for several fish species in fresh water in the wild. Coho salmon, Oncorhynchus kisutch, from a small stream in Owego County, New York, USA, showed a peak of feeding between 1600h-2400h whilst steelhead, O. mykiss, from the same river, showed a peak between 1200h-2000h (Johnson & Johnson, 1981). The steelhead's diet composition was relatively uniform over a 24 hr period, whilst that of the coho salmon varied between 4 hr samples (Johnson & Johnson, 1981). The diets of the two species rarely overlapped and the abundance of their preferred diets at specific times of the day appear to account for the varability of the two feeding patterns.

Hoar (1942) studied Atlantic salmon held in wooden boxes in the Moser River, Nova Scotia, in August, and found that little food was taken between 2200h and 0500h, as in the present study. In Hoar's study the salmon had 2 periods of active feeding while in the present study only one peak of stomach fullness occurred during the summer. The salmon were kept hungry in Hoar's study "to keep the physiological factor of hunger relatively constant", and this may have accounted for the extra peak of feeding activity after 0500h "when it is getting quite light". The fish in the present study were fed in excess of their daily requirements so any peaks in gut fullness were due to natural feeding rhythms

and not enforced by food shortage.

8.1.2 Sea water

The feeding rhythms of fish in sea water in the wild, may differ from those in fresh water due to the differences in flow patterns. Also, the method of obtaining food differs, namely active searching as opposed to sit-and-wait tactics. Also, more mature fish require increased amounts of prey. Some Scottish Atlantic salmon travel considerable distances into the Norwegian or Greenland seas to feed before returning to their natal rivers to spawn. As there are relatively few studies on wild Atlantic salmon during this part of their life history, we must rely on salmon held in sea water cages and tanks for information on their feeding behaviour. Karpenko (1982) studying chum, Oncorhynchus keta, and pink salmon, O. gorbuscha in the mouth of the Anapka River, Russia, concluded that - "young salmon in the initial period of their marine life [in the wild] may have various periods of active feeding per day, determined by environmental conditions". Karpenko referred to the food availability which varies not only daily but also between regions and also between temperatures and tidal flows. Farmed salmon, due to their captivity are not so greatly influenced by these parameters, but given the opportunity to feed naturally they may reflect these patterns, since it was under these influences that the feeding rhythms originally evolved. Farmed fish may feed under the feeding regimes

imposed upon them in captivity, however their hormonal and physiological functions may not adapt as easily. This may result in less than efficient food utilization and growth may be limited (Eriksson & Alanara, 1992).

To achieve optimum growth, it is desirable to establish what are the natural feeding rhythms for the fish species being cultured. Kadri et al (1991) recorded a peak in appetite in both the morning and afternoon for Atlantic salmon in sea cages. In the present study a peak in rhythmic stomach fullness was recorded in the late afternoon but not in the morning (Chapter 4). The fish in Kadri's study were not fed at night, thus this initial peak may be a reaction to the starvation period, since it has now been established that salmon may attempt to feed at night (Fraser et al, 1993). In summer, the feeding pattern for salmon held in sea cages was similar to that for fish in fresh water in tanks. This may have been due to the fish being newly transferred to sea water. Fish of similar age in the wild would only just have left the river and might not yet have developed new feeding patterns.

8.2 SEASONAL EFFECTS

It is unlikely that a single feeding rhythm would meet the changing requirements of a single fish species, since feeding is influenced by a complex interaction of both biotic and abiotic variables (Brodeur, 1992) whose ability

to influence feeding changes seasonally. Therefore the feeding patterns or stomach fullness levels of fish need to be monitored each season, to give a clear indication of the rhythm. This also helps to establish which variables are influential and their roles throughout the year. The temporal feeding patterns for brook trout, Salvelinus fontinalis, in a mountain stream, Colorado, were monitored seasonally (Allan, 1981). In June and July feeding was greatest in early evening, shifting to late afternoon by August, becoming aperiodic in early September and maximal at midday in late September. Feeding near dusk shifted to during the day, also in the arctic charr, Salvelinus alpinus, held in tanks at the University of Tromso, Norway (Jorgensen & Jobling, 1989). As in the present study x-radiography was used to monitor stomach fullness. Arctic char showed a significant increase in food intake during late afternoon/early evening during spring, autumn and winter. The pattern differed in the summer when intake increased at midday with a small rise in the evening, again showing a seasonal change in the temporal feeding pattern. The pattern of stomach fullness for Atlantic salmon throughout the year differed from both of the above species (Chapter 3). In the present experiments, also carried out in fresh water, the salmon showed a peak of stomach fullness in late afternoon in the summer. In autumn and winter there were no significant diel changes in stomach

fullness. Then in the spring again a peak was observed in late afternoon. Thus, preferential periods of feeding were only apparent in spring and summer. The amount of food taken decreased from summer through autumn to reach the lowest levels during winter before again rising during spring.

8.3 INFLUENCE OF TEMPERATURE

It is difficult to isolate and monitor the influence of temperature on feeding. Grove *et al* (1978) reported that rainbow trout, *Oncorhynchus mykiss*, consumed food in relation to their body weight and that intake increased with temperature. Seymour (1989) found similar relationships for eels, *Anguilla anguilla*, as did Farmer *et al* (1983) for Atlantic salmon. As temperature increases so does evacuation rate, permitting further intake (Brett & Higgs, 1970; Flowerdew & Grove, 1979; Persson, 1982; Kaushik, 1986).

