FEEDING EFFICIENCY AND AGGRESSION IN JUVENILE ATLANTIC SALMON, *SALMO SALAR* (L.) UNDER ALTERNATIVE FEEDING REGIMES

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Candidate's Declaration

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated and that it is of my own composition. No part of this work has been submitted for any other degree.

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This thesis is dedicated to the loving memory of my mam.

Summary

This thesis is a compilation of three long-term studies of on-demand feeding in Atlantic salmon parr and post-smolts in an aquacultural cage environment. The feeding rhythm studies are concerned with whether fish show a daily rhythm of appetite in cage culture. The feeding regime chapters evaluate the effect of an on-demand feeding regime upon growth performance and production when compared with existing fixed ration regimes. Two studies assess the impact of feeding regime upon social interactions around meal times, examining aggression and competition in relation to food presentation. My aim was to show how feeding systems that preferentially distribute feed in relation to changes in appetite could affect fish production and welfare in a cage rearing facility.

The AKVAsmart AQ1 adaptive feeding system is the on-demand feeder used throughout this thesis. It is a system that has been designed principally for cage culture and can manage several feeding programs, depending upon farmers' requirements. Chapter 2 reports on the general configuration of the on-demand feeders for each of cage trials in this thesis, outlining the behavioural and production implications of the parameters chosen.

Chapter 3 is split into two parts: 3a reports upon the feeding rhythms of Atlantic salmon post-smolts in sea cages; 3b examines the effect of an on-demand feeding regime upon growth performance and social interactions between conspecifics. The results of chapter 3a show that there was a significant peak in feed delivery immediately after first light for two months following smolt transfer. There was no significant morning peak in feed delivery during June, three months after transfer. Salinity and daylength were the best predictors of daily feed requirements, although the univariate regression model only accounted for around 20% of daily feeding variability. Feeding did not appear to be regulated by aggression after first light. Chapter 3b suggests that an on-demand feeding regime reduced aggression and competition between conspecifics in sea cages. It also reduced the incidence of fin injury, which may be attributable to reduced aggression, decreased stress levels, or a combination of both. A feed regime that matches feed delivery to daily feed requirements and appetite also improves performance, in terms of uniformity of growth and production efficiency of post-smolts, when compared with an existing fixed ration feeding strategy.

Chapter 4 is divided into three sections: 4a provides information on feeding rhythms in freshwater production cages under ambient conditions from autumn until spring; 4b evaluates the effects of an on-demand feeding regime on growth performance and production of parr in comparison with an existing fixed ration regime; and 4c examines how a feeding regime can affect the prevalence of fin damage and behavioural interactions between conspecifics around meal times.

Chapter 4a reports that the feeding rhythms of cage held Atlantic salmon parr shift with season. Although there was marginal disagreement between replicates, general trends in feeding patterns were observed. During late summer and autumn, parr exhibited a significant peak in appetite after dawn. In winter, the fish showed a tendency to feed in the afternoon until smoltification in March, where the fish retained an afternoon peak in appetite whilst extending their feeding throughout the daylight period outwith dawn and dusk. Daily feed requirements decreased during autumn and remained low during winter, before increasing in spring. Daylength was a significant predictor of daily feed delivery for two of the three replicates, with the model accounting for around 70% of the daily feed variability. Daily variation in feed delivery is absorbed over longer time periods, where on-demand fed fish select similar daily rations. Feeding commenced at dawn in all cages within the on-demand treatment during autumn, suggesting that fish do not refrain from feeding at first light due to aggression, as reported by Kadri et al. (1997*a*).

Chapter 4b reports the effect of an on-demand feeding regime on growth performance and production in comparison with a fixed ration regime. There was generally no significant difference in growth performance and the uniformity of growth between regimes; any differences observed should be interpreted with caution due to sampling error (see chapter 4b results). The feeding efficiency of the on-demand feeding system was slightly better than the imposed regime, suggesting that feeding to tables may overfeed fish at certain times of the year, by offering food in excess of daily feed requirements. The lower feeding efficiency suggests there was increased wastage in the fixed ration regime. This pellet wastage can contribute to environmental degradation, and is therefore undesirable in aquaculture.

Chapter 4c examines the effects of feeding regime upon the prevalence of fin injury and competition around meal times. Direct behavioural observations suggest on-demand feeding systems can reduce levels of intraspecific competition around meal times, within large-scale freshwater production cages. Fish exhibited escalated and more variable swimming manoeuvres during a meal under an imposed feeding regime, when compared with fish fed on-demand. There are also higher levels of overt aggression during meals in fish fed a fixed ration. The prevalence of fin injury was significantly higher in fish fed predetermined rations when compared with those fed on-demand. This study also suggests a feed regime can influence which fish are affected by fin injury within a group; in late winter the smallest fish under the imposed regime exhibited a significantly greater incidence of fin damage, which was not evident in fish fed on-demand.

The final experimental chapter was split into two segments: 5a investigated the feeding rhythms of cage held Atlantic salmon parr in production cages under an artificial photoperiod (as part of an accelerated smoltification strategy); 5b examines the effect of an on-demand feeding regime on growth and production in comparison with an in-house fixed ration regime.

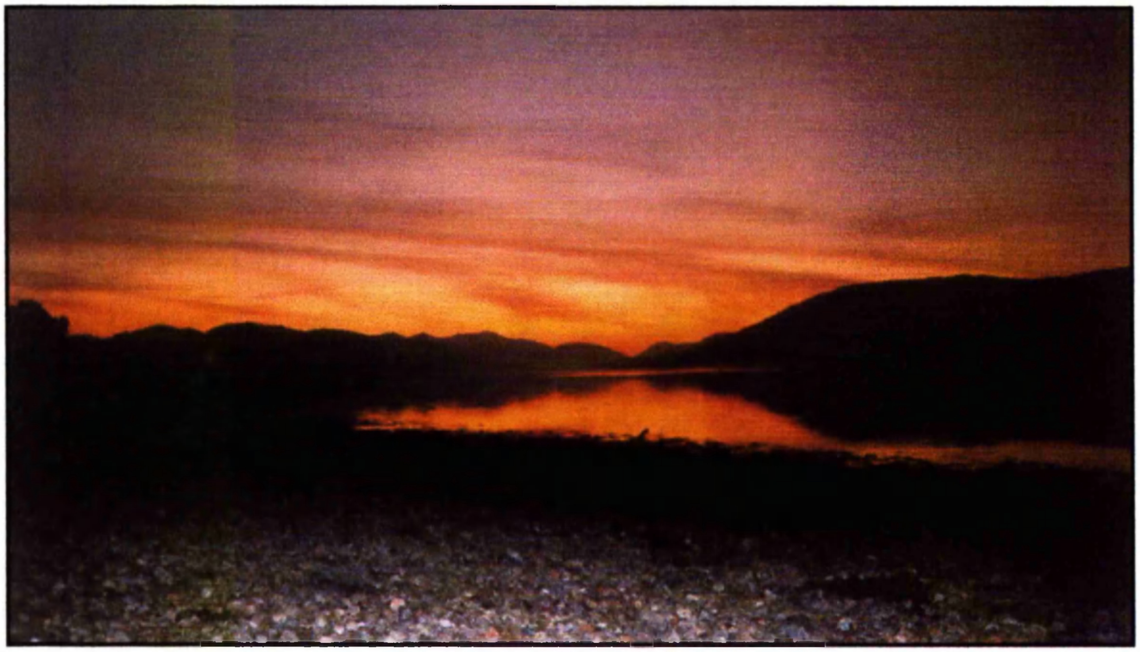
Chapter 5a shows Atlantic salmon did not exhibit a propensity to feed at all times of the day when fed on-demand. There were again some disagreements between on-demand fed replicates, but general trends did emerge. During September under ambient lighting conditions, fish in all replicates showed a significant morning peak in appetite. This finding was similar to that of chapter 4a, even though the fish used in that trial were of a different size, strain and held under different stocking densities. Under constant lighting conditions in October and early November, the fish generally exhibited no peaks in feeding activity and fish consume around 30-40% of their daily ration during the illuminated nocturnal period. The best predictor of daily feed requirements was natural daylength in two of the replicates. Artificially increasing daylength in autumn did not elicit a corresponding increase in daily appetite.

Chapter 5b examines the effect of on-demand feeding upon growth performance and production in comparison with an in-house regime delivering predetermined fixed rations. There was no significant difference in growth performance after one month of the trial, but fish fed on-demand grew significantly better during the second month, when the fish fed to an imposed regime were underfed. There was no significant difference in the uniformity of growth between feeding regimes, which is a similar finding to chapter 4b. Again, on-demand feeding improves feeding efficiency in the month where the fish fed a fixed ration were underfed.

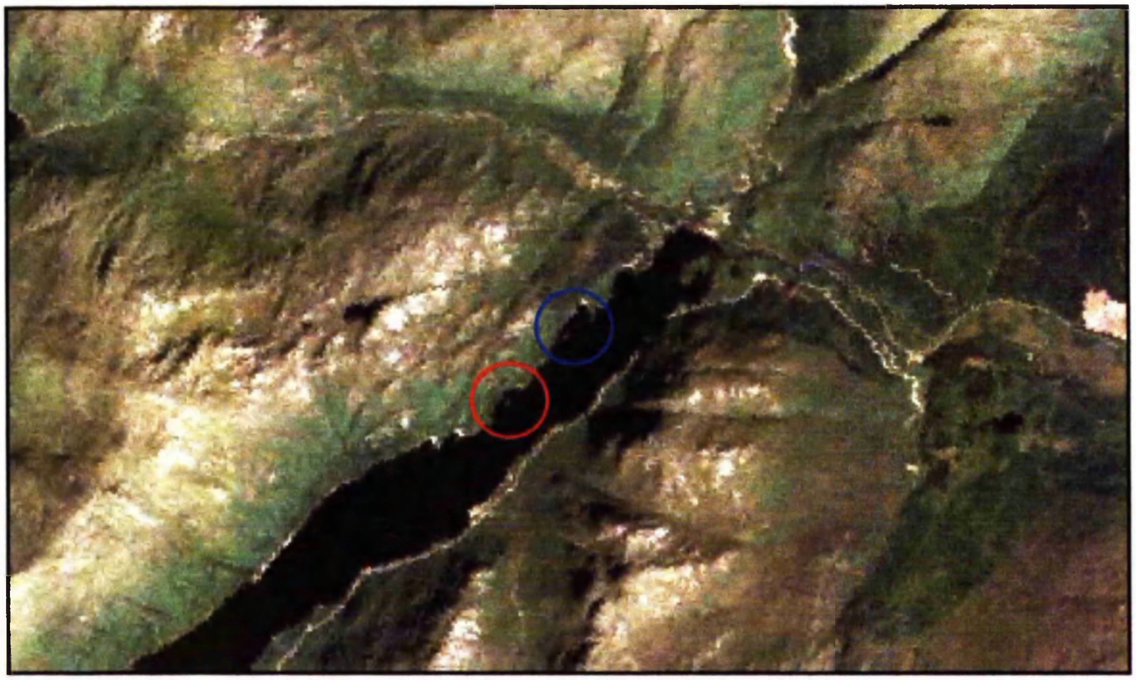
Chapter 6 reviews the main conclusions and findings of the previous chapters, outlining their implications for aquacultural feed management strategies.

Contents

Aerial image of Loch Eil. Circle shows location of farm and experimental location (Chapter 3a and 3b).



Autumnal sunset on Loch Eil. *Photograph by Chris Noble*



Aerial image of Loch Shiel. Red circle shows location of experimental cages for Chapter 4, whereas blue circle shows location of cages for Chapter 5.

Loch Shiel during winter. *Photograph by Chris Noble*

Chapter 1: General Introduction

1.1 The Atlantic salmon

The Atlantic salmon, *Salmo salar* L., is a widely distributed, anadromous teleost that exhibits an ontogenetic shift between two discrete habitats: the freshwater and the marine environment. The freshwater habitat is the spawning and nursery ground, whilst the marine phase is the principal feeding and growth stage of the life cycle (see Thorpe, 1988). Juvenile salmonids have a flexible life history and can spend between one and seven years in freshwater before making the migration to sea (Thorpe et al., 1992). Prior to this migration, the young salmon undergo smoltification, where individuals transform from freshwater dwelling parr to smolts, which have the capacity to osmoregulate in seawater.

During the first summer in freshwater, potential migrating and resident individuals differ in their size trajectories, and by autumn there is a distinct, bimodal difference in metabolic rates and length frequency distribution (see Thorpe, 1977). The lower modal group (LMG), with diminished appetite and growth rates (Metcalfe et al., 1986, 1988) contains individuals that delay migration and will remain in freshwater for at least another year. The upper modal group (UMG) incorporates individuals that have retained relatively high growth and metabolic rates and will complete smoltification the following spring. It has been suggested that these individuals adopt a fast growth strategy (Metcalfe et al., 1988); a characteristic which is desirable for the aquaculture industry as there is minimal residence time in freshwater, shortening the growth cycle.

Atlantic salmon exhibit behavioural adaptations that are frequently exclusive in other teleosts. In the natural freshwater environment, agonistic behaviour is judged to be the fundamental mechanism for the acquisition of food and territory (Kalleberg, 1958; Keenleyside and

Yanamoto, 1962; Wankowski and Thorpe, 1979). However, it has been reported that during their migratory phase in the marine environment, salmon normally form non-aggressive schools (Kalleberg, 1958) and their governing social behaviour is mutual attraction.

1.2 Atlantic salmon aquaculture

There is a growing recognition of the nutritional advantages of fish protein in the human diet (Monahan, 1993) and the global decline of ocean fisheries stocks has meant aquaculture is receiving increasing attention as a source of fresh fish. Atlantic salmon has historically had a high commercial value as a food source and recent years have seen increasing interest in its commercial culture. This interest is coupled with increased salmon catches worldwide during the late 80's and early 90's (Johnson, 1998). There are two types of salmon culture (after Laird and Needham, 1991):

- **Total culture.** Fish are reared from egg to market under controlled and supervised conditions and are kept in captivity for the duration of their life cycle. This rearing method is used in commercial Atlantic salmon aquaculture.
- **Partial culture.** This is where fish are reared in captivity for only a part of their life cycle. This may be part of a commercial ranching operation, or as part of a strategy to enhance or replace depleted wild stocks.

Atlantic salmon aquaculture was first developed as a commercial venture in Norway in the 1960's with the deployment of floating cages (Monahan, 1993). There was a huge expansion of farming in the mid-eighties, with 75% of Atlantic salmon production coming from farming in 1985 (Laird and Needham, 1991). Atlantic salmon farming is now found in areas that were previously outwith the fish's native range, such as Chile and Tasmania. The market for salmon is becoming highly competitive, and fish no longer command an attractive market price for the aquaculturist (Rasmussen and Ostenfeld, 2000). Producers are turning their attention to improving the efficiency of existing rearing facilities, to maximise profits and reduce loss. This can be achieved by reviewing and evaluating feed management.

1.3 Feeding management

Feed management strategies in aquaculture govern feed delivery rate, frequency and duration; ration size (both within and between days) and the spatial and temporal distribution of feed (Talbot 1993; Talbot et al., 1999). Erroneous design and implementation of feed management strategies can have a considerable impact upon the profitability of an aquaculture venture, as feed costs account for 40% of the outlay of a freshwater farm (D. Mitchell, pers. comm.) and up to 60% of a seawater farm (Blyth et al., 1993). If a farmer does not match feed delivery to the appetite requirements of fish, it can lead to poor feed conversion ratios (FCR: which is expressed as the amount of food offered (kg), divided by wet weight gain (kg)). This can lead to a culturist either over-feeding or under-feeding fish within a rearing facility.

Overfeeding fish is uneconomical because it promotes pellet wastage (Thorpe and Cho, 1995) *and* pollutes the environment. In trout farming, reducing FCR from 1.5 to 1.0 reduces phosphorus discharge from 10-13kgs to 5-7kg, per tonne of fish produced (Alanärä, 1994b). This phosphorus discharge can potentially enhance primary production in freshwater bodies and lead to eutrophication (see Cho and Bureau, 1997). Dissolved wastes within the rearing facility can decrease water quality and be detrimental to fish health (Wedemeyer, 1997). Underfeeding can also increase FCR (Cho, 1992), reduce growth and increase competition and aggression (Lundstrom et al., 1990; M^{c}Carthy et al., 1992). Restrictive amounts of food can also promote growth depensation (Miglavs and Jobling, 1989; M^{c}Carthy et al., 1992) and increase stress levels due to competition and aggression from conspecifics (Wedemeyer, 1997). These behavioural stressors are detrimental to animal welfare and can be deleterious upon growth (Pickering, 1993) and feed conversion efficiency (Abbot and Dill, 1989).

Ration size is difficult for a farmer to assess, as it can vary both within and between days (MacLeod, 1977; Juell et al., 1993; Blyth et al., 1993; 1999). Many farmers rely upon feed charts and tables relating to food type and fish size when calculating daily feed requirements for fish. These tables are based upon extensive research into fish nutrition and have been developed and revised by commercial feed companies for many years, but they assume fish consume food whenever it is offered and do not account for diel changes in appetite. In fact, fish do not show a propensity to feed at all times of the day (Jobling, 1993) and may exhibit preferential peaks in feed consumption and appetite throughout a day. If feed delivery can be matched to these peaks in appetite a farmer may be able to improve growth performance. This has been demonstrated in many commercially cultured species such as: goldfish, *Carassius auratus* (Noeske et al., 1981; Noeske and Spieler, 1984), Indian catfish, *Heteropneustes fossilis* (Sundaraj et al., 1982), channel catfish, *Ictalurus punctatus* (Noeske-Hallin et al., 1985), African catfish, *Heterobranchus longifilis* (Kerdchuen and Legendre, 1991) and rainbow trout, *Oncorhynchus mykiss* (Reddy et al., 1994; Boujard et al., 1995; Gélineau et al., 1996).

Environmental variables can also affect the feed intake of cultured species, especially in cage culture where a farmer has little control over abiotic parameters. Even with this apparent lack of regulation, some variables such as daylength and water temperature can be relatively predictable and a culturist can integrate their effects into feed management. Light and temperature are considered the primary environmental factors that govern how much food is consumed by fish (Brett, 1979; Boujard and Leatherland, 1992*b*). Other environmental factors that can influence feed intake are: wave action (Juell, 1995); increased wind speed and rainfall (Bégout and Lagardere, 1993; Juell, 1995); turbidity (Berg and Northcote, 1985; Ang and Petrell, 1997; Mallekh et al., 1998); oxygen depletion (Thetmeyer et al., 1999) and salinity, after smolt transfer

(Jørgensen and Jobling, 1994; Damsgård and Arnesen, 1998). These variables are very difficult to manipulate in cage culture, but if they are monitored and an aquaculturist is aware of their impacts, they can be accommodated in feeding practices.

Other biotic factors can influence feed intake, such as disease (Bloch and Larsen, 1993) or competition. The feeding rhythms and feed intake of adult Atlantic salmon can be influenced by the relative size of individuals within the group (Kadri et al., 1997*b*). Atlantic salmon parr have also been reported to refrain from feeding at first light because of aggression (Kadri et al., 1997*a*). In addition to influencing which fish gain access to a feed resource, aggression can cause fin damage (Turnbull et al., 1998; MacLean et al., 2000), which decreases the market appeal of whole fish.

1.4 Choice of feeding technique

The techniques used when feeding fish can have an impact on commercial variables and social interactions between fish in cages. This section will focus on some of the feeding techniques that are available to the aquaculture industry.

1.4.1 Hand feeding

Hand feeding is one of the most straightforward feeding techniques employed on a farm. The farmer has daily contact with the fish, and an experienced observer can alter the amount fed to match the appetite variability within and between cages. This is done by using cessation in surface feeding activity as an indicator of falling group appetite. However, there are a number of problems: on large farms it is an extremely labour intensive process and the rate of feed delivery is entirely dependent upon the individual involved; the fish can only be fed during the hours that farm staff are present so feeding times are imposed by farm management and not dictated by the needs of the fish. The presence of staff around the cages may disturb the fish and cause premature cessation of feeding, and if the species is not a surface feeder, it can be difficult to assess when the fish have finished eating. This technique can be enhanced using a mechanical water cannon or blower that can greatly improve feed delivery rate and distribution, and is less labour intensive.

1.4.2. Automated fixed ration systems

Many larger farms use automatic feeding systems that distribute a fixed ration of food at certain times of the day. Although these systems may seem costly at the time of purchase, the overall site costs are lower because they are less labour intensive. The computerised versions have the option of a number of different feeding methods, depending on the preferences of the farmer but they do require a certain degree of computer literacy on the behalf of farm staff. Some larger systems can also cope with the bulk handling of feed, and allow staff to concentrate on other essential husbandry jobs. A major benefit for the larger more intensive farm is that these systems can be used outwith normal working hours. Again there are limitations: there is no consideration of within/between day appetite variation as this technique is based upon commercially available or in-house feed tables. It is also difficult to ascertain if all fish have access to the feed resource and to maintain low pellet wastage (Thomassen and Fjæra, 1996). The system removes the need for daily contact with the fish, although this can have a negative aspect as many farmers use this period to assess their fish populations for early indicators of health problems, for example. Furthermore, the farmer may not get the opportunity to detect problems whilst they are in their infancy and easily manageable.

Both hand feeding and automated fixed ration feeding have been recently coupled with feedbackloops such as cameras, airlift systems or waste cones in order to minimise pellet wastage. Such feedbackloops can present a video image, store uneaten food or return food to the surface, where a farmer can alter feed delivery in relation to the amount of wasted pellets. These systems do require an operator present and can be time consuming for the individuals involved.

1.4.3 Automated on-demand feeding systems

Several appetite-based systems have been recently developed whereby farmed fish can be fed on-demand. With these systems the feeding regime is regulated by the appetite of the fish and not imposed by farm management; the systems act as an interface between the farmer and the feeding requirements of fish. Since these systems can continually log the amount of food delivered as well as managing feed delivery, they can offer biologists the opportunity to investigate the performance of on-demand fed salmon for comparison with those fed under standard regimes.

There have been numerous studies of fish fed by operant self-feeders (see, for example, Landless, 1976; Sánchez-Vázquez et al., 1994; Alanärä, 1994*a*, 1996; Paspatis and Boujard, 1996). As these systems have had a limited application in current Atlantic salmon net culture (Alanärä, pers. comm.) due to scaling up problems, a brief overview of their constituents and application in culture systems will be given here. Self-feeders rely on fish either pressing or biting a trigger which releases feed from either a mechanical or automated dispenser. This arrangement can lead to monopolisation of the trigger by a number of socially dominant fish and reinforce social hierarchies (Alanärä, 1996). There are also energetic costs involved with these on-demand systems, as the activation of triggers requires a direct input from the fish. These systems generally release a fixed ration size per feeder actuation and it is therefore important to adjust the reward level in accordance with fish size, stocking density, environmental parameters and the feeding behaviour of the cultured species.

Interactive feedback systems indirectly control feed delivery by monitoring pellet wastage, or fish activity assessed by video footage or echosounding technology. The experiments throughout this thesis will use a commercially available system where fish indirectly control their feeding regime via their appetite: the AKVAsmart AQ1 adaptive feeding system (AKVAsmart UK Ltd, Inverness). The rationale behind its development is (after Blyth et al., 1993) "to regulate feed input into salmon cages without human intervention". This is attained by using an underwater infra-red sensor that can detect and count uneaten pellets. This data is inputted to feeding software, which regulates the amount of food offered in relation to pellet wastage (refer to chapter 2 for a detailed description of the system). The system matches the time of feed delivery to appetite and gives the farmer an opportunity to integrate feeding variability into feed management. This is coupled with the ability to change ration size throughout the day according to changes in appetite. The system logs data on feeding time, frequency and duration and can identify feeding rhythms; generating information that a farmer can use to adapt *and* evaluate existing feeding strategies. Furthermore, the fish themselves do not have to instigate feeder activation as feed delivery is under the passive control of a feedback loop. This appetite-based feeding system was used throughout this study, as it has been successfully deployed in Atlantic salmon sea cage culture to investigate the feeding rhythms of adult fish (Blyth et al., 1993, 1997, 1999).

1.5 Aims and objectives

To my knowledge, there have been no large-scale investigations into the feeding rhythms of parr in freshwater cage conditions. This thesis is a compilation of three long-term investigations into the on-demand feeding behaviour of juvenile Atlantic salmon in an aquacultural cage environment. Chapter 2 outlines the configuration of the AKVAsmart AQ1 systems. Chapter 3 is concerned with the feed management of post-smolts, Chapter 4 addresses feeding in parr and Chapter 5 addresses the feeding of parr under accelerated smoltification regimes. Each chapter is broken down into sections that firstly investigate the feeding patterns of the fish and daily feed intake, and secondly evaluate the effect of on-demand feeding upon growth and social interactions in comparison to standard feeding regimes. The layout of the thesis follows the chronological order in which the experiments were carried out, not the life history of the fish.

Commercial aquaculture needs information on the feeding rhythms of fish and how feeding on-demand can affect aggression, food acquisition and growth. This information can help a farmer develop husbandry and feed management practices that promote productivity and welfare, whilst minimising adverse environmental effects. The feeding rhythm studies (Chapters 3a, 4a and 5a) are variations on a theme and address whether fish show a daily rhythm of appetite in cage culture, and what factors may control these rhythms and daily feed intake. This will provide the aquaculturist with information on daily feed requirements and the best times to feed fish in relation to changes in appetite. The feeding regime chapters (3b, 4b and 5b) assess the effect of an on-demand feeding regime upon growth performance and production when compared with existing fixed ration regimes. The behavioural sections of this thesis (Chapter 3b and 4c) investigate the impact of feeding regime upon social interactions between conspecifics around meal times, examining levels of competition and aggression in relation to food presentation. Competition and aggression can affect fish stress and welfare, and data obtained from on-demand feeding may help a farmer circumvent some of these problems.

Chapter 2: The AKVAsmart AQ1 Adaptive Feed System

2.1 Introduction

2.1.1 Aims and content

This chapter will describe and outline the commercially available 'AKVAsmart AQ1 adaptive feeding system', the on-demand feeding system that is used throughout the present study. The general configuration of the system for each of the production trials, including an outline of the biological and economical significance of each parameter, will be described in the latter part of the chapter.

2.1.2 The AKVAsmart AQ1 adaptive feeding system

Several appetite-based feeding systems have been recently deployed where farmed fish can be fed on-demand. The fundamental principle behind the development of on-demand feeding systems is that feed delivery is regulated by the needs of the fish and not imposed by the fish farmer. There are two types of demand feeders (see Alanärä et al., 2001):

- **Self-feeders** are active on-demand feeders, where fish regulate feed delivery by activating a trigger.
- **Interactive feedback systems** are passive on-demand feeders, where feed delivery is indirectly controlled by monitoring uneaten food or evaluating feeding activity, using the appetite of the whole group as an index of satiation.

Since these systems continually log information on the amount of food delivered, they can offer biologists the opportunity to investigate the impact of on-demand feeding upon the growth performance and behaviour of fish in a commercial aquaculture environment. Throughout this study I will assume that the data obtained from the on-demand feeders is an accurate reflection of group appetite.

The AKVAsmart AQ1 adaptive feeding system (AKVAsmart UK Ltd, Inverness) is a system predominantly designed for cage aquaculture that monitors uneaten food within a rearing unit (although it has recently been deployed in tank culture). This is attained by using an underwater infra-red sensor linked to feeding software, which regulates the amount of food offered in relation to pellet wastage. The system consists of three components: an underwater sensor, a computerised control unit (AQ1) and feed delivery hardware. A simplification of the algorithm that regulates the feeding software is: if the sensor detects pellets after feeder activation the fish are not hungry, cease or reduce feed delivery; if no pellets are detected after feeder actuation the fish require food, maintain or increase feed delivery. The system can deliver a small amount of food at user-defined intervals throughout the day to test whether the fish are hungry. The system matches the time of feed delivery to appetite and gives the farmer an opportunity to integrate feeding variability into feed management. This is coupled with the ability to change ration size throughout the day according to changes in appetite. Furthermore, the fish themselves do not have to instigate feeder activation as feed delivery is under the passive control of a feedback loop. All information on pellet wastage and feed delivery is stored in the control unit for analysis with PC based feeding software.

In all trials the AQ1 control unit was linked to a centralised feed hopper with a vibrator feeder attached to a circular spreader (Sterner AS; Aquatess Ltd, Inverness, UK) and these spread pellets in a circle across the surface of the cage. The AQ1 was linked to a conical pellet trap and underwater pellet sensor, which was suspended in the centre of the cage beneath the group of fish (Fig 2.1). The cone and sensor were positioned directly beneath the feed hopper although they could drift along a fixed line with tidal or wind driven currents. The sensor was calibrated in clean water before the experiments commenced, to facilitate pellet recognition. The sensor must count 40 pellets before it is calibrated. After calibration the control unit could distinguish between pellets and foreign bodies that were present in the cages such as algae or faeces. It was also capable of single pellet precision and could recognise individual pellets as small as 1.5mm in length.

The control unit could manage several feeding programs with or without the sensor as a waste feedback loop. Throughout the thesis the system was primarily utilised as an adaptive on-demand feeder, regulating the feeder output according to waste detected by the sensor, except where stated in the text. There were a number of secondary parameters within the adaptive feeding program that could refine the system to the biological and economic requirements of each trial, which will be outlined.

2.1.3 The set-up for the AKVAsmart on-demand feeding systems for each trial

The AQ1's for each trial were set up using a combination of system specific parameters (see Table 2.1). Their biological and economical significance will be outlined in this section. Each on-demand feeder was programmed to begin feeding at dawn and cease at dusk unless stated in each chapter. When feeding commenced the system delivered a pre-set amount of food as a test to see if the fish were hungry. This was defined as the **Minimum Feed Delivery** and was the smallest amount of food delivered per feeder actuation. This setting delivered only a small amount of food in an attempt to reduce pellet wastage if the fish were not hungry. **Maximum Feed Delivery** was the maximum amount of food delivered per feeder actuation. These two values established the upper and lower feed output thresholds within which the AQ1 operated. The **Maximum Feed Delivery** should not limit feed intake. The feed output of the feed distributor is governed by how much pellet waste the sensor detects. A primary objective of all three production trials was to reduce pellet wastage and the thresholds for measuring waste (**Pellet Sense High** and **Low**) were set to minimal values. **Sensor Depth** is the depth (in metres) from the water surface to the sensor. The sensor was positioned near the base of the net to allow fish access to the feed resource before it passed through the waste detection cone. **Sense-time** was the time (seconds) that the sensor operated after feed delivery. It commenced when pellets reached the sensor based on **Sensor Depth** and **Pellet Sink Rate** (cm s^{-1}). If the AQ1 sensed a number of pellets that were above the **Pellet Sense High** threshold, the system decreased or ceased feed delivery. **Minimum Sleep Time** was the shortest time that the feeder remained inactive after the **Pellet Sense High** threshold was breached. This parameter ensured fish were not offered food when satiated and minimised waste feed due to feeder actuation tests. **Maximum Sleep Time** was the longest that feeding operations were suspended after a feeding episode. The AQ1 could auto-range between these two settings depending upon the number of pellets sensed after the **Minimum Feed Delivery** feeder actuation. Using these parameters I attempted to allow the fish to feed efficiently to daily satiation. If it appeared that the fish were being under or over-fed the relevant settings were altered accordingly.

Table 2.1 An outline of the AQ1 on-demand feeding parameters at the beginning of each trial. Each on-demand feeding replicate was programmed with the same criteria throughout each trial

		Trial:	
AQ1 Parameter	**Loch Eil post-smolts**	**Glenfinnan S1 parr**	**Glenfinnan S1/2 parr**
Minimum Feed Delivery (g)	10	10	30
Maximum Feed Delivery (g)	20	120	230
Min. Sleep time (min.)	30	30	30
Max. Sleep time (min.)	60	30	30
Pellet Sense High	3	2	2
Pellet Sense Low	1	1	1
Sensor Depth (m)	3	3.8	3.8
Sense-time (s)	40	45	45
Pellet Size (mm)	2.3	2	2
Pellet sink rate (cm s^{-1})	10	7.0	7.0
Cone diameter (m)	1	0.5	0.5

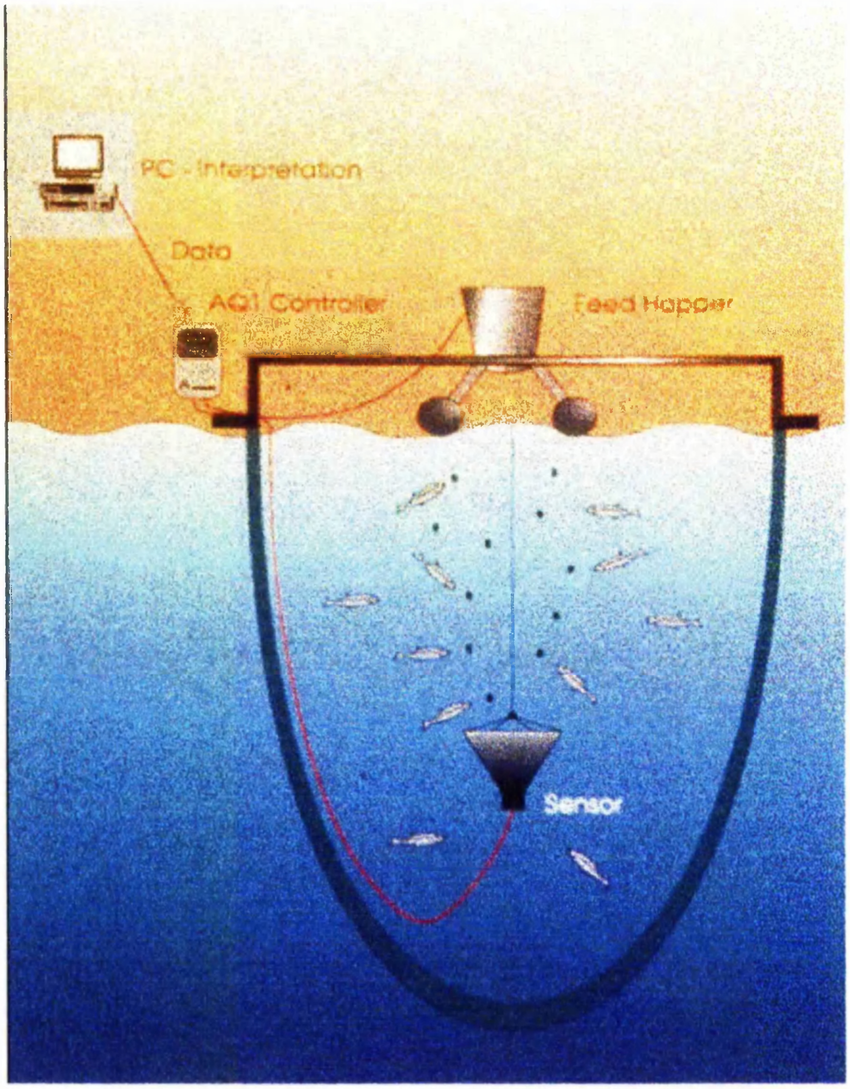


Fig. 2.1 The cage set-up of the AKVAsmart AQ1 adaptive feeding system. Reproduced with permission from AKVAsmart UK Ltd.