In five experiments Atlantic salmon grew better under rhythmically alternating water temperature than under constant conditions (Berg *et al*, 1990), as did rainbow trout (Hokanson *et al*, 1977). Therefore the effect of temperature must be evaluated by altering it from ambient without removing all the fluctuations. In the present experiments temperature synchronized feeding peaks when light was constant (i.e. no periods of darkness), but also when light only changed for short periods during dawn and dusk. Earlier experiments (Rawlings, 1989) suggested that

temperature had a secondary effect on synchronizing the timing of a peak in stomach fullness and was not apparent when fish were held under ambient light. This observation on the role of temperature is supported by others, for example in subarctic areas (e.g. River Kaltisjokk, Northern Sweden), water temperature was constant ($^{+}/_{-}$ 0.4 ^{o}C) from November to May, but several fish species showed distinct changes between activity and rest indicative of feeding bouts (Muller, 1978a). Thus temperature is considered to be of secondary importance determining the amount of activity but not the phase positioning of that activity (Muller, 1978a). Further Nelson et al, (1975) noted that "fluctuations in environmental temperature had only a secondary effect". Additional experiments investigating the effects of light and photoperiod (Chapter 6, and below) yielded results consistent with these hypotheses.

8.4 INFLUENCE OF LIGHT AND PHOTOPERIOD

Most fish species are nocturnal, diurnal or crepuscular in their activities (Harker 1964). This suggests light or photoperiod is one of the most important environmental variables influencing these activities. It is difficult to assess which feature of the day-night cycle may be important, particularly in the rhythmic stomach fullness of Atlantic salmon. The two major factors are light intensity and its variation, and absolute day-length.

The initial experiment (Chapter 5), concluded light was not of primary importance in the timing of peaks in stomach fullness when light was constant throughout 24 hours or constant between simulated dawn and dusk. Under both conditions light did not synchronize feeding rhythms.

The effect of excluding a dark period resulting in different feeding rhythms has been observed with other species. The dab, Limanda limanda, exhibits different feeding rhythms when held under constant light as opposed to ambient light (Gwyther & Grove, 1981), thus indicating that the fish require either periods of darkness or constantly changing light levels to synchronize its feeding and therefore to exhibit the feeding rhythms observed for those fish held under ambient light. Krakenes et al, (1991) held Atlantic salmon post-smolts in sea cages under two light regimes, natural light and continuous light (additional light from halogen lamps) with two feeding regimes - either unrestricted food or food only during daylight. Therefore both cages experienced changing light levels during daylight, but the cage with additional light never experienced total darkness only a drop in light levels to a minimum of 5 lux at the bottom of the cage. No difference in growth rate for the two feeding regimes was noted, suggesting an extended period of available food does not result in an increased growth rate. Either because additional food is not taken during the extra period,

therefore the feeding rhythm is unchanged or that food is consumed during this period but results in little extra growth. If it is the former, then feeding rhythms are synchronized by the changing light levels and not the presence or absence of a period of darkness. However, since this study did not monitor feeding rhythms this cannot be a conclusion only a supposition. Growth rate and grilsing were highest in fish held under continuous light, indicating that the change in photoperiod (i.e. the absence of a period of darkness) affected maturation as described by the model put forward by Thorpe (1986, 1989). This was confirmed by Hansen et al (1992), Atlantic salmon held under additional light had an increased growth rate indicating a seasonal growth pattern influenced by photoperiod.

To investigate further the effect of light on feeding patterns, additional experiments were carried out (Chapter 6). These indicated that fish under natural light and those under simulated light and photoperiod showed the same patterns of rhythmic stomach fullness. This confirmed the results of the initial experiment that a particular number of hours of daylight alone were insufficient to synchronize feeding patterns. Harker (1964) was of the same opinion: "phases of a rhythm are by no means set by the change from absolute darkness to light" although "very few experiments use slowly changing light intensites". The absolute levels above a certain light intensity do not seem to be important

in feeding, since natural light levels exceeded at least twice those obtained under the simulated light conditions, and stomach fullness levels were similar for all light regimes. Stefansson et al (1990) found light intensity did not affect growth rates of Atlantic salmon, although these intensities were at constant levels.

8.5 TEMPORAL DIFFERENCES

Since Atlantic salmon show feeding patterns, feeding them at different times of the day will result in different responses. As in other organisms, salmon physiology is dielly rhythmic (Spieler, 1977), it is logical to assume that peaks in feeding will coincide with the best physiological state to receive and process the food. For example, in channel catfish (Ictalurus punctatus) feeding schedule is important in determining the metabolic fate of nutrients (Noeske- Hallin et al, 1985). It is unlikely therefore that food will be taken at a time that will result in an unwanted outcome, for instance growth at the wrong time of the year. Therefore if fish are offered discrete meals more will be eaten at those coinciding with favourable physiological conditions, than at those outwith these periods, regardless of the number of meals offered. In the present study Atlantic salmon postsmolts fed only in the afternoon showed greater stomach fullness than those fed

only in the morning and when fed twice daily the afternoon meal was always larger than the morning meal (Chapter 7). Goldfish, Carassius auratus, also show differences in the amount of food taken depending on the time of day of feeding (Noeske et al, 1981). This indicates that by offering food at peaks of the feeding rhythm shown by the patterns of rhythmic stomach fullness more food is consumed and therefore less food falls as uneaten waste to the seabed.