Chapter 3a: The effect of biological and environmental variables on the feeding rhythms and daily food intake of Atlantic salmon post-smolts in sea cages

3a.1 Introduction

3a.1.1 Aims and content

This chapter will outline the feeding activity of post smolt Atlantic salmon, *Salmo salar*, in the three month period following smolt transfer. I will discuss the variability in appetite within and between days when a group of fish are allowed to feed on-demand using a commercially available interactive feedback system. The aim of this study was to see if fish exhibited any patterns of feed intake in an aquaculture environment and to assess how such patterns were affected by changes in abiotic variables. This information may help a farmer improve existing feeding techniques.

3a.1.2 What can affect feed intake?

There can be considerable variability in the feed intake of fish between days (MacLeod, 1977; Grove et al., 1978; Juell et al., 1993; Blyth et al., 1993), and seasons (Higgins and Talbot, 1985; Rowe and Thorpe, 1990; Jobling and Baardvik, 1991; Blyth et al 1993,1999). This circadian and circannual variability in feeding is generally assumed to be under endogenous control, but can be synchronised and entrained by a number of environmental variables (Boujard and Leatherland, 1992*b*). Light is considered to be the most important external factor that entrains feeding rhythms, with the principal entraining agent considered to be changes in the daily light/dark cycle (Gibson and Keenleyside, 1966; Boujard and Leatherland, 1992*b*; Eriksson and Alanärä, 1992). A secondary factor that may govern when a fish chooses to feed is temperature. Fraser et al., (1993, 1995) reported that juvenile Atlantic salmon switch their feeding patterns from diurnal to nocturnal when temperature drops below 10°c during the winter months. Feeding intensity may also be affected by temperature. Metabolic rate is governed by temperature (Brett, 1979) and fish regulate their feed response according to their metabolism (Wurtsbaugh and Davis, 1977; Jobling 1994, 1997). Long term increases in temperature can stimulate increases in feed intake within a fish's tolerance range, although acute or substantial changes may lead to a suppression of appetite (Alanärä, 1992*b*).

Other environmental variables can have a negative impact upon feed intake and feeding patterns. These include: oxygen depletion (Thetmeyer et al., 1999); increased ammonium concentrations from waste products (Beamish and Tandler, 1990); wave action (Bégout Anras, 1995; Juell, 1995; Mallekh et al., 1998); increased wind speed and rainfall (Bégout and Lagardère, 1993; Juell, 1995); turbidity (Berg and Northcote, 1985; Ang and Petrell, 1997; Mallekh et al., 1998); and salinity, immediately following smolt transfer (Jørgensen and Jobling, 1994; Stradmeyer, 1994; Damsgård and Arnesen, 1998). Although sea lochs can be subject to considerable hydrological and meteorological fluctuations, changes in some environmental variables such as light or water temperature are predictable and can be integrated into feed management strategies. Other variables are harder to predict in cage culture systems, but if a farmer is aware of their short term impacts upon feed intake it may be possible to accommodate these within feeding practices.

Biotic factors such as disease or competition can also govern the expression of feeding rhythms. Disease or increased parasite loads are potential stressors for fish and may reduce feed intake and appetite (Bloch and Larsen, 1993; Roberts and Shepherd, 1997). For example, adult Atlantic salmon held in sea cages may exhibit decreased feed intake and appetite when infested with the sea lice, *Lepeophtheirus salmonis* (S. Kadri, unpublished). Intraspecific competition can influence the expression of feeding patterns when fish are held in groups. There can be high inter-individual variability in the feeding patterns within groups of rainbow trout, *Oncorhynchus* *mykiss* (Brännäs and Alanärä, 1997). Kadri et al., (1997*b*) found feeding rhythms of adult Atlantic salmon in tanks were associated with the relative size of individuals within the group. The same authors also found that Atlantic salmon parr defer feeding at first light because of a high incidence of aggressive encounters (Kadri et al., 1997*a*). This study investigated whether this deferment of feeding persists in post smolts under commercial rearing densities. Feeding management strategies may also influence feeding rhythms. Ration size and reward level at each feeder actuation can modify when a fish chooses to feed (Brännäs and Alanärä, 1994; Gélineau et al., 1998). Increased awareness of behavioural impacts upon feeding may be useful information for farmers when planning feeding strategies.

3a.1.3 The application of feeding rhythm studies to aquaculture

Many commercial feed management strategies are based upon in-house or commercially available feed tables. These feed tables contain recommendations on a fish's daily food requirements, but do not account for changes in daily appetite and assume fish will consume food whenever it is offered. Many fish species have a distinct daily rhythm in appetite and do not feed continually (see Kadri et al., 1991), which can lead to food wastage. Feed costs are a critical outlay in an aquaculture venture and can account for up to 60% of the running costs of a seawater farm (D. Mitchell, pers. comm; Blyth et al., 1993). An improvement in feeding efficiency on farms can have considerable influence upon the profitability of a farm and therefore such improvements have received increased attention throughout the industry. A greater understanding of feeding rhythms can help a farmer determine when food is eaten and when it is wasted, which also reduces environmental impacts as wasted feed can contribute to environmental degradation in cage culture (Cho and Bureau, 1998).

The time of day which fish receive food has been reported to affect growth performance, nutrient utilisation and proximate composition of many cultured species (see Chapter 1). By matching the timing of feed delivery to appetite a farmer may be able to improve the efficiency of feed management strategies (Spieler, 1977, 1990). One could account for this using existing feeding techniques by delivering feed prescribed by tables differentially across the day in relation to changes in appetite, but this is difficult to achieve and labour intensive. An attractive alternative is to employ commercially available interactive on-demand feeding systems that accurately match feed delivery to appetite.

3a.1.4 Objectives of the study

In the following study I will examine the feeding of post smolt Atlantic salmon for any diel rhythms within cage culture, and assess the influence of changes in environmental variables. The questions I will address during this study are: -

i) Are there any patterns in group appetite and how do they vary within a day?

ii) Are these feeding patterns retained over longer time intervals?

iii) Is there any variability in feed intake between days and over longer time periods? To what extent can it be explained by environmental variables?

iv) What time of day did feeding commence in relation to sunrise?

If there is a delay in feeding after sunrise it may support the hypothesis of Kadri et al, (1997*a*) that fish refrain from feeding due to aggression between conspecifics after first light. The present study will investigate whether this behaviour is retained under high stocking densities and after seawater transfer. The results of this chapter are based upon a one-sample study, but I hope the detailed nature of the data set can provide information on the best way to temporally distribute the feed resource within a day.

3a.2 Materials and methods

3a.2.1 Fish husbandry

Fish used in this study were from a pooled, medium maturing hatchery stock of 1+ upper modal group (UMG) Atlantic salmon that were identified as fish that migrate to sea after one year in freshwater (Thorpe, 1977). On the 27th March 1998 two groups of approximately 800 fish (mean weight *ca.* 55g ± 10g S.D.) were transferred from a smolt production site at Glenfinnan (Grid ref: NM 887 790) to two seawater cages at the Loch Eil Feed Trial Unit (Grid ref: NM 989 778). Marine Harvest Ltd owned both facilities. Fish were transferred by oxygenated tank and it took approximately one hour to transfer each group. The trial began on the 1st April 1998 to allow sufficient recovery time from transportation stress. Fish were stocked in 5x5x4m deep cages at an initial stocking density of approximately 0.4kg m^{-3}. At the end of the three-month trial stocking density had increased to approximately 1.3kg m^{-3}. Fig. 3a.1 contains further details and an overview of the study site.

Both cages were fitted with a centrally mounted 25kg feed hopper with a vibrator feeder attached to a circular spreader (Sterner AS; Aquatess Ltd, Inverness, UK) which spread pellets (Trouw Royale, Trouw UK Ltd, Northwich, sizes 2.3-3mm according to manufacturers recommendations for fish size) in a 4 – 4.5m diameter circle. Both feed hoppers were controlled by an AKVAsmart AQ1 Adaptive Feed System (AKVAsmart UK Ltd, Inverness). (Refer to chapter 2 for a detailed description of this on-demand feeding system).

Fish in one cage were fed on-demand from dawn until dusk (times defined by the Royal Observatory, Edinburgh, UK) and fish in the other cage were fed six meals per day starting just after dawn and ceasing at dusk. At the start of the study ration for the imposed regime was determined using a combination of sub-surface cameras and visual observation of a cessation of surface activity as an estimate of satiation. Ration was then divided equally among six meals and updated when the fish did not appear to be satiated at the end of a meal. Meal duration in the imposed regime was five minutes. This six meal, long day feeding strategy was chosen to mimic current husbandry practices for achieving the best growth performance from automatic feeders in post-smolts (D. Mitchell pers. comm.). In the on-demand fed group discrete meals were observed across the day (Boujard and Leatherland, 1992*a*), but these meals were selected by the group of fish and were not imposed by feed management protocols.

3a.2.2 Measuring environmental variables

The times of sunrise and sunset for Fort William (Grid ref: NN 108 742) were obtained from the Royal Observatory (Edinburgh, UK). Daylength (hours) was then obtained by working out the difference (hours) between the beginning of civil twilight and the end of civil twilight. The Royal Observatory (Edinburgh, UK) defines these times as the beginning and end of twilight when the outer edge of the sun's disc is six degrees below the horizon. The change in daylength from the previous day (hours) was also recorded (after Smith et al., 1993)

Salinity and water temperature were measured on the farm at approximately 8.00am each morning using an electronic monitor (Partech Electronics Ltd, St. Austell, Cornwall), at 4m and 2m depth respectively. Water clarity (metres) was also measured at 8.00am daily using a secchi disc.

The British Atmospheric Data Centre (Chilton, Didcot, UK) kindly provided meteorological and hydrological data, compiled from weather stations in the vicinity of the study site. Data on daily rainfall were obtained from the Keil weather station (Grid ref: NN 008 650), and data on daily wind speed and direction were obtained from the Tulloch Bridge Saws weather station (Grid ref:

NN 350 783). Daily tidal range data (metres between high and low water) were compiled from Admiralty Tide Tables (1998) using data from Loch Eil head (Grid ref: NM 972 785).

3a.2.3 Manipulation of the on-demand feeding data

On-demand feeding data were manipulated in two ways. Rather than look at absolute time of day, which lacks a fixed position relative to the light-dark cycle (Spieler, 2001), data were broken down into fractions of the feeding day (to the nearest minute). To increase the resolution of the data 10 fractions of the day were selected.

Secondly, it was observed that there were variations in group appetite between days. As the trial looked at how feeding differed across a day, a Relative Feed Delivery Index was created. This is a function of the mean of the total amount fed per day, divided by the amount fed during a given portion of the feeding day. This is expressed as: -

$\mathbf{R}_{i,d} = [\mathbf{F}_{d} / \bar{\mathbf{F}}_{i,d}]$

Where: R = Relative Feed Delivery Index

i = ith of the portion of the feeding day (number of feeding increments: 10)

d = dth of day (specified portion of the feeding day e.g. 1 = first increment after sunrise)

F = feed delivery (feed delivery for a given portion of day)

$\bar{F}$ = mean of daily feed delivery (total feed delivery for day, divided by number of portions)

For example: **1 = [80 / (800/10)]**

Where: 1 = RFDI

80 = Feed delivery for a given portion of the feeding day (e.g. first portion after sunrise)

800 = Total feed delivery for day

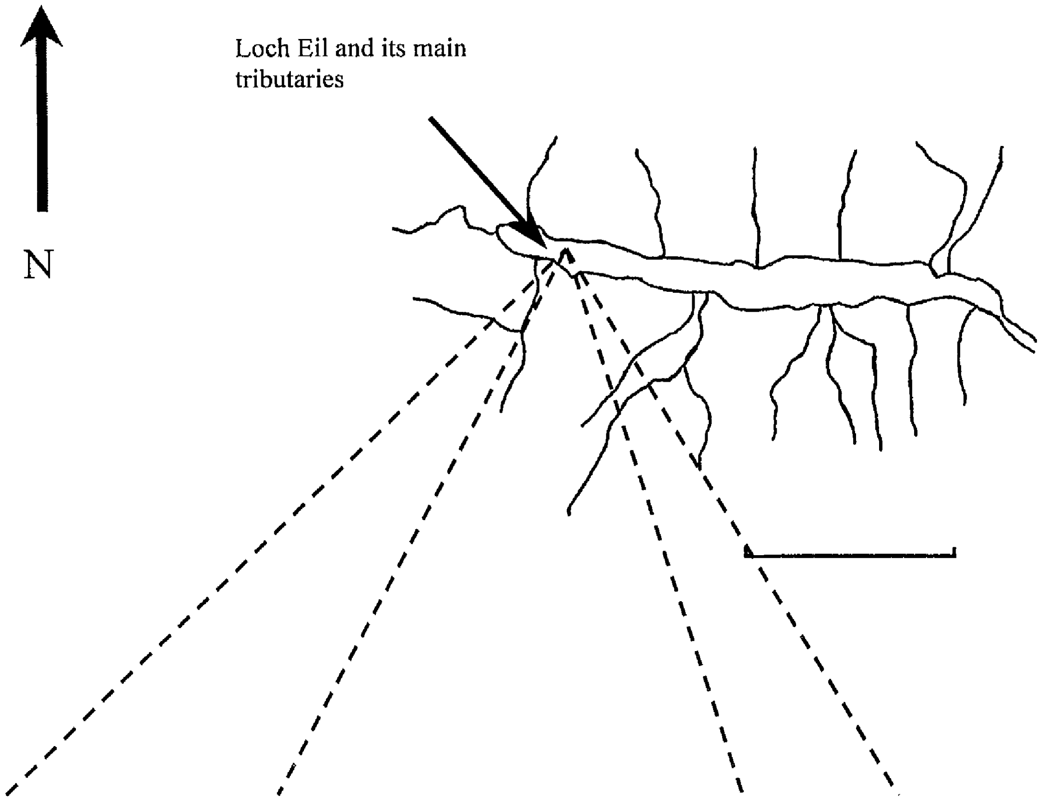
10 = Number of increments for day

Total daily feed delivery was transformed into amount fed as a percentage of body weight day^{-1}, to correct for increases in the amount of food consumed as fish grew. The time feeding commenced and ceased in relation to twilight was also recorded.

3a.2.4 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0, Minitab Inc., Philadelphia) or SPSS (v. 10-0, SPSS Inc., Chicago) for MS Windows. A non-parametric ANOVA (Kruskal-Wallis test) was used to test for possible differences in appetite across a day in the on-demand fed treatment. A post hoc multiple comparison test (Siegel and Castellan, 1988) was used to locate any differences observed. Data are presented as median values ± first and third interquartile ranges.

Differences in appetite between days were tested with a Univariate General Linear Model (GLM) model in SPSS (v. 10-0). Independent variables were: day, daylength (h), change in daylength from previous day (h), salinity at 4m depth (ppm), water temperature at 2m depth (°C), water clarity (m), tidal range (m), total daily rainfall (mm d^{-1}), average daily wind speed (knots). All variables were also included in the model with day as an interaction. The dependent variable was daily ration, which was transformed into amount fed as a percentage of body weight day^{-1}. This percentage data was further transformed using the arcsin square root procedure to satisfy normality and homogeneity of variance requirements. A significance level of $P < 0.05$ was used for all statistical tests.



Imposed cage	**On-demand cage**
Mean wet weight on 22.3.98 = 54.72g	Mean wet weight on 23.3.98 = 53.70g
Stocking density approx. = 0.4 kg m^{-3}	Stocking density approx. = 0.4 kg m^{-3}
No. of fish = 800	No. of fish = 800

Fig. 3a.1. The location of the experimental production cages on Loch Eil, showing initial fish numbers, stocking densities and average weights at the beginning of the study. Scale bar = 2 miles.

3a.3 Results

3a.3.1 Patterns of feeding across a day

The caged group of on-demand fed salmon in this trial exhibited distinct peaks in appetite across the day, although this diurnal pattern of feeding was not consistent between months (Fig. 3a.2 and Fig. 3a.3). During the first month after smolt transfer (April) there was a significant morning peak in appetite after first light when compared with appetite at dusk (Kruskal-Wallis H_{adj} = 26.61, d.f. = 9, P = 0.002). This peak was also apparent during May (Kruskal-Wallis H_{adj} = 51.44, d.f. = 9, $P < 0.001$) in relation to midday, but was not retained during June, when there was no significant peak in feeding and activity was spread equally across the day (Kruskal-Wallis H_{adj} = 14.99, d.f. = 9, P = 0.091).

3a.3.2 Differences in feed delivery between days

Differences in daily on-demand feed delivery for the whole group of fish were analysed in relation to a number of abiotic variables using the GLM model in SPSS. It should be noted that the daily food demand variables are not independent of each other as the data is taken from a single cage over an extended period of time. The daily variation in feed delivery is shown in Fig. 3a.4. along with manufacturers recommended ration size and the environmental variables measured. Univariate GLM analysis showed salinity and daylength significantly affect appetite and this relationship was positive. The other variables did not significantly affect daily food demand (see Table 3a.1). Overall, the model accounted for only 18.9% of the variability in daily food intake.

3a.3.3 Time that feeding commences and ceases in relation to twilight

Feeding in post-smolt Atlantic salmon fed on-demand in a sea cage commenced at sunrise for the three-month period following smolt transfer (Fig. 3a.5). There was no clear relationship between the time of twilight and the cessation of feeding at dusk.

Table 3a.1 The results of univariate General Linear Model comparing daily feed intake with environmental factors for Atlantic salmon post-smolts in sea cages. $R_{2\ \mathrm{adj.}} = 0.189$; the model accounts for 18.9% of the daily variability in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Salinity at 4m	5.243	6.102	0.017
Daylength	4.083	4.752	0.034
Excluded from model			
Change in daylength	2.27	2.736	0.105
Turbidity	1.924	2.385	0.129
Windspeed	1.528	1.932	0.171
Tidal Range	1.805	2.421	0.127
Temperature	0.586	0.783	0.381
Rainfall	0.267	0.352	0.556

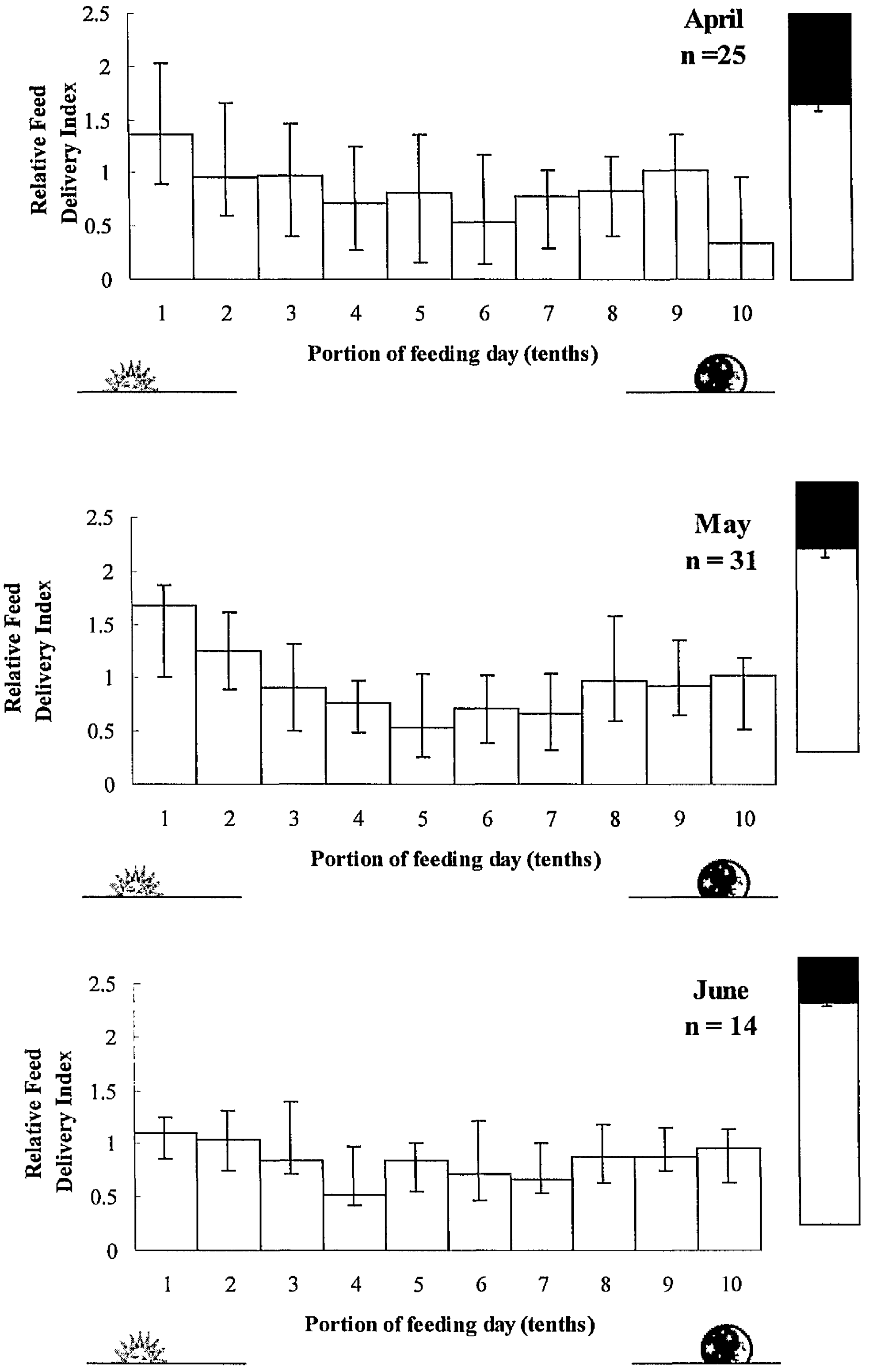


Fig. 3a.2 The relative daily feed delivery of *Salmo salar* post-smolts fed on-demand in a sea cage. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. n = sampling days month^{-1}. Block on right represents mean daylength month^{-1}, and its error bar SE of the mean. The images of the sun and moon represent sunrise and sunset.

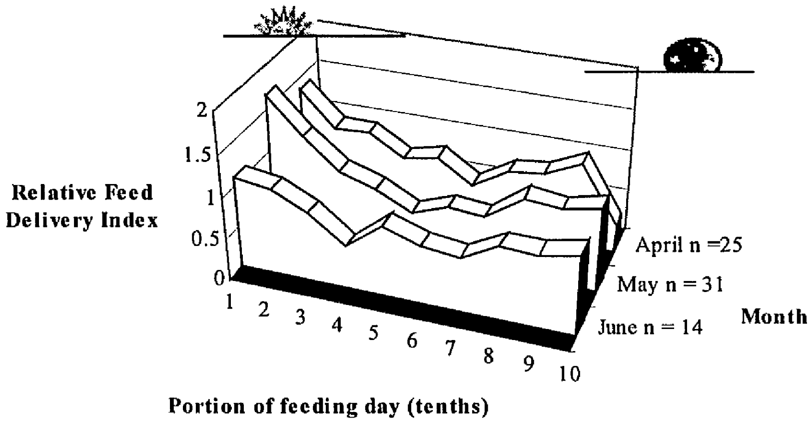


Fig. 3a.3 The relative daily feed delivery of *Salmo salar* post-smolts fed on-demand in a sea cage. Feed delivery began at dawn and ceased at dusk. n = sampling days month^{-1}. The images of the sun and moon represent sunrise and sunset.

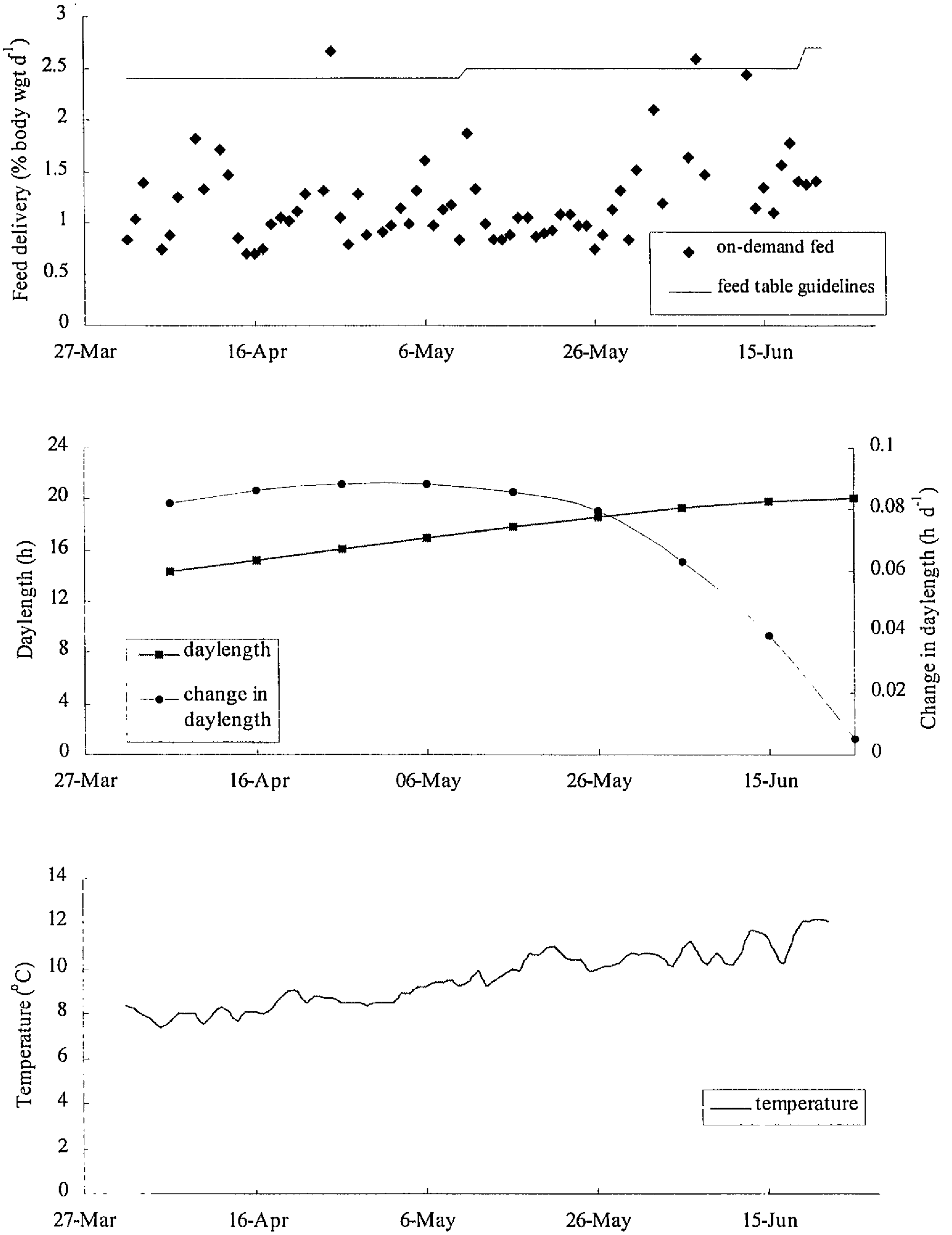


Fig. 3a.4 Daily variation in appetite of on-demand fed post-smolts, following seawater transfer (as percentage body weight day^{-1}). Also shown is the daily variation in daylength and temperature.

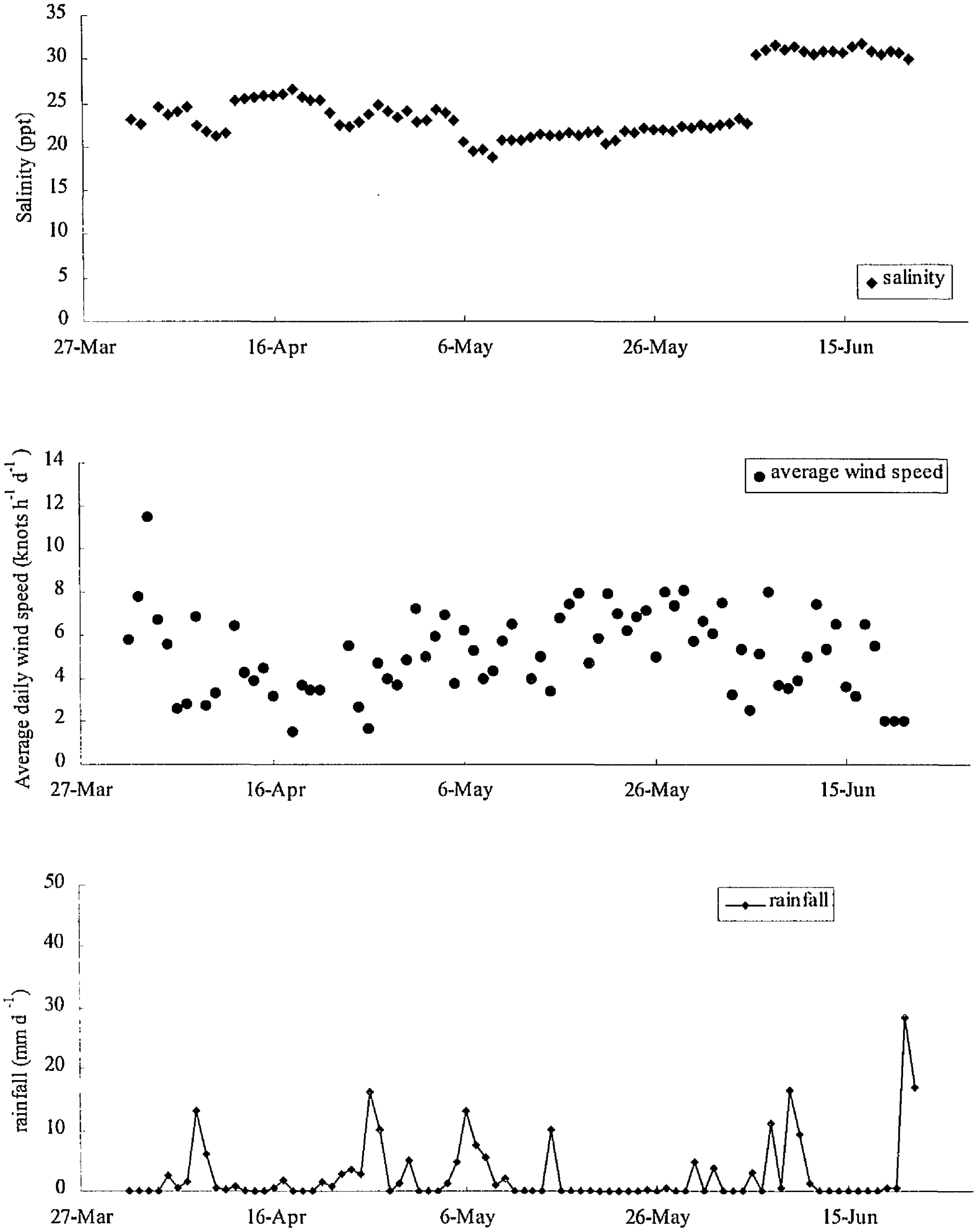


Fig. 3a.4 cont. Daily variation in salinity, windspeed and rainfall.