8.6 IMPORTANCE FOR WILD FISH POPULATIONS

Many salmonids occupy two types of habitats in the wild, running water of burns and rivers and still water of lochs and seas. In running water they may defend their chosen territory and select food as it enters their domain, whilst in still water they forage for prey. Potential prey is seen in organic drift which is either dispersed throughout the water column or is trapped in the surface film. The diel and seasonal occurrence of organic drift is relatively predictable, and its abundance depends on productivity of individual habitats (Eriksson & Alanara, 1992). During summer large drift items are relatively abundant, and increasingly available from late afternoon towards dusk as summer progresses. During autumn smaller drift items are available and the food for young salmonids is made up of sub-optimally sized items, as in winter, although their abundance is much reduced. Again in spring, large items

occur in the drift although reduced in abundance compared to summer. Surface drift is readily available in summer and to a lesser extent in autumn (Muller, 1978a). In the present experiments, peaks of gut fullness were observed in late afternoon only in summer and spring in fresh water (Chapter 3). This corresponds to the occurence of large prey items in the organic drift and to the presence of surface drift. In autumn and winter no significant peaks in foregut fullness could be observed in Atlantic salmon. This corresponds to periods in the wild when only small drift items are available and therefore when feeding would need to be frequent to meet maintenance requirements. Therefore feeding patterns of Atlantic salmon held in freshwater tanks corresponds to availability of prey of different sizes in the wild. In still water (i.e. lochs) the situation is some what different since salmon search for prey more actively where as in running water potential prey often comes to the fish. Due to their habitats fish in still water are also limited in both spatial and temporal access to their potential prey. As the fish used in our experiments orignated from stock taken from the river Almond and are held in tanks with a recordable water flow the situation encountered in running water would seem more relevant.

8.7 IMPORTANCE TO COMMERCIAL PRODUCTION OF ATLANTIC SALMON

Salmon culture relies on a good knowledge of basic salmonid behaviour and of the fishes reponses to different

environmental conditions and management techniques. Salmon have evolved under constraints imposed by their natural habitat, and are now expected to develop as well if not better, under a second set of constraints imposed by man. The present work has shown that if innate feeding patterns are copied in production environments, the meal sizes taken are substantially larger than if these patterns are ignored. Hence if more food is eaten by the fish then less must fall uneaten to the sea bed. This was supported by Seymour (1991) who wrote "immediate reduction in feed wastage could be made by presenting feed when the fish can use it most efficiently". Many feeding tables have been produced, not just for Atlantic salmon but for several fish species, these recommend feeding rates for different weight of fish. This alters at different temperatures. However as this present research indicates temperature is not the major factor effecting food intake of fish. More importantly is the photoperiod, some tables give changes in recommended food intake depending on fish size, water temperature and month. However, the month is often the parameter ignored or taken least notice of, proably because its significant in relation to photoperiod and food intake has not been well illustrated. Most problems occur in indoor production units where artifical lighting is used. In these situations not only is the light on for a relatively constant length of time throughout the year, but often no sunset or

sunrise is observed or if there is then this is the only period of changing light levels throughout the day. This will result in an altered feeding rhythm as shown in Chapter 6. Feeding guides should be produced taking into account these points and production manager should be made aware of the effects lighting regimes may have on the feeding behaviour of the fish. Since feeding behaviour is not a random process but predictable and periodic (Armstrong, 1980) we should use this information to increase the amount of food consumed by the fish at the most efficient times, thereby increasing the efficiency of production and reducing the impact of fish farming on the environment by reducing pollution of the sea bed by uneaten waste food.

REFERENCES

Ali, M. A. (1961). Histophysiological studies on the juvenile Atlantic salmon (Salmo salar) retina. II Responses to light intensities, wavelengths, temperature and continuous light and dark. Can. J. Zool. 39, 511-526.

Allan, I. R. H. & Ritter, J. A. (1975). Salmonid terminology. J. Cons. int. Explor. Mer. 37, 293-299.

Allan, J. D. (1978). Trout predation and the size composition of stream drift. Limnol. Oceanogr. 23, 1231-1237.

Allan J. D. (1981). Determinants of diet of brook trout (Salvelinus fontinalis) in a mountain stream. Can. J. Fish. Aquat. Sci. 38, 184-192.

Allen, D. M., Loew, D. M. & McFarland, W. N. (1982). Seasonal change in the amount of visual pigment in the retinae of fish. Can. J. Zool. 60, 281-287.

Armstrong, S. (1980). A chronometric approach to the study of feeding behaviour. Neurosci. and Biobehav. Rev. 4, 27-53.

Aschoff, J. (1960). Exogenous and endogenous components in circadian rhythms. In Biological Clocks. Cold Spring Harbour Symp. Quant. Biol. 11-28.

Axelrod, J. (1974). The pineal gland: A neurochemical transducer. Science. 184, 1341-1384.

Baggerman, B. (1980). Photoperiodic and endogenous control of the annual reproductive cycle in teleost fishes. In

Environmental Physiology of Fishes (Ali, M. A. ed.) pp. 533-567. New York, Plenum Press.

Berg, O. K., Finstand, B., Grande, G. & Wathne, E. (1990). Growth of Atlantic salmon (Salmo salar L.) in a variable diel temperature regime. Aquaculture. 90, 261-266.

Beukema, J. J. (1968). Predation by the three-spined stickleback (Gasterosteus aculeatus): The influence of hunger and experience. Behaviour 31, 1-126.

Blyth, P. J., Purser, G. J. & Russell, J. F. (1993). Detection of feeding rhythms in seacaged Atlantic salmon using new feeder technology. In 1st Int. Fish Farming Tech. Conf. Trondheim Aug. '93.

Boujard, T., Keith, P. & Luquet, P. (1990). Diel cycle in Hoplosternum littorale (Teleostei): evidence for synchronization of locomotor, air breathing and feeding activity by circadian alternation of light and dark. J. Fish Biol. 36, 133-140.

Boujard, T., Moreau M. & Luquet P. (1991). Entrainment of the circadian rhythm of food demand by infradian cycles of light-dark alternation in Hoplosternum littorale (Teleostei). Aquat. Living Resour. 4, 221-225.

Boujard, T. & Leatherland, J. F. (1992). Circadian rhythms and feeding time in fishes. Env. Biol. of Fish. 35, 109-131.

Braaten, B., Aure, J., Ervik, A. & Boge, E. (1983). Pollution problems in Norwegian fish farming. ICES, CM 1983/F:26. 1-12.

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

Brett, J. R. (1979). Environmental factors and growth. In Fish Physiology. (Hoar, W. S., Randall, D. J. & Brett, J. R. ed.). Academic Press, New York, San Franciso and London. 8, 599-675.

Brett, J. R. & Higgs, D. A. (1970). Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Bd. Canada 27, 1767-1779.

Brodeur, R. D. (1992). Factors related to variability in feeding intensity of juvenile coho salmon and chinook salmon. Trans. Am. Fish. Soc. 121, 104-114.

Brodeur, R. D. & Pearcy, W. G. (1987). Diel feeding chronology, gastric evacuation and estimated daily ration of juvenile coho salmon, Oncorhynchus kisutch (Walbaum), in the coastal marine environment. J. Fish Biol. 31, 465-477.

Brown, J. R., Gowen, R. J. & McLusky, D.S. (1987). The effects of salmon farming on the benthos of a Scottish sea loch. J. Exp. Mar. Biol. and Ecol. 109, 39-51.

Bunning, E. (1960). Circadian rhythms and the time measurement in photoperiodism. Cold Spring Harbour Symp. Quant. Biol. 25, 1-9.

Bunning, E. (1967). The physiological clock. 2nd rev. ed. Springer Berlin.

Campbell, R. C. (1989). Statistics for biologists. University Press, Cambridge. 3rd ed.

Chaston, I. (1968). Influence of light on activity of brown trout (Salmo trutta). J. Fish. Res. Bd. Can. 25 (6), 1285-1289.

Clarke, W. C., Shelbourn, J. E. & Brett, J. R. (1978). Growth and adaptation to sea water in 'underyearling' sockeye (Oncorhynchus nerka) and coho (O. kisutch) salmon subjected to regimes of constant or changing temperature and day length. Can. J. Zool. 56, 2413-2421.

Cloudsley-Thompson, J. L. (1961). Rhythmic Activity in animal physiology and behaviour. Academic Press. New York.

Cohn, C. & Joseph, D. (1960). Role of rate of ingestion of diet on regulation of intermediary metabolism ("Meal eating" vs. "Nibbling"). Metabolism. 9, 492-500.

Eriksson, L. -O. (1978). Nocturnalism versus diurnalism; dualism within fish individuals. In Rhythmic Activity of Fishes. (Thorpe, J. E. eds.) 69-90. Academic Press, London.

Eriksson, L.-O. & Alanara, A. (1992). Timing of feeding behaviour of salmonids. In The Importance of Feeding Behaviour for the Efficient Culture of Salmonid Fishes. (Thorpe, J. E. & Huntingford, F. A. eds.) World Aquac. Workshops 2, 41-48.

Eriksson, L.-O. & Lundqvist, H. (1982). Circannual rhythms

and photoperiod regulation of growth and smolting in baltic salmon (Salmo salar L.). Aquaculture. 28, 113-121.

Eriksson, L.-O. & Veen, T. V. (1980). Circadian rhythms in the brown bullhead, Ictalurus nebulosus (Teleostei). Evidence for an endogenous rhythm in feeding, locomotor, and reaction time behaviour. Can. J. Zool. 58, 1899-1907.

Fange, R. & Grove, D. (1979). Digestion. In Fish Physiology (Hoar, W. S., Randall, D.J. & Brett, J.R. ed.) . VIII. 163-260. Academic Press, New York.

Farmer, G. J., Ashfield, D. & Goff, T. R. (1983). A feeding guide for juvenile Atlantic salmon. Can. Man. Rep. Fish. Aquat. Sci. 1718, 1-13.

Farner, D. S. (1970). Predictive functions in the control of annual cycles. Envir. Res. 3, 119-131.

Fauconneau, B., Choubert, G., Blanc, D., Breque, J. & Luquet, P. (1983). Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. Aquaculture. 34, 27-39.

Fayette, A. R. & Haines, T. A. (1980). Effects of chronic exposure to hydrogen sulphideon newly hatched brown trout Salmo trutta. Envir. Pollu. 22 (A), 11-17.

Flowerdew, M. W. & 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. J. Fish Biol. 14, 229-238.

Fraser, N. H. C., Metcalfe, N. B. & Thorpe, J. E. (1993). Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. Roy. Soc. 252, 135-139.

Furnell, D. (1990). THe feed squeeze - How to make every pellet pay. Can. Aquac. 6, 37-39.

Gibson, R. J. & Keenleyside, M. H. A. (1966). Responses to light of young Atlantic salmon (Salmo salar) and brook trout (Salvelinus fontinalis). J. Fish. Res. Bd. Can. 23 1007-1021.

Godin, J.-G. J. (1981). Daily patterns of feeding behaviour, daily rations, and diets of juvenile coho salmon (Oncorhynchus gorbuscha) in two marine bays in British Columbia. Can. J. Fish. Aquat. Sci. 38, 10-15.

Gowen, R. J. & McLusky, D. S. (1988). How farms affect their surroundings. Fish Farmer. Sept/Oct. 33-34.

Gowen, R. J., Brown, J. R. & McLusky, D. S. (1988). Investigations into benthic fauna hypernutrification and eutrophication associated with mariculture in Scottish coastal waters. Dept. of Biol. Sci., University Stirling. 1-289.