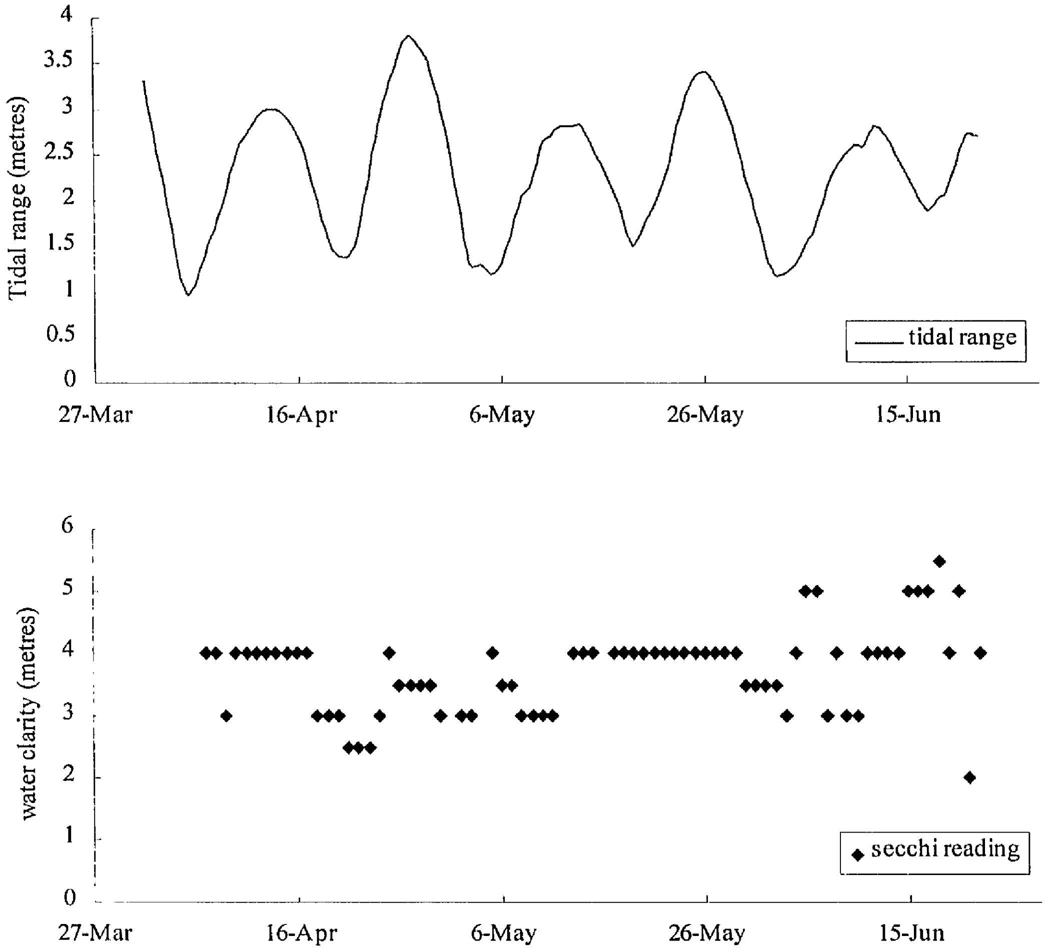


Fig. 3a.4 cont. Daily variation in tidal range and water clarity.

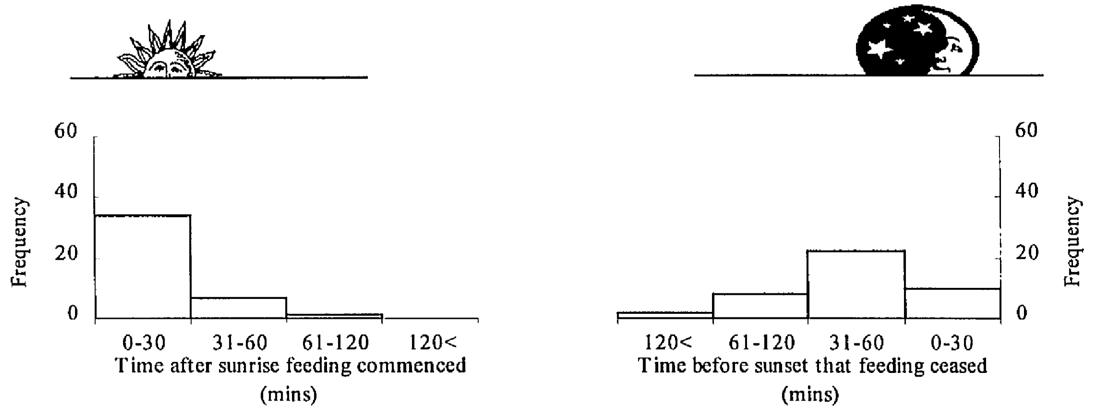


Fig. 3a.5 The effect of the beginning and end of twilight upon the time feeding commenced and ceased in post-smolts fed on-demand in a sea cage. The images of the sun and moon represent sunrise and sunset.

3a.4 Discussion

Fish do not feed with equal intensity at all times of the day (see Jobling, 1993). Feed management strategies based upon feed tables may not account for this circadian variability in appetite and may overfeed fish at certain times of the day whilst underfeeding fish at other times (Kadri et al., 1991).

3a.4.1 Daily and monthly feeding patterns

This study demonstrates that a cage-held group of Atlantic salmon post-smolts exhibit daily rhythms of feeding activity. This pattern of feed delivery changed as the months progressed. During April, the first month after smolt transfer, on-demand fed fish exhibited a significant morning peak in feeding after first light when compared with feeding at dusk. During May there was a significant difference in feeding between morning and midday, although significant differences in the timing of feeding were not observed during June. Many other studies on the feeding behaviour of adult Atlantic salmon in sea cages have reported similar findings; feeding patterns that are generally crepuscular with a significant morning peak during summer (Hoar, 1942; Kadri et al., 1991; Blyth et al., 1997; Blyth et al., 1999). This pattern has also been observed at other times of year (Blyth et al., 1993; Juell et al., 1994). In a study on juvenile Florida pompano, *Trachinotus carolinus*, Heilman and Spieler (1999) also observed a morning peak in feeding activity and suggested this peak quickly replenished anabolic substrates and energy reserves because the fish did not feed at night. The fish in the present trial were not given an opportunity to feed at night as adult Atlantic salmon are generally considered to be diurnal feeders (Kadri et al., 1991, 1997*b*; Blyth et al., 1993, 1997; Juell et al., 1994).

The lack of a significant morning peak in feeding activity during June may be due to a number of factors. Many authors have found appetite can be suppressed after smolt transfer and that it can take up to fifty days for all fish to completely resume feeding (Arnesen et al., 1998). Fish can increase their feed intake to compensate for this initial depression in appetite (Damsgård and Arnesen, 1998 and references therein) and may extend their period of feeding throughout the daylight hours during the third month following smolt transfer in response to this. Water temperature and salinity also increased during June and these factors can enhance the growth performance of Atlantic salmon in sea cages (Austreng et al., 1987). Variability in feed intake can also be influenced by stomach fullness and gastric emptying time (Grove et al., 1978; Ruohonen et al., 1997) and increased temperature can increase rates of digestion (Ruohonen et al., 1997). At the higher temperatures encountered during June appetite may return more rapidly after a feeding bout and the fish may modify their feeding time in relation to this. With a few exceptions (Noeske-Hallin et al., 1985; Baras et al., 1995; Heilman and Spieler, 1999), a farmer may be able to improve production efficiency and profits by implementing feed management strategies which synchronise daily feeding to peaks in appetite, as this can improve growth performance (Spieler, 1977; Hogendoorn, 1981; Boujard et al, 1995; Azzaydi et al., 1999).

3a.4.2 Variability in daily feed intake in relation to environmental factors

There can be marked variations in feed intake between days (Grove et al., 1978; Juell et al., 1993; Blyth et al., 1993) and several biological or environmental factors may contribute to this variability. As stated earlier, some of this variation may be related to changes in stomach fullness and appetite (Ruohonen et al., 1997), but it may also be affected by changes in environmental variables (see Bégout Anras, 1995).

This study showed that there was a clear and distinct difference in overall feed intake between days in a cage held group of on-demand fed Atlantic salmon post smolts. Daily feed intake increased with time. GLM analysis showed the best predictors of daily feed intake were salinity and daylength, but the model only accounted for around 20% of the variability in feed intake. It is not surprising that daylength can govern appetite as it is considered a primary Zeitgeber (an external periodic influence) in entraining feeding rhythms (Eriksson and Alanärä, 1992; Boujard and Leatherland, 1992*b*). Appetite also increases with daylength (Komourdjian et al., 1976; Higgins and Talbot, 1985; Villarreal et al., 1988). In a similar study in larger Atlantic salmon in sea cages (2-5kgs), Smith et al. (1993) found daylength and the change in daylength regulated the appetite of cage-held salmon more than other factors, although the authors did not measure salinity. When sea bass, *Dicentrarchus labrax*, were held in ponds, the main abiotic factors that governed appetite were daylength, wind direction and rainfall (Bégout Anras, 1995).

The significant effect of salinity upon the appetite of post-smolts was initially surprising and may mask other factors that are responsible for low feed intake after smolt transfer. As stated earlier, appetite and growth performance may be suppressed after smolt transfer for prolonged periods (see Usher et al., 1991; Jørgensen and Jobling, 1994; Stradmeyer, 1994; Stead et al., 1996; Arnesen et al., 1998), even though osmoregulatory adaptation only takes up to ten days (Usher et al., 1991). The fish may increase their feed intake following this period of appetite suppression (Damsgård and Arnesen, 1998) and this increase may also correspond with the increased salinity. After smolt transfer the salinity of the water in the cages was around 25ppt, which is less than full strength seawater (33-34ppt). Smolts may initially benefit from being transferred to lower salinity seawater whilst they are undergoing osmoregulatory adaptation (Bjerknes et al., 1992). After this initial ten day adaptation period (Usher et al., 1991) lower salinities may be detrimental to appetite. In salmonids there have been many conflicting findings of the effect of salinity upon appetite. Usher et al., (1991) and Duston (1994) concluded differing salinities had no effect on the growth of Atlantic salmon smolts following smolt transfer, whereas Austreng et al., (1987) suggested smolts grow better with increased salinity. In rainbow trout, *Oncorhynchus mykiss*, low salinities can reduce appetite (McKay and Gjerde, 1985) and evacuation of gut contents is more rapid at higher salinities (MacLeod, 1977). In addition, there was optimal feed intake at intermediate salinities in coho salmon, *Oncorhynchus kisutch*, (Otto, 1971). In other species, feed intake increases with salinity (for example, red Florida Tilapia, a hybrid tilapia (Watanabe et al., 1988); grass carp, *Ctenopharyngodon idella* and common carp, *Cyprinus carpio* (Wang et al., 1997)).

Cages can be a highly unpredictable environment and many abiotic factors that can affect feed intake cannot be governed or controlled by an aquaculturist. An aim of studying feeding rhythms and feed intake of fish in cages is to identify factors that can affect feed intake so a farmer can integrate these into feed management strategies. Many existing strategies are based upon feed tables which state fish size and temperature are primary factors governing food intake (see Austreng et al., 1987), whereas the model in the current study dismissed temperature as a predictor of daily feed intake. Temperature may become more important as a predictor of daily feed intake over longer periods, where there is a greater range of temperatures.

3a.4.3 Timing of feed delivery in relation to sunrise and sunset

Kadri et al. (1997*a*) reported that aggression in groups of Atlantic salmon parr was highest after first light and this delayed the feeding response of parr in both spring and autumn. Using the time feed delivery commenced in relation to sunrise it is possible to investigate whether this phenomenon existed after smolt transfer in a larger group of Atlantic salmon. In this trial feeding generally commenced at sunrise (see Figure 3a.5) and the results of this study suggest that there was no abatement in feeding due to aggression. This may have been due to a lack of aggression after smolt transfer, or it may be a group size effect (Li and Brocksen, 1977). The study by Kadri et al. (1997*a*) used 15 fish whereas the group in this trial consisted of approximately 800 fish. Aggressive behaviour can be curtailed within larger groups and at increased stocking densities (Kalleberg, 1958; Fenderson and Carpenter, 1971).

3a.4.4 Conclusions

This study supports the hypothesis that fish do not feed at all times of the day. Post-smolt Atlantic salmon fed on-demand show a significant peak in appetite after first light for two months following smolt transfer in relation to later periods of the day. This peak in appetite is not retained during the third month. If a farmer can modify existing feed management strategies to account for this peak in appetite it may improve production efficiency and the growth performance of the fish (see Spieler, 1977, 1990; Azzaydi et al., 1999). The peak in morning appetite commenced immediately after dawn and did not appear to be affected by high levels of aggression. In this study the best predictors of daily feed intake were salinity and daylength and the relationship was positive. Although a farmer has difficulty controlling abiotic parameters in cage culture, knowledge of their effects may improve feed management strategies if an aquaculturist can monitor these variables.

Chapter 3b: How feeding regime can affect the behaviour and growth performance of post-smolt Atlantic salmon in sea cages

3b.1 Introduction

3b.1.1 Aims and content

In this chapter I will review some of the current feeding management strategies employed in commercial aquaculture. I will then discuss some aspects of behavioural interactions between Atlantic salmon conspecifics, and the circumstances in which they can arise in both the wild and aquacultural systems in relation to food presentation. The aim of the study described in this chapter was to examine possible impacts that different feeding regimes can have upon the social interactions between individuals within a cage, exploring how they may affect growth performance and production.

3b.1.2 Feeding management strategies used in aquaculture

Feed management has three primary constituents; there is firstly the amount of food the fish should be given, and then the temporal and spatial presentation of this food to the fish (Talbot et al., 1999). In addition, there is the question of which technique or feeding method to choose for its implementation. A farmer has to give each of these options serious consideration, as feed management can have a considerable impact on the profitability of an aquaculture venture, with food expenditure accounting for up to 60% of the total running costs of a seawater farm (D. Mitchell, pers. comm; Blyth et al., 1993).

3b.1.2.1 Ration size

Commercial fish farmers tend to rely upon empirical data relating to feed charts and food type when implementing a feeding regime and calculating daily ration. Although feed tables are based on extensive research into fish nutrition, they assume fish will consume food whenever it is offered, irrespective of time of day or feed regime and do not account for changes in appetite within a day. As a consequence, fish farmers generally impose meal times that are suitable for the operation of the farm, rather than the demands of the fish, and this can increase their feed conversion ratios (FCR) because of pellet wastage (Thorpe and Cho, 1995).

Diel and seasonal variation in fish feeding patterns is of particular interest to the aquaculture industry, because it determines when food is eaten and when it is wasted. Feed intake can vary both within and between days, at an array of levels from the individual upwards (Grove et al., 1978; Cui and Wooton, 1988; Jobling and Baardvik, 1991; Juell et al., 1993; Blyth et al., 1993). This variation is difficult to quantify and control for farmers using existing feed tables. Overfeeding fish is not only uneconomical in terms of feed wastage, but it also pollutes the environment (Thorpe and Cho, 1995) and under current environmental protection regulations, may decrease the sustainability of a farm. Deteriorating water quality may also be detrimental for the health of fish within the culture system (Wedemeyer, 1997). From a commercial viewpoint underfeeding reduces the growth potential of fish and increases growth disparity as competition for food increases (see for example Magnuson, 1962; Kadri et al., 1996). Furthermore, restrictive amounts of food have been shown to increase competitive ability and aggression (Symons, 1968; McCarthy et al., 1999) and can also affect proximate body composition (Hung et al., 1993).

3b.1.2.2. Temporal presentation of food

Fish may not show an inclination to feed at all times of the day and if allowed to feed voluntarily may exhibit preferential peaks of appetite within a 24-hour period (Jobling, 1993). The effective design of feeding regimes requires recognition of patterns in appetite and competition for food amongst fish, as feeding out of phase with voluntary rhythms can have a deleterious effect on potential growth performance. The time of day that fish receive food has been reported to affect growth performance and food intake in many cultured species (see earlier chapters). The timing and frequency of food delivery can also influence a number of other commercially important production characteristics, such as feed conversion ratio and growth depensation (Boujard et al., 1995; Jobling, 1995; Sveier and Lied, 1998).

3b.1.2.3 Spatial presentation of food

Successful feed management strategies must integrate the timing, quantity and spatial components of feed delivery. Food may be distributed across the water surface in a number of ways ranging from spatially clumped to highly dispersed, depending on the individual or technical apparatus involved. When food is delivered in a spatially localised fashion, an individual can restrict its movement to a small region of high resource density (Grant, 1993; Jørgensen et al., 1996), which may promote competition. Highly competitive individuals can exclude less competitive individuals, which may lead to resource monopolisation and disproportionate food acquisition (McCarthy et al., 1992; 1999). Further, a visual forager such as Atlantic salmon may miss a feeding opportunity because the prey item does not fall within its reactive distance (Dill, 1978). Increasing food dispersal can proliferate the feeding response by offering more fish a feeding stimulus and can render a food source indefensible by stronger competitors, making the resource more accessible (Magnuson 1962; Ryer and Olla, 1996*b*). This is supported by a study on Atlantic salmon in a sea cage (Thorpe et al., 1990), where more individuals had access to the food resource when it was spatially dispersed.

3b.1.3 Competition

Feed management strategies may not only influence commercial production issues such as weight gain or FCR; they may also affect behavioural interactions between conspecifics within a rearing system by stimulating competition. Behavioural variation amongst individuals of a species can have far reaching effects on intraspecific interactions in ecological communities (Schindler et al., 1997 and references therein). This variation can be evident during feeding and is widespread in aquacultural rearing systems (Kadri et al., 1997*a*).

There are a number of ways this behavioural variation can be expressed. It can occur during scramble competition, when a poor forager is non-aggressively excluded from feeding by a faster reacting competitor (see Gill and Hart, 1996) during a feeding bout. It can also manifest itself in interference competition, when an individual is aggressively thwarted by a conspecific in its attempts to feed (Ryer and Olla, 1998). Atlantic salmon parr may refrain from feeding when a competitor is present (Metcalfe, 1989; Huntingford et al., 1993) due to intimidation (Huntingford et al., 1993). This form of antagonistic exclusion becomes increasingly important when food is inadequately dispersed among a group of competing individuals, or is delivered in insufficient amounts.

Inappropriate feeding regimes can lead to increased competition (Kadri et al., 1997*a*), which can be energetically costly (Godin and Rangeley, 1989). During feeding a fish can exhibit increased swimming activity and swimming costs are suggested to increase with manoeuvre complexity (Boisclair and Tang, 1993; Tang and Boisclair, 1993; Krohn and Boisclair, 1994).

3b.1.4 Aggression in Atlantic salmon

Aggression is common in many species of teleost fish (see for example, Jones, 1983; Grant and Kramer, 1992; Grand and Grant, 1994; Hecht and Uys, 1997; Robb and Grant, 1998) and is a well documented behaviour between juvenile Atlantic salmon conspecifics in the wild (Kalleberg, 1958; Keenleyside and Yamamoto1962; Wankowski and Thorpe, 1979). It can involve ritualised threat behaviour in the form of displays, as well as overt chases and attacks and it is often over resources such as food or shelter.

A consequence of aggressive behaviour in salmonids can be injury to the fins, primarily the dorsal fin (Abbott and Dill, 1985; Christiansen et al., 1992; Turnbull et al., 1998). This form of injury is usually termed fin damage and is common in aquaculture (Turnbull et al., 1996; Turnbull et al., 1998). In aquaculture this damage is considered a sign of inappropriate rearing conditions (Alanärä and Brännäs, 1996) and may encourage pathogenic infection by *Aeromonas salmonicida,* which causes furunculosis (Turnbull et al., 1996). Furthermore, it can decrease the market appeal of whole fish and is thus receiving increasing attention from the aquaculture industry.

3b.1.5 Implications of both aggression and variation in competitive ability

Recipients of overt aggression are encumbered with increased metabolism and tissue repair (Nicieza and Metcalfe, 1999). Fish may also become stressed due to aggression from conspecifics (Wedemeyer, 1997) and this can be detrimental to growth (Pickering, 1993), immuno-competence (Peters et al., 1988; Salonius and Iwama, 1993), tissue repair (Gregory and Wood, 1999), appetite and food conversion efficiency (Jobling and Wandsvik, 1983; Abbot and Dill, 1989). Competition can skew the distribution of food amongst a population and can cause growth depensation, where increased variation in size within a population is related to differences in growth rate (Jobling, 1995; Kadri et al., 1996). Fish that are competitively excluded from a feed resource will grow at sub-optimal rates. There is increasing commercial interest in reducing competition and aggression as the industry endeavours to maximise growth and produce fish of a more uniform size (Noakes and Grant, 1992).

Interactive on-demand feeding systems, which accurately match feed delivery to appetite, have the potential to provide solutions to some of these problems by allowing fish to regulate the size and timing of feed delivery. An aquaculturist may be able to produce fish of a more uniform size as it has been inferred that growth variation within a population can be reduced when fish have increased access to a feed resource (M^{c}Carthy et al., 1992).

3b.1.6 Objectives of the study

The questions I will address during this chapter are: -

i) Does feeding regime affect growth performance and other production results?

ii) How does a feeding regime, amongst other multiple factors, affect the social environment within a cage?

iii) Do feeding strategies have any impact on the injury levels of farmed fish?

The experiment was conducted in a production cage environment where farmers strive to maximise efficiency, product quality and growth. This production-orientated approach will be used to address how feeding strategies can be improved to produce data for more acceptable feeding practice. Although the results are based upon a one-sample experiment I hope they can give an insight into what is happening within a cage in relation to methods of food presentation.

3b.2 Materials and methods

3b.2.1 Fish husbandry

For an outline of fish husbandry used in this study, refer to Chapter 3a.

3b.2.2 Sampling methods for growth and morphological studies

A sample of fish was taken from each group just prior to smolt transfer on the 23rd and 24th March, and then on a monthly basis for three months. The entire group of fish was crowded into a small area of the cage and netted out at random. This procedure was carried out by the same person using the same equipment at each sampling operation to reduce variation. The sample size was either 100 or 200.

Fish were anaesthetised using benzocaine and excess water was drained before they were weighed (wet weight to nearest 0.5g) and assessed for morphological injury. The number of individuals with dorsal fin damage (defined as fin splitting: Turnbull, 1992) was noted prior to smolt transfer and monitored monthly thereafter.

3b.2.3 Observations of social behaviour

An underwater camera (Model RS410, Rovscan UK Ltd, Perth) positioned 2m below the water surface and 1m from the cage wall was used to record the behaviour of fish before, during and after a meal. The camera was connected to a video recording device (either a Sony Video Walkman or a 12v Panasonic 1070 video recorder).

Whenever possible fish in each cage were filmed once weekly, over a period of nine weeks. Filming took place only on calm days. The fish under the imposed regime were filmed during the second feed of the day at 8.00am; the on-demand fed fish were filmed during their closest meal to that time. The on-demand fed fish selected the duration of a meal, so the length of the feeding bout was variable and the entire feeding episode was analysed. The meal duration of the imposed regime was five minutes. Data from both treatments was transformed into the number of aggressive interactions fish^{-1} min^{-1}. From the video recordings, the number of aggressive interactions in the five minutes prior, during and five minutes after the meal were counted. These were defined as displays, chases or nips. When an aggressive encounter was observed the video was paused and the number of fish on the screen was counted. If there were any overt interactions the attack site on the assaulted individual was scored.

Swimming speeds were also examined from the video recordings. The time taken for each of 10 fish to swim one body length was measured for five minutes prior, during and five minutes after a meal using video analysis. If there were more than 10 fish on the screen, measured fish were chosen at random by recording the first fish that swam across the centre of the screen. This minimised observer bias towards faster moving fish. The time to swim one body length was converted into bodylengths per second for subsequent analysis. Thirty fish from each of five filmed meals were selected at random during the feeding period and any observed feeding events were recorded.

Variable swimming manoeuvres are known to be more energetically costly than swimming in a straight line (Krohn and Boisclair, 1994). Turning angle was therefore used as an index of the amount of energy an individual was willing to expend to acquire food pellets under each feeding regime, using the hypothesis that the choice of turning angle may reflect feeding motivation. During a feed attempt the degree of turn an individual executed was classified as follows (see Fig. 3b.1):

1 - an individual makes a turn of 0 – 45 degrees during a feed attempt.

2 - an individual makes a turn of 46 – 90 degrees.

3 - an individual makes a turn of 91 – 135 degrees.

4 - an individual makes a turn of 136 – 180 degrees.

The extent of each turn was assessed using slow motion video analysis. Feed attempts were classified only where they could be easily observed from the video footage.

3b.2.4 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0, Minitab Inc., Philadelphia) or SPSS (v. 10-0, SPSS Inc., Chicago) for MS Windows. Data were natural log transformed to satisfy normality and homogeneity of variance requirements. As this was a one-sample experiment, a two-tailed Kolmogorov-Smirnov was used to test the significance of any differences between the distributions of wet weight (grams) for each treatment.

A repeated measures ANOVA was used to test for possible differences between swimming speeds both within and between treatments. Differences between the number of aggressive interactions around meal times within each treatment were tested for using a non-parametric Kruskal-Wallis test. Differences in the degree of turn executed by individuals during feeding between treatments were tested for using a non-parametric Mann Whitney test. A Chi^2 test was used to test for differences in feeding intensity between treatments. Further statistical testing was prohibited as the study was based upon a single replicate. Fish that showed evidence of dorsal fin damage were split into five relative size categories (wet weight) using percentiles. Percentiles divided each monthly weight-frequency distribution at 20, 40, 60 and 80, which divided the group into five categories (0-20 being the smallest fish, 81-100 being the largest).

Specific Growth Rate (SGR, a function of % body weight per day) was calculated using [(ln Final weight (g) – ln Initial weight (g))/ Time in days] x 100, where ln is natural log. Within-group variability in size was calculated using Coefficient of Variation: CV = (S.D. / Mean wet weight (g)) x 100, where S.D. is standard deviation. Feed Conversion Ratio was calculated using [Amount fed (kg)/ Wet weight gain (kg)] for each growth period. Data are presented as means ± S.E unless stated. A significance level of $P < 0.05$ was used for all statistical tests.

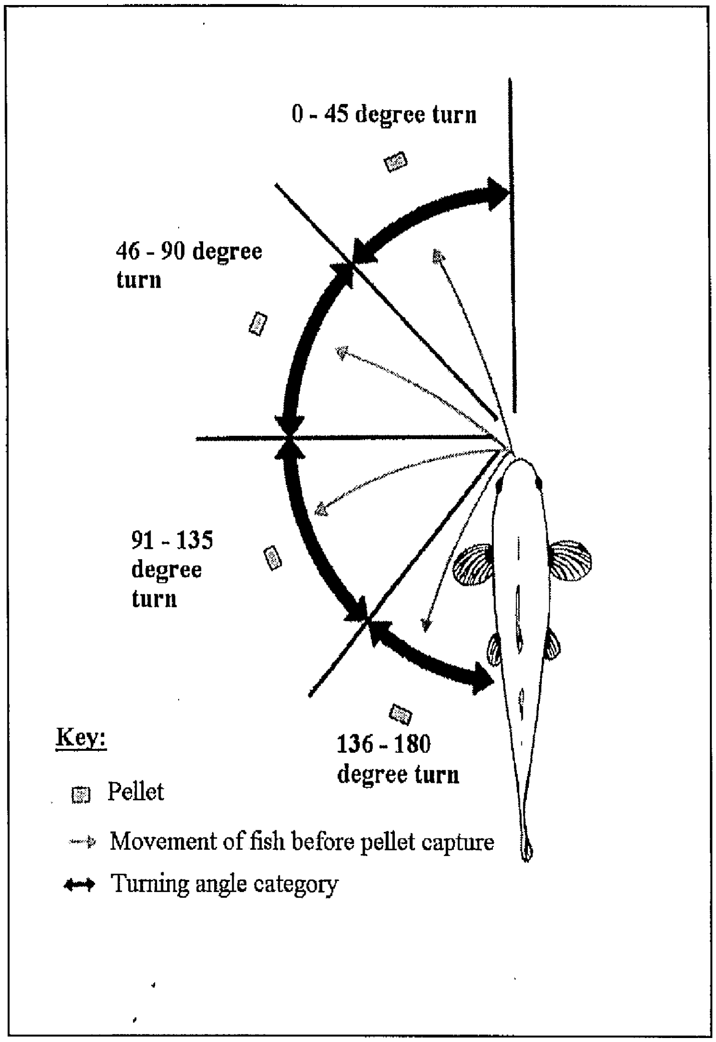


Fig. 3b.1 Classification of degree of turn executed during a feed attempt in post-smolts. The turn was executed before pellet capture.

3b.3 Results

3b.3.1 Fin injury

To evaluate the hypothesis that feed regime can have an effect upon fin injury, the occurrence of fin damage (splitting) was noted during sampling periods. Prior to smolt transfer there was no difference in the numbers of fish affected by dorsal fin injury for each treatment group (Fig. 3b.2). One month after smolt transfer there was a reduction in the number of fish affected by dorsal fin damage in on-demand fed fish to 33%, whereas the level of fin damage in the imposed group remained at 57%. Two months after smolt transfer neither group showed evidence of dorsal fin injury and no further evidence was noted for the duration of the trial.

Prior to smolt transfer there was evidence of greater dorsal fin damage in the smallest fish under the imposed feeding regime, whereas there was no distinct pattern for on-demand fed fish (refer to Figs. 3b.3a). The size of fish affected by fin damage was then assessed one month after smolt transfer (see also Figure 3b.3b). The smallest fish were most affected by fin injury for both treatments; after smolt transfer it appears that only the overall prevalence of fin injury is affected by feed regime.

3b.3.2 Aggressive interactions within the sea cages

Aggressive behaviour in the form of displays, chases or nips was rarely observed in either the imposed or on-demand fed group. Any overt aggression resulted in the aggressor attacking the opercular region, pectoral fin or lower segment of the caudal fin. Overt aggression in the form of nips or chases accounted for 78% of aggressive interactions in the imposed feeding group and all aggression observed in the on-demand fed group was overt. No reciprocal acts of aggression or biting of the dorsal fin were observed in either treatment. In the imposed feeding treatment the number of aggressive interactions that took place before and after a meal was significantly less than during a feeding bout (Kruskal-Wallis H_{adj} = 13.30, d.f. = 2, P = 0.001), whereas in the on-demand feeding treatment there was no significant difference (Kruskal-Wallis H_{adj} = 4.13, d.f. = 2, P = 0.127). Aggressive interactions during feeding were less frequent in the on-demand fed group. No aggressive interactions were observed after feeding in either treatment group (refer to Fig. 3b.4). Only a single incidence of an openly contested pellet was witnessed during the filmed meals and this was in the imposed feeding regime. The results suggest on-demand feeding reduces the incidence and frequency of aggression per capita around meal times. There were also other behavioural differences between groups of fish held under different feeding regimes.

3b.3.3 Swimming speeds and turning angle

To examine the hypothesis that feed regime can affect swimming speeds (presumed to reflect hunger levels), the swimming activity of fish was compared before, during and after a meal for each treatment using repeated measures analysis of variance. There was a quadratic relationship between swimming speed and time around a meal (repeated measures ANOVA: effect of time $F_{2,336}$ = 35.67, $P < 0.001$; quadratic term of polynomial contrasts: $F_{1,168}$ = 73.00, $P < 0.001$). This relationship was not consistent between feeding regimes (interaction between time around a meal and regime: $F_{2,336}$ = 17.06, $P < 0.001$). There was a significant effect of feed regime upon swimming speed (effect of regime: $F_{1,168}$ = 28.95, $P < 0.001$), see also Fig.3b.5. From Figure 3b.6 it can be seen that a greater proportion of feeding events (mean percentage of fish reacting to pellets during a meal) were observed in the imposed feeding regime cage than those fed on-demand (χ^2 = 8.33, d.f. = 1, P=0.004), implying a greater feeding intensity during meals when fed by normal practice.

Another variable, which may be related to competition levels, is the degree of turn an individual is prepared to undertake to obtain food items. It was noted that the turn was executed prior to pellet capture. The results in this study suggest that fish fed to the imposed regime incur increased costs in pellet capture, with turning angles being significantly greater prior to pellet capture than in on-demand fed fish (imposed median = 2, on-demand median = 1, Mann-Whitney $W = 790.0$, $P = 0.0014$). This means fish under the imposed treatment undertook a turn of on average 46-90°, whereas on-demand fed fish turned on average 0-45° to obtain pellets (Fig. 3b.7).

3b.3.4 Effect of feeding regime on weight gain and production

Feeding regime did have an effect on wet weight gain during the trial (Fig. 3b.8). There was no significant difference in the size of fish when they were transferred to seawater cages (Two-sample Kolmogorov-Smirnov test, $D = 1.273$, $P = 0.078$). On-demand fed fish showed significantly better weight gain than imposed fed fish after one month in seawater, (Two-sample Kolmogorov-Smirnov test, $D = 1.556$, $P = 0.016$). Differences between the weight distributions for each treatment was not noted in May, although there was a marginally significant difference in mean weight between treatments, with fish fed on-demand weighing more, (Two-sample Kolmogorov-Smirnov test, $D = 1.344$, $P = 0.054$). In June on-demand fed fish again showed significantly better weight gain, (Two-sample Kolmogorov-Smirnov test, $D = 2.121$, $P < 0.001$). There was a slight difference in Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) during the trial, with the on-demand fed group having better growth and feeding efficiency (Fig. 3b.9).

These differences in weight gain and FCR may have been the result of differences in monthly ration between regimes (Fig. 3b.10). Monthly rations show that fish under the imposed regime were consistently underfed in comparison to the on-demand fed fish. This suggests that the existing technique of using cessation in surface feeding activity to determine satiation is inaccurate in sea cage culture. There was also an increase in the size heterogeneity of fish under the imposed regime with time, after an initial decrease one month after smolt transfer. The size variation in on-demand fed fish remained relatively homogenous in comparison (Fig. 3b.11).