Grove, D. J., Loizides, L. G. & Nott, J. (1978). Satiation amount, frequency of feeding and gastric emptying rate in Salmo gairdneri. J. Fish Biol. 12, 507-516.

Gwyther, D. & Grove, D. J. (1981). Gastric emptying in Limanda limanda and the return of appetite. J. Fish Biol. 18, 245-259.

Hansen, T., Stefansson, S. & Taranger, G. L. (1992). Growth and sexual maturation in Atlantic salmon, Salmo salar L., reared in sea cages at two different light regimes. Aquacult. Fish. Mgmt. 23, 275-280.

Harker, J. E. (1964). The environmental control of phase-timing. In The Physiology of Diurnal Rhythms (Bennet, T. A., Salt, G. & Wigglesworth V. B. ed.) Cambridge Monographs in Experiemntal Biology. 13, 6-18.

Hastings, W. H., Hinson, B., Tackett, D. & Simco, B. (1972). Monitoring channel catfish use of a demand feeder. Prog. Fish-Cult. 34, 204-206.

Higgins, P. J. (1985). Growth, feeding and metabolism in juvenile Atlantic salmon (Salmo salar L.). Ph.D. Thesis, University of Aberdeen.

Higgins, P. J. & Talbot, C. (1985). Growth and feeding in juvenile Atlantic salmon (Salmo salar L.). In Nutrition and feeding in fish. (Cowey, C. B., Mackie, A. M. & Bell, J.G. eds.) Academic Press, London. 243-263.

Hoar, W. S. (1942). Diurnal variation in feeding activity of salmon and trout. J. Fish. Res. Bd. Can. 6, 90-101.

Hoar, W. S. (1965). The endocrine system as a chemical link between the organism and its environment. Trans. Roy. Soc. Can. 3, 175-200.

Hokanson, K. E. F., Kleiner, C. F. & Thorslund, T. W.

(1977). Effects of constant temperature and diel temperature fluctuations on specific growth and mortality rates and yield of juvenile rainbow trout, Salmo gairdneri. J. Fish. Res. Bd. Canada 34, 639-648.

Holmgren, S., Grove, D. J. & Fletcher, D. J. (1983). Digestion and the control of gastrointestinal motility. In Control Processes in Fish Physiology (Rankin, R. C., Pitcher, T. J. & Duggan, R. T. eds.) 23-41. Croom Helm, London. 22-40.

Ingram, M. (1990). What price a 'green' farm? Fish Farmer Sept/Oct. 49-50.

Jacob, S. S. & Nair, N. I. S. (1983). Periodicity in feeding activity in the larvivorous fish Aplocheilus lineatus (Cuv. & Val.) and Macropodus cupanus (Cuv. & Val.). Proc. Indian Natn. Sci. Acad. 49B, 1-8.

Jenkins, B. W. & Green, J. M. (1977). A critique of field methology for determining fish feeding periodicity. Env. Biol. Fish. 1, 209-214

Jobling, M. (1984). Mythical models of gastric emptying and implications for food consumption studies. In Gut Shop '84. (Simenstad, C. A. & Cailliet, G. M. eds.) 35-50. Dordrecht, Dr W. Junk Publishers.

Jobling, M. (1987). Gowth of arctic charr (Salvelinus alpinus L.) under conditions of constant light and temperature. Aquaculture. 60, 243-249.

Johnson, J. H. & Johnson, E. Z. (1981). Feeding periodicity

and diel variation in diet composition of subyearling coho salmon, Oncorhynchus kisutch, and steelhead , salmo gairdneri, in a small stream during summer. Fishery Bulletin 79, 370-376.

Jones, J. W. (1959). The salmon. Collins, London. 1-192.

Jorgensen, E. H. & Jobling, M. (1989). Patterns of food intake in arctic charr, Salvelinus alpinus, monitored by radiography. Aquaculture. 81, 155-160.

Jorgensen, E. H. & Jobling, M. (1992). Feeding behaviour and effects of feeding regime on growth of Atlantic salmon, Salmo salar. Aquaculture. 101, 135-146.

Kadri, S., Metcalfe, N. B., Huntingford, F. A. & Thorpe, J. E. (1991). Daily feeding rhythms in Atlantic salmon in sea cages. Aquaculture. 92, 219-224.

Karpenko, V. I. (1982). Diurnal feeding rhythm of young salmon during the intial stage of marine life. J. Ichthyol. 22, 131-134.

Kaushik, S. J. (1986). Environmental effects on feed utilization. Fish Physiol. Biochem. 2, 131-140.

Kohler, C. C. & Ney, J. J. (1980). Piscivory in a land-locked alewife (Alosa pseudoharengus) population. Can. J. Fish. Aquat. Sci. 37, 1314-1317.

Knights, B. (1985). Feeding behaviour and fish culture. In Nutrition and Feeding in Fish. (Cowey, C. B., Mackie, A. M. & Bell, J. G. eds.) 223-241. Academic Press, London.

Krakenes, R., Hansen, T., Stefansson, S. O. & Taranger, G. L. (1991). Continuous light increases growth rate of

Atlantic salmon (Salmo salar L.) postsmolts in seacages. Aquaculture 95, 281-287.

Kreiberg, H. Withler, R. E. & Clarke, W. C. (1988). Early seawater growth of chinook salmon strains at rearing sites in British Columbia. Aquacult. Assoc. Can. Bull. 88 (4), 16-18.

Laird, L. & Needham, T. (eds.) (1988). Salmon and Trout Farming. Ellis Horwood Ltd, Chichester.