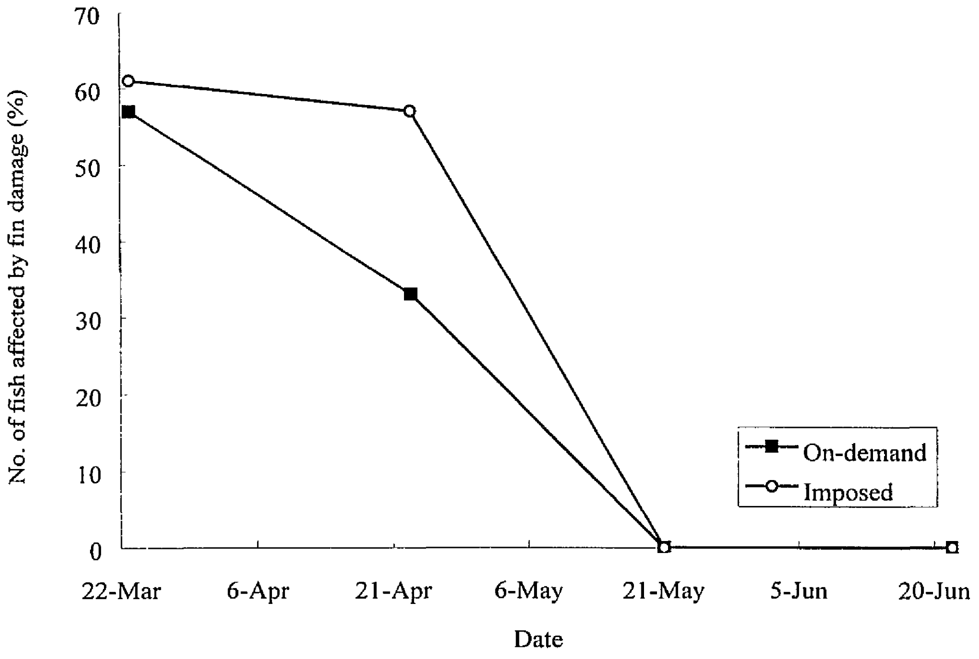


Figure 3b.2 The relationship between feeding regime and the number of fish (%) affected by dorsal fin damage following smolt transfer. NB. Error bars are not applicable for this data set.

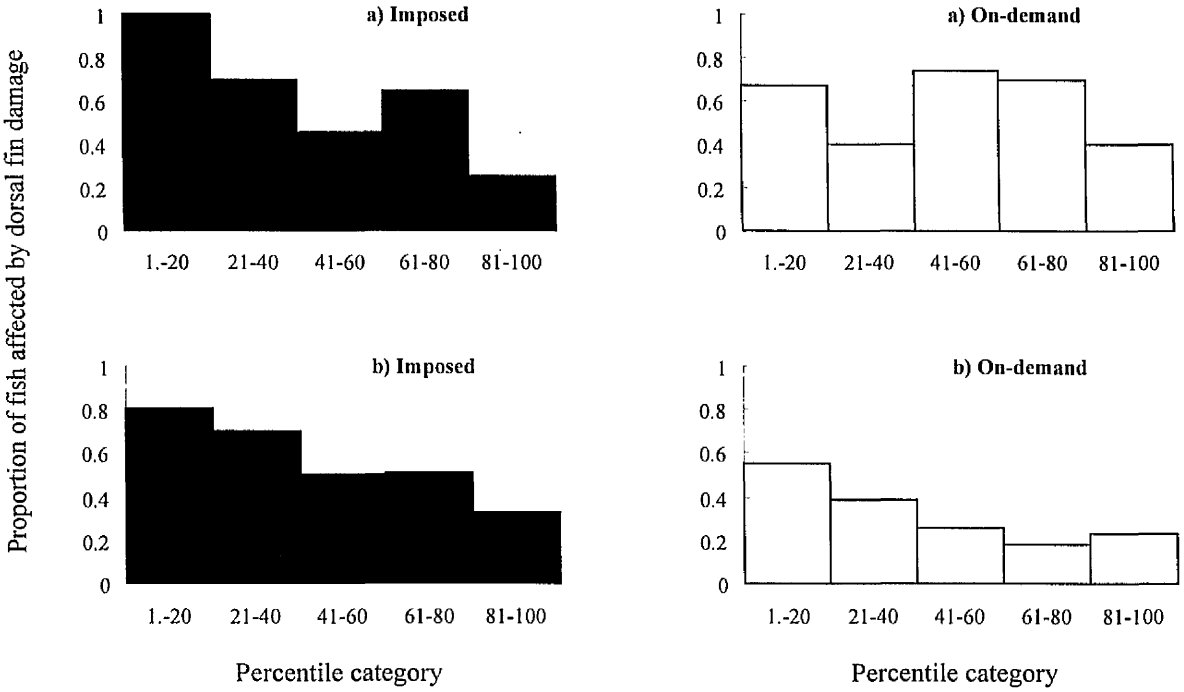


Fig. 3b.3a,b The effect of feeding regime upon the prevalence of fin damage according to size category. Fish that showed evidence of fin damage were split into five relative size categories using percentiles at 20, 40, 60 and 80 (0-20 being the smallest fish, 81-100 being the largest). Samples were a) two days prior to smolt transfer, and b) one month after smolt transfer. NB. Error bars are not applicable for this data set.

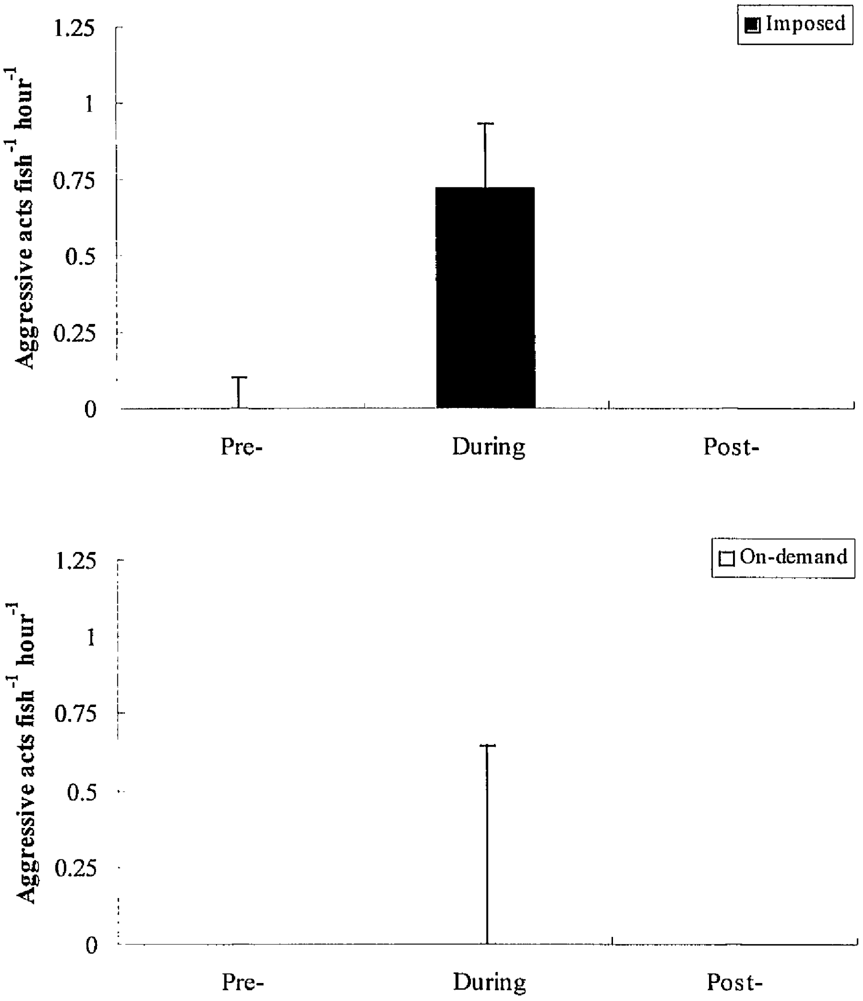


Fig. 3b.4 The effect feed regime upon aggressive interactions around meal times of post-smolts held in sea cages. Main bars indicate median values and error bars represent interquartile range.

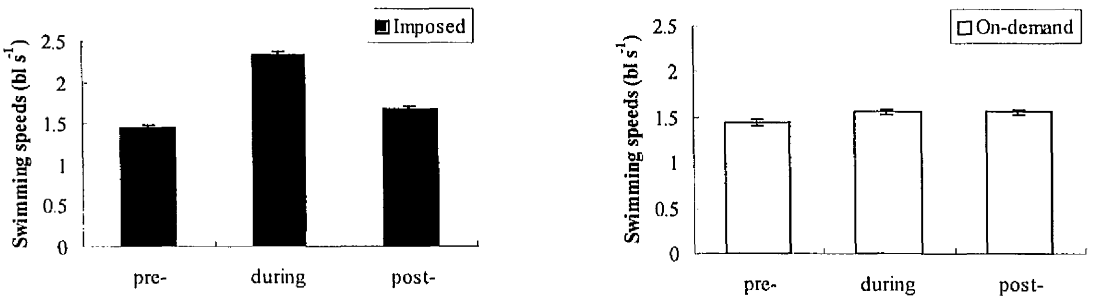


Fig. 3b.5 The effect of feeding regime upon the swimming speeds of post-smolts around meal times. Main bars indicate mean values and error bars represent standard error of the mean.

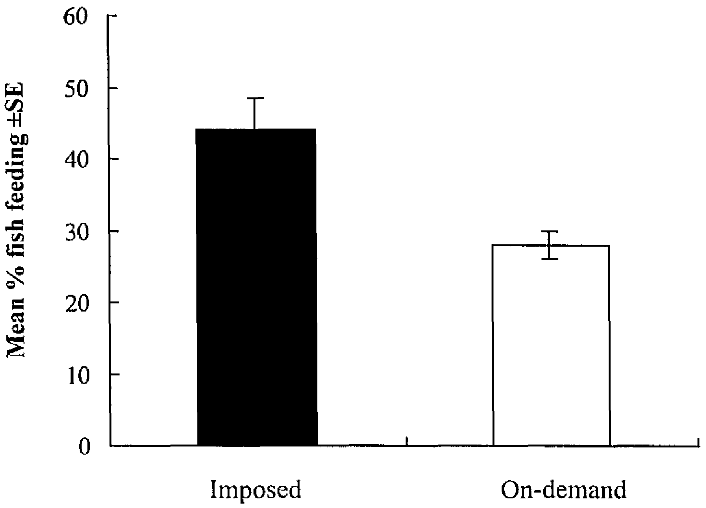


Fig. 3b.6 The effect of feeding regime upon feeding intensity (the % of fish reacting to pellets during a feeding bout) in post-smolts. Main bars indicate mean values and error bars represent standard error of the mean.

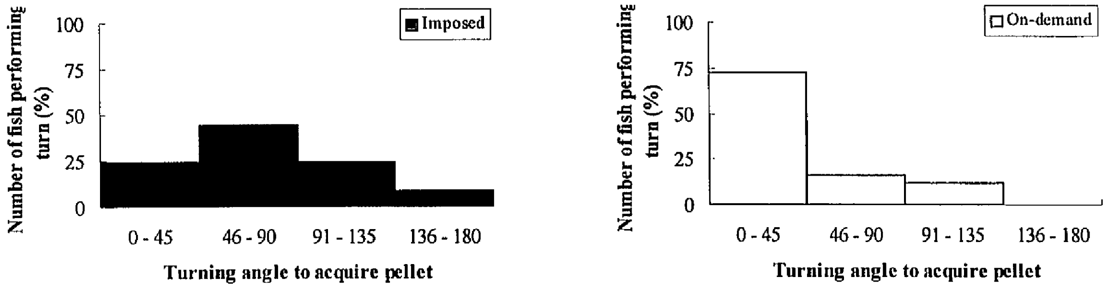


Fig. 3b.7 The relationship between feeding regime and turning angle executed during feed attempts in post-smolts. Note that the turn was executed before pellet capture (see text).

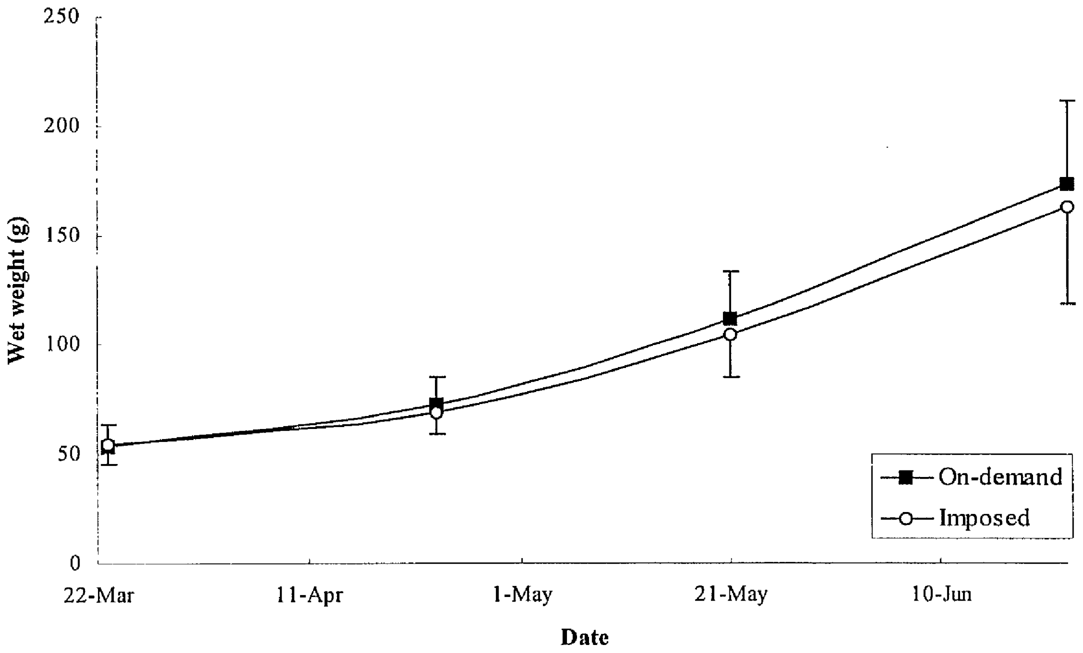


Fig. 3b.8 The effect of feeding regime upon the wet weight gain of post-smolts following seawater transfer. Growth is expressed as mean wet weight (grams) and error bars indicate standard deviation. Either + or - error bars are shown to aid clarity.

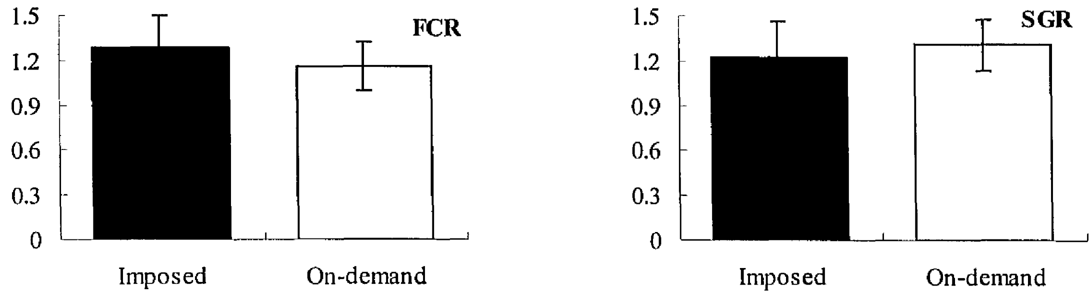


Fig. 3b.9 The effect of feeding regime upon feed conversion ratio (FCR) and specific growth rate (SGR) in cage held post-smolts following seawater transfer. Error bars indicate standard error of the mean. For an explanation of calculations refer to text in methods section (this chapter).

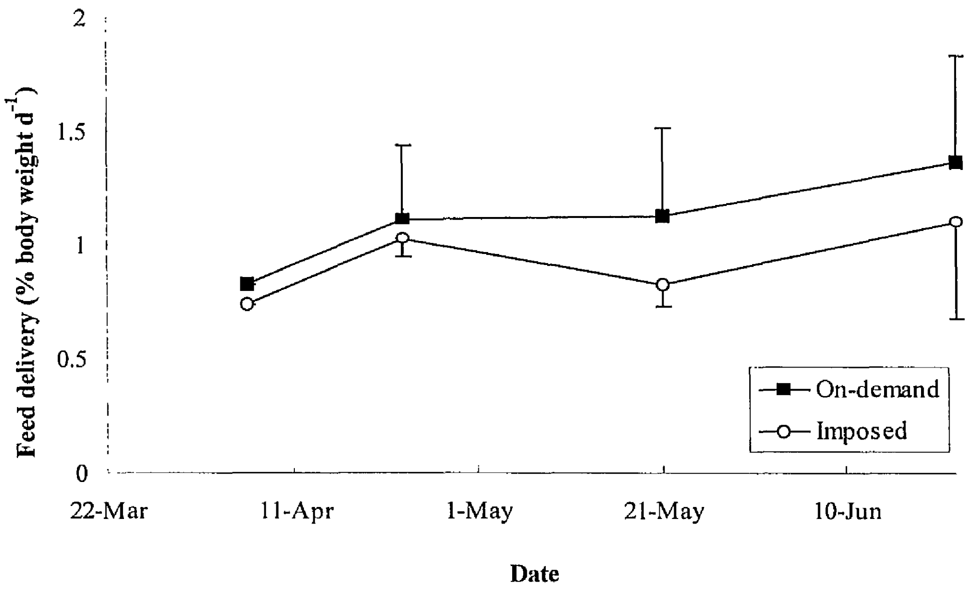


Fig. 3b.10 The differences in mean monthly ration between the on-demand and imposed feeding regimes. Error bars indicate standard deviation (either + or – error bars are shown to aid clarity).