Landless, P. J. (1976). Demand-feeding behaviour of rainbow trout. Aquaculture. 7, 11-25.

Lees, A. D. (1966). Photoperiodic timing in insects. Nature 210, 986-989.

Malloch, P. D. (1975). Life-history and habits of the salmon, sea-trout, trout and other freshwater fishes. P. D. Malloch. Perth.

Manteifel, B. P., Girsa, I. I. & Pavlov, D. S. (1978). On rhythms of fish behaviour. In Rhythmic Activity Of Fishes. (Thorpe, J. E. ed.) 215-224. Academic Press, London. 215-224.

Meier, A. H., Trobec, T. N., Haymaker, H. G., Macgregor, R. & Russo, A. C. (1973). Daily variations in the effects of handling on fat storage and testicular weights of several vertebrates. J. of Exper. Zool. 184, 281-288.

Metcalfe, N. B., Huntingford, F. A. & Thorpe, J. E. (1986). Seasonal changes in the feeding motivation of juvenile Atlantic salmon (Salmo salar). Can. J. Zool. 64 (11),

2439-2446.

Metcalfe, N. B., Huntungford, F. A. & Thorpe, J. E. (1987). Feeding intensity, growth rates, and the establishment of life-history patterns in juvenile Atlantic salmon Salmo salar. J. Anim. Ecol. 57, 463-474.

Meyer, J. H. (1980). Gastric emptying of ordinary food: effect of antrum on particle size. Amer. J. Physiol. 239, G133-G135.

Mills, D. & Graeser, N. (1981). The salmon rivers of Scotland. Cassel, London. 1-339.

Mills, D. H. & Piggins, D. J. (1988). Atlantic salmon: planning for the future. London, Croom helm.

Minaur, J. (1973). Smolt rearing at Almondbank, Perthshire. J. Inst. Fish. Mgmt. 4, 65-68.

Minors, D. S. & Waterhouse, J. M. (1986). Circadian rhythms and their mechanisms. Experentia. 42, 1-108.

Molin, B. M., Perez, E., Pupier, R. & Buisson, B. (1990). Entrainment of the circadian activity rhythm in the juvenile trout, Salmo trutta L., by red light. J. Interdisc. Cycle Res. 21, 81-89.

Monk, T. H. (1982). Research methods of Chronobiology. In Biological Rhythms, Sleep and Performance. (Webb, W. B. ed.) 27-57. John Wiley & Sons, New York.

Moore, R. Y. & Card, J. P. (1985). Visual pathways and the entrainment of circadian systems. In Medical and Biological Effects of Light (Wurtman, R. J., Baum, M. J. & Potts, J. T. Jr. eds.) pp. 123-133. New York, New York

Academy of Sciences.

Moore-Ede, M. C. (1981). Light: An information source for circadian clocks. Photochem. Photobiol. 34, 237-238.

Muller, K. (1978a). Locomotor activity of fish and environmental oscillations. In Rhythmic Activity of Fishes. (Thorpe, J. E. ed.) 1-19. Academic Press, London.

Muller, K. (1978b). The flexibility of the circadian system of fish at different latitudes. In Rhythmic Activity of Fishes. (Thorpe, J. E. ed.) 91-104. Academic Press, London.

Muntz, W. R. A. & Wainwright, A. W. (1978). Annual cycles in the light environmnets and visual mechanisms of fishes. In Rhythmic Activity of Fishes. (Thorpe, J. E. ed.) Academic Press, London. 105-129.

Murphy, K. (1992). Is there madness in the feeding method ? Fish Farmer. May/June, 18 & 39.

Nelson, W., Cadotte, L. & Halberg, F. (1973). Circadian timing of single daily "meal" affects survival of mice. Proc. Soc. Exp. Biol. Med. 144, 766-769.

Nelson, W. L., Scheving, L. & Halberg F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. J. Nutr. 105, 171-184.

Netboy, A. (1980). Salmon: the worlds most harrassed fish. London, Deutsch. 1-304.

Noeske, T. A., Erickson, D. A. & Spieler, R. E. (1981). The

time-of-day goldfish receive a single daily meal affects growth. J. World Maricul. Soc. 12, 73-77.

Noeske, T. A. & Spieler, R. E. (1984). Circadian feeding time affects growth of fish. Trans. Am. Fish. Soc. 113, 540-544.

Noeske-Hallin, T. A., Spieler, R. E., Parker, N. C. & Suttle, M. A. (1985). Feeing time differentially affects fattening and growth of channel catfish. J. Nutr. 115, 1228-1232.

Palsson, J. O., Jobling, M. & Jorgensen, E. H. (1992). Temporal changs in daily food intake of arctic charr, Salvelinus alpinus L., of diffeent sizes monitored by radiography. Aquaculture 106, 51-56.

Pardo, R. J. & de Vlaming, V. L. (1976). In vivo and in vitro effects of prolactin on lipid metabolism in the cyprinid teleost Notemigous crysoleucas. Copeia 563-573.

Parker, N. C. (1984). Chronological approach to Aquaculture. Trans. Am. Fish. Soc. 113, 545-552.

Pavlov, D. S. & Kasumyan, A. O. (1990). Sensory principles of the feeding behaviour of fishes. J. of Ichthyology 30, 77-93.

Persson, L. (1984). Food evacuation and models for multiple meals in fishes. Env. Biol. Fish. 10, 305-309.

Philippens, K. M. H., von Mayersbach, H. & Scheving, E. (1977). Effects of the scheduling of meal-feeding at different phases of the circadian system in rats. J.

Nutr. 107, 176-193.

Rawlings, C. E. (1989). Feeding patterns of Atlantic salmon parr. MSc Thesis, Plmouth Polytechnic.

Rawlings, C. E., Talbot, C. Thorpe, J. E. & Bromage, N. B. (1991). Feeding rhythms of Atlantic salmon smolts (Salmo salar L.) in sea cages in summer. In Aquaculture and the Environment. EAS Spec. Publ. 14, 271-272.

Reiter, R. J. (1988). Comparative aspects of pineal melatonin rhythms in mammals. Animal and Plant Sci. 1, 111-116.

Reynolds, W. W. & Casterlin, M. E. (1980). The role of temperature in the environmental physiology of fishes. In Environmental Physiology of fishes. (Ali, M. A. ed.) 497-518. Plenum Press, New York and London.

Richkus, W. A. & Winn, H. E. (1979). Activty cycles of adult and juvenile alewifes, Alosa pseudoharengus, recorded by two methods. Trans. Nat. Acad. Sci. U.S.A. 75, 6276-6280.

Richardson, N. E. & McCleave, J. D. (1974). Locomotor activity rhythms of juvenile Atlantic salmon (Salmo salar) in various light conditions. Biol. Bull. 147, 422-432.

Rosenthal, J. H., Weston, D. P., Gowen, R. J. & Black, E. (1988). Report of the ad hoc stdy group on environmental impact of mariculture. ICES Coop. Res. Rep. 154, 1-81.

Ruggerone, G. T. (1989). Gastric evacuation of single and multiple meals by piscivorous coho salmon, Oncorhynchus

kisutch. Env. Biol. Fish. 26, 143-147.

Sagar, P. M. & Glova, G. J. (1988). Diel feeding periodicity, daily ration and prey selection of a riverine population of juvenile chinook salmon, Oncorhynchus tshawytscha (Walbaum). J. Fish Biol. 33, 643-653.

Saito, M. (1972). Daily rhythmic changes in brush border enzymes of the small intestine and kidney in rat. Biochem. Biophys. Acta. 286, 212-215.

Saunders, R. L. & Harmon, P. R. (1988). Extended daylength increases postsmolt growth of Atlantic salmon. World Aquac. 19, 72-73.

Saunders, R. L. & Henderson, E. B. (1970). Influence of photoperiod on smolt development and growth of Atlantic salmon (Salmo salar). J. Fish. Res. Bd. Can. 27, 1295-1311.

Saunders, R. L., Henderson, E. B. & Harmon, P. R. (1985). Effects of photoperiod on juvenile growth and smolting of Atlantic salmon and subsequent survival and growth in sea cages. Aquaculture. 45, 55-66.

Saunders, R. L., Henderson, E. B. & Harmon, P. R. (1987). Extended daylength during autumn enhances growth of juvenile Atlantic salmon. Proc. Ann. Meet. Aquacult. Assoc. Can. 1, 32-33.

Schwassmann, H. O. (1971). Biological rhythms. In Fish Physiology (Hoar, W. S. & Randall D. J., eds.) VI, 371-

428. New York: Academic Press.

Schwassmann, H. O. (1980). Biological rhythms: their adaptive significance. In Environmental Physiology of Fishes. (Ali, M. A. ed.) 613-630. Plenum Press, London.

Sedgwick, S. D. (1988). Salmon farming handbook. Dorset Press, Dorchester.

Seimen, M. J. & Carline, R. F. (1989). Effect of temperature cycles on growth of Atlantic salmon (Salmo salar). In Abstracts Fisheries Management Controversies: 119th Ann. Meet. Am. Fish. Soc. Anchorage, Alaska 4-8 Sept. 9.

Seymour, E. A. (1989). Devising optimum feeding regimes and temperatures for the warmwater culture of eel, Anguilla anguilla L. Aquac. and Fish. Mgt. 20, 311-323.

Seymour, E. A. & Bergheim, A. (1991). Towards a reduction of pollution from intensive aquaculture with reference to the farming of salmonids in Norway. Aquac. Eng. 10, 73-88.

Shrearer, W. S. (1992). The Atlantic Salmon: Natural History, Exploitation and Future Management. Fishing News, Oxford.

Simpson, A. (1993). Investigtion of the factors influencing maturation in Atlantic salmon, Salmo salar L., parr. Ph.D. Thesis, University of Glasgow.

Spieler, R. E., Meier, A. H. & Loesch, H. C. (1976). SEaonal variations in circadian levels of serum prolactin in stripped mullet, Mugil cephalus. General and Comparative Endocrinology 29,156-160.

Spieler, R. E. (1977). Diel and seasonal changes in response to stimuli: a plaque and a promise to mariculture. Proc. of the Eighth Ann. Meet. World Mariculture Soc. 865-882.

Spieler, R. E. (1979). Diel rhythms of circulating prolactin, cortisol, thyroxine, and triiodothyronine levels in fishes: A review. Rev. Can. Biol. 38 (4), 301-315.

Spieler, R. E. & Noeske, T. A. (1981). Timing of a single daily meal and diel variations of serum thyroxine, triiodothyronine and cortisolin goldfish, Carassius auratus. Life Sci. 28, 2939-2944.

Spieler, R. E. & Noeske, T. A. (1984). Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol and thyroxine in goldfish. Trans. Am. Fish. Soc. 113, 528-539.

Stefansson, S. O. & Hansen, T. J. (1989). The effect of spectral composition on growth and smolting in Atlantic salmon (Salmo salar) and subsequent growth in sea cages. Aquaculture. 82, 155-162.