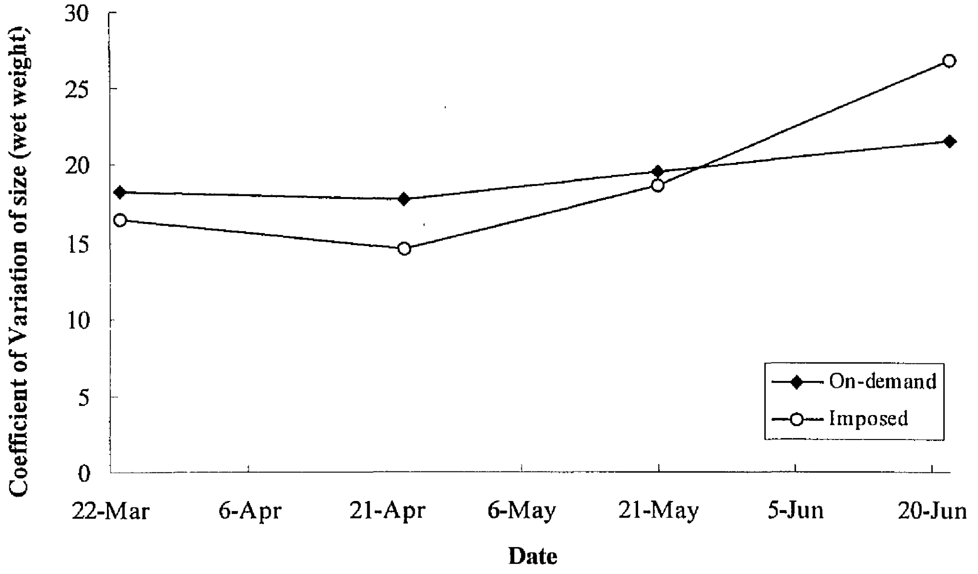


Fig. 3b.11 The effect of feeding regime upon the size variation of cage held post-smolts. Size variation is expressed as coefficient of variation (wet weight).

3b.4 Discussion

3b.4.1 Ration differences between feeding regimes

By evaluating differences in ration size between on-demand and imposed feeding regimes a farmer can appraise the accuracy of current feeding techniques in determining satiation. Although current husbandry methods were used to determine satiation (visual cues of surface activity and camera work), the fish under the imposed regime were underfed for long periods in comparison with on-demand fed fish (which selected the size of ration in relation to changes in group appetite). This is not entirely unexpected, as monitoring surface feeding activity is known to be an inaccurate method for determining satiation (Juell, 1995; Ang and Petrell, 1997; Talbot et al., 1999). Fish under the imposed feeding regime received a daily ration that was below commercial feed table recommendations but interestingly, the daily ration selected by the on-demand fed fish was also consistently below manufacturer's guidelines (see Fig. 3a.4).

Food availability can primarily affect growth performance and the uniformity of growth (see earlier). In this present study the fish fed on-demand grew better than fish under the imposed regime. It is not considered appropriate to surmise from this study that feeding fish within their preferred phase improves weight gain, as fish under the imposed regime received a restrictive amount in comparison to the ration selected by fish using the on-demand feeder. The predetermined ration of the imposed regime offered restrictive amounts of food for a prolonged period of time. Food restriction can have a negative effect on growth (Weatherley and Gill, 1987; Quinton and Blake, 1990; Thorpe and Huntingford, 1992; Jobling, 1994,1995; Nakagawa et al., 1999) and in an experiment on post-smolt Atlantic salmon, Stead et al. (1996) reported that fish which received the highest ration had better growth performance.

Following a slight decrease after one month in seawater, growth depensation increased in both groups of salmon during the course of the trial. Size variation increased rapidly amongst fish under the imposed regime. In contrast, the size variation within the on-demand regime remained relatively homogenous but increased slightly towards the end of the trial; this may be a reflection of reduced competition and increased uniformity in access to feed. Competition for food amongst fish increases under conditions of resource restriction (Symons, 1968; Grant, 1993; Jobling and Koskela, 1996). As a consequence inter-individual differences in feed intake become more pronounced (Thorpe et al., 1990; Thorpe and Huntingford, 1992) and this heterogeneity in access to a feed resource can cause growth depensation (Jobling, 1995; Kadri et al., 1996). This study suggests that behavioural interactions have a greater influence on the regulation of feed intake of fish under the imposed regime than those fed on-demand. Similar results have also been reported in 1.5kg cage-held Atlantic salmon by Johansen and Jobling (1998), who reported less growth disparity in groups of salmon fed to satiation rather than to a predetermined ration. Feed conversion ratio (FCR) and specific growth rates (SGR) were also slightly better in fish fed on-demand in the present study. Underfeeding in the imposed feeding regime may have caused this as it suppresses weight gain and can increase FCR (Cho, 1992). The on-demand feeding system can also reduce pellet wastage as it terminates feed delivery when the fish are satiated, improving FCR (Thorpe and Cho, 1995). Azzaydi et al. (1999) have also reported that sea bass, *Dicentrarchus labrax*, fed on-demand using self-feeders had a better FCR than fish fed to an imposed regime.

3b.4.2 Effect of feeding regime upon swimming speeds and manoeuvre complexity

In this study swimming speeds differed between feeding regimes and also between feeding and non-feeding periods. Swimming activity prior to feed delivery did not differ between treatment groups. Fish held under the imposed regime increased their swimming activity during a meal, whereas fish fed on-demand did not. Increased locomotor activity during foraging can improve an individual's prey encounter rate (Boisclair, 1992) and has been observed in juvenile turbot, *Scophthalmus maximus*, where swimming activity escalates with food delivery (Champalbert and Le Direach-Boursier, 1998). This increased activity can be energetically costly, with individuals expending up to fifteen times more energy during sustained swimming than when at rest (Beamish, 1978). Swimming costs are also suggested to increase with manoeuvre complexity (Boisclair and Tang, 1993; Krohn and Boisclair, 1994) and can be detrimental to growth performance (Niimi and Beamish, 1974). This negative relationship between swimming activity and growth has been reported in a number of salmonids (see for example, Farrell et al., 1997; Gregory and Wood, 1999) and may be another reason for the reduced weight gain capacity of fish held under the imposed regime.

Fish held under imposed feeding conditions exhibited more complex swimming manoeuvres during feeding attempts, which is again indicative of increased energy expenditure. This study suggests the imposed feeding regime increases competition levels in comparison with an on-demand regime. This escalated competition may be associated with the imposed regime delivering a sub-optimal daily ration for prolonged periods. Atlantic salmon become more competitive when deprived of food (Nicieza and Metcalfe, 1997) and reduced rations increase swimming activity in turbot, *S. maximus* (Huse and Skiftesvik, 1985), and cod, *Gadus morhua* (Hammer, 1997).

3b.4.3 Aggression under differing feeding regimes

Aggression in Atlantic salmon is closely related to feeding and food availability (Wankowski and Thorpe, 1979; Kadri, 1997*a*). There is an increased commercial need to develop husbandry practices to reduce this. This direct behavioural study of cage held groups of post-smolt Atlantic salmon demonstrated low levels of aggression around meal times regardless of feeding regime. This is in accordance with the work of Kjartanson et al. (1988), who found there was minimal aggression amongst post smolts held in sea cages, and also with Thomassen and Fjæra (1996) who observed little aggression during feeding in 0.7-1.2kg Atlantic salmon post-smolts. If aquacultural feed management strategies can feed a group of post-smolt fish to near satiation it may reduce aggression, which can reduce the potential for injury.

In this study, low levels of observed aggression might be related to the life history stage of the fish involved. In freshwater it has been demonstrated that Atlantic salmon parr are aggressive and territorial, defending feeding sites from conspecifics (Kalleberg, 1958; Keenleyside and Yamamoto, 1962). After smoltification these parr normally form non-aggressive schools (Kalleberg, 1958) and this behavioural shift may be responsible for the observed low levels of aggressive interactions. However, more recent work on Atlantic salmon (Gibson, 1983; Damsgård and Arnesen, 1998) found hatchery reared parr and smolts exhibited similar levels of aggression. Furthermore, facultative schoolers may exhibit intense aggressive behaviour when competing for food (Ryer and Olla, 1995, 1996b). Spontaneous activity associated with aggression can elevate metabolic rate in salmonids (Brett, 1964), which may reduce the efficiency of food utilisation and increase FCR (Fagerlund et al., 1981). In both feeding regimes food was delivered in a dispersed, spatially unpredictable manner. Grand and Grant (1994) have suggested interference competition utilising resource defence is only effective when food is spatially predictable; scramble competition is favoured when resources are spatially unpredictable. The low incidence of aggression suggests that scramble competition, rather than interference competition may be the predominant mechanism in gaining access to food in this study. This is supported by the earlier section on swimming behaviour in this chapter, where underfed fish exhibit increased swimming activity when foraging, and by Kadri et al., (1996) who observed that salmon often scramble for food when held in sea cages.

Furthermore, this study has shown that an on-demand feeding regime in cage held Atlantic salmon post-smolts can further reduce this level of aggression. It is inferred that on-demand feeding reduces the resource value of feed by reducing competition, which allows more individuals access to food without being displaced by conspecifics. Competition can regulate temporal access to feed in post-smolts (Kadri et al., 1997*b*) and improving access to a feed resource can reduce the intensity of competition (Davis and Olla, 1987). The higher incidence of aggression in the fish fed to an imposed regime may be an intrinsic constituent of the feeding strategy, as a uniform and predictable rate of feed delivery can stimulate aggression (Grant and Kramer, 1992). There was no aggressive behaviour observed after a meal in either regime, indicating that fish do not aggressively defend a spent feed resource against conspecifics. This is in agreement with the economic defensibility theory proposed by Brown (1964), which suggests animals will defend a feeding site only when the economic benefits of defence exceed the costs (time, energy, potential for injury).

3b.4.4 Effect of feeding regime upon fin damage

This study suggests an on-demand feeding regime can reduce the competition for food around meal times. Can this reduced competition have any effect upon the incidence of fin injury? Fin damage is a known consequence of overt aggression in salmonids (Abbott and Dill, 1985; Christiansen et al., 1992; Turnbull et al., 1998) and its presence can be a useful indicator of how successful a feeding regime can be in reducing aggression (MacLean et al., 2000). In this study there was no difference in the incidence of dorsal fin damage between the two groups prior to smolt transfer. After one month in seawater, the on-demand fed fish showed a reduction in the incidence of fin injury in comparison to fish fed under the imposed regime. After two months in seawater there was no evidence of fin damage in either group. The results of this study therefore suggest that cage held post-smolts either recover more rapidly from fin injury when fed on-demand, or may be subject to fewer overt aggressive attacks, or it may be a combination of both.

The higher incidence of fin damage in fish fed by an imposed regime may be due to increased levels of aggression. Restrictive rations (such as those encountered under the imposed feeding regime) may increase competitive ability and aggression (Symons, 1968; Thorpe and Huntingford, 1992). A restrictive ration can increase the incidence of fin damage in salmonids (Storebakken and Austreng, 1987; Dåmsgard et al., 1997) and other species (in juvenile greenback flounder, *Rhomosolea tapirina*, Shelverton and Carter, 1998). Interestingly, on-demand feeding does not reduce the incidence of fin damage relative to fish that were hand fed in cutthroat trout, *Oncorhynchus clarki* (Wagner, 1995). It was suggested that the hand feeding regime closely matched feed delivery to appetite, reducing aggression but the on-demand feeding system may have promoted aggression due to its design. The system used by the author was an operant self-feeder, which can be a defensible food source, and Landless, (1976) observed aggressive behaviour around the area of the trigger in the rainbow trout, *Oncorhynchus mykiss*.

The dorsal fin was not an observed attack site during this trial, so it is possible that fin damage is a result of aggressive interactions that occurred during the fish's freshwater phase. It is tentatively suggested that fish fed on-demand in this study recovered more rapidly from historical fin injury than fish fed by an imposed regime. As stated earlier, feed restriction promotes competition, which is a potential stressor to fish (M^{c}Carthy et al., 1992; Wedemeyer, 1997). Stressors cause a rapid and severe elevation of plasma corticosteroids such as cortisol (see for example Ejike and Schreck, 1980; Pottinger and Pickering, 1992). Plasma cortisol levels are already heightened in smolting salmonids as an intrinsic part of the smoltification process (Barton et al., 1985). The resting plasma cortisol level of smolts is three times that of parr, whereas their stress response is 25 times greater than that of parr (Carey and M^{c}Cormick, 1998). Increased levels of cortisol can reduce growth rates and appetite (Barton et al., 1987; Gregory and Wood, 1999), and can influence tissue repair. Gregory and Wood, (1999) found cortisol treated rainbow trout exhibited greater fin damage than their corresponding controls and that this may not necessarily be associated with increases in aggression. This suggests that further to reducing immuno-competence (Peters et al., 1988; Pottinger and Pickering, 1992; Salonius and Iwama, 1993) stress is deleterious upon tissue repair. If fish under the imposed regime are subject to chronic stress their capacity for tissue repair may be impaired in comparison with those fed on-demand. To my knowledge no studies have investigated this hypothesis. Furthermore, it cannot be discounted that dorsal fin damage was caused by overt aggression during this trial, as only a few bites are required for a fin to exhibit splitting (J. Turnbull, pers. comm.).

After two months in seawater the low levels of fin injury, irrespective of feeding regime, may have been caused by an increase in ambient water temperature, which increases the healing response (Turnbull, 1992). Another suggestion for the absence of fin damage may be that conspecifics become habituated and decrease levels of aggression. There was no evidence that this occurred during the present study period and Dåmsgard and Arnesen (1998) also found no evidence of habituation in their trial on cage-held Atlantic salmon. When present, fin damage was closely related to relative fish size. The smallest fish in both regimes showed the greater incidence of fin damage. This is in agreement with previous findings, where smaller subordinate fish received most fin damage (Abbott and Dill, 1989; Gregory and Griffith, 1996; Dåmsgard et al., 1997; Moutou et al., 1998).

3b.4.5 Conclusions

A major challenge for aquaculture is to supply a correct ration size and minimise competition at feeding times. Commercially available on-demand feeding systems can help a farmer achieve this. Overall, this study suggests that in cage held Atlantic salmon post-smolts, on-demand feeding reduces competition for food and the incidence of injuries that are caused by agonistic behaviour. In post-smolt Atlantic salmon, appetite-based systems may improve weight gain, production efficiency and the uniformity of growth compared with an imposed feeding regime. A strategy of six meals per day (which on a farm without automated feeding would be very labour intensive) does not match the performance of feed management strategies based upon adapting feed delivery to appetite. On-demand systems can reduce competition within a cage environment, which in turn may improve weight gain, stress and fin injury levels. The lack of replication in this study (due to production constraints) means the results of this trial should be interpreted as indicative of how feeding regimes can affect feeding behaviour and weight gain, rather than conclusive. Further studies could be carried out to test whether the time of feeding affects growth, or to investigate the stress physiology of fish fed to differing feed regimes.

Chapter 4a: The effect of biological and environmental variables on the feeding rhythms and daily feed intake of Atlantic salmon parr in production cages

4a.1 Introduction

4a.1.1 Aims and content

In this chapter I will review some of the current literature on feeding rhythms and the factors that may affect the timing of feed intake in fishes. Then I will discuss how the timing of feeding can vary both within and between days in relation to production cage aquaculture to provide information for improved husbandry practice. The aim of this study was to investigate feeding patterns in juvenile salmon in a freshwater cage environment and assess how such patterns are affected by environmental variables.

4a.1.2 Definitions of rhythms

A biological rhythm can be described as an event or sequence of events that are repeated in time, and are involved in regulating the spatial and temporal activity of animals (Boujard and Leatherland, 1992*b*). Rhythms co-ordinate the internal activity of an organism with its external environment (Kavaliers, 1986) and can be influenced by a number of parameters that are generally classified into two categories:

- **Exogenous.** These are directly influenced by environmental or other external periodic factors, for