Stefansson, S. O., Hansen, T. & Taranger, G. L. (1989). The influence of light on growth and smolting of Atlantic salmon (Salmo salar): effects of spectral composition, intensity and photoperiod. In Proceedings of Canada-Norway finfish aquaculture workshop. 11-14 Sept. (Saunders R. L. ed.) Can. Tech. Rep. Fish. Aquat. Sci. 1761, 79-84.

Sundararaj, B. I. & Vasal S. (1973). Photoperiodic regulation of reproductive cycles in the catfish, Heteropneustes fossilis (Bloch). In Endocrinology. 180-184. Proceedings of the IV International Congress of Endocrinology, Washington D.C. 1972. Amsterdam, Excerpta Medica.

Sundararaj, B. I. & Vasal S. (1976). Photoperiod and temperature control in the regulation of reproduction in the female catfish, Heteropneustes fossilis. Journal of the Fisheries Research Board of Canada. 33, 959-973.

Sundararaj, B. I., Nath, P. & Halberg, F. (1982). Circadian meal timing in relation to lighting schedule optimizes catfish body weight gain. J. Nutr. 12, 1085-1097.

Swift, D. R. (1962). Activity cycles in the brown trout (Salmo trutta Lin.) 1. Fish feeding naturally. Hydrobiologia. 20, 241-246.

Swift, D. R. (1964). Activity cycles in the brown trout (Salmo trutta L.) 2. Fish artificially fed. J. Fish. Res. Bd. Can. 21, 133-138.

Talbot, C. & Higgins, P. J. (1983). A radiographic method for feeding studies on fish using metallic iron powder as a marker. J. Fish Biol. 23, 211-220.

Talbot, C., Higgins, P. J. & Shanks, A. M. (1984). Effects of pre- and post-prandial starvation on meal size and evacuation rate of juvenile Atlantic salmon, Salmo salar L. J. Fish Biol. 25, 551-560.

Thonney, J.-P. & Gibson, R. J. (1989). Feeding strategies of

Brook trout, Salvelinus fontinalis, and juvenile Atlantic salmon, Salmo salar, in a Newfoundland river. Can. Field-Nat. 103, 48-56.

Thorpe, J. E. (1977). Daily ration of adult perch, Perca fluviatilis L. during summer in Loch Leven, Scotland. J. Fish Biol. 11, 55-68.

Thorpe, J. E. (1986). Age at first maturity in Atlantic salmon Salmo salar: freshwater period influences and conflicts with smolting. Can. Spec. Publ. Fish. Aquat. Sci. 89, 7-14.

Thorpe, J. E. & Huntingford, F. A. (eds.) (1992). The importance of feeding behaviour for the efficient culture of salmonid fishes. World Aquaculture Workshops 2.

Thorpe, J. E., Metcalfe, N. B. & Huntingford, J. A. (1992). Behavioural influences on life-history variation in juvenile Atlantic salmon Salmo salar. Environ. Biol. of Fishes. 33, 331-340.

Thorpe, J. E., Morgan, R. I. G., Pretswell, D. & Higgins, P. J. (1988). Movement rhythms in juvenile Atlantic salmon, Salmo salar L. J. Fish Biol. 33, 931-940.

Thorpe, J. E., Talbot, C., Miles, M. S. & Keay, D. S. (1990a). Control of maturation in cultured Atlantic salmon, Salmo salar, in pumped seawater tanks, by restricting food intake. Aquaculture. 86, 315-326.

Thorpe, J. E., Talbot, C., Miles, M. S., Rawlings, C. &

Keay, D. S. (1990b). Food consumption in 24 hours by Atlantic salmon (Salmo salar L.) in a sea cage. Aquaculture 90, 41-49.

Toates, F. M. (1981). The control of ingestive behaviour by internal and external stimuli - a theoretical review. Appetite 2, 35-50.

Tugendhat, B. (1960). The normal feeding behaviour of the three-spined stickleback Gasterosteus aculeatus. Behaviour 15, 284-318.

Usher, M. L. (1988). Feeding, drinking and digestion in Atlantic salmon Salmo salar L. adapted to seawater and freshwater. Ph.D. Thesis, Dundee University.

Villareal, C. A., Thorpe, J. E. & Miles, M. S. (1988). Influence of photoperiod on growth changes in juvenile salmon (Salmo salar L.). J. Fish Biol. 33, 15-30.

Wagner, H. A. (1974). Photoperiod and temperature regulation of smolting in steelhead trout (Salmo gairdneri). Can. J. Zool. 52, 219-234.

Wallace, J. C., Kolbeinshavn, A. & Aasjord, D. (1988). Observations on the effect of light intensity on the growth of arctic charr fingerlings (Salvelinus alpinus) and salmon fry (Salmo salar). Aquaculture 72, 81-84.

Wankowski, J. W. J. & Thorpe, J. E. (1979). The role of food particle size in the growth of juvenile Atlantic salmon, Salmo salar L. J. Fish Biol. 14, 351-370.

Wedemeyer, G. A., Saunders, R. L. & Clarke, W. C. (1980). Environmental factors affecting smoltification and early

marine survival of andromous salmonids. Mar. Fish. Rev. 42 (6), 1-14.

Whitmore, A. V. & Bowmaker, J. K. (1989). Seasonal variations in cone sensitivity and short-wave absorbing visual pigments in the rudd Scardinius erythropthalmus. J. Comp. Physiol. A. 166, 103-115.

Yallop, D. B. (1986). Ground illumination . Science and Engineering Research Council, NAO Technical Note 57.

APPENDIX 1

Plate 5: This is a positive print of a delevoped x-ray plate indicating iron powder as the inert marker used to label fish food, which has been consumed by the fish. The area estimated to represent the foregut is indicated between the two demarcation lines, highlighted by the arrows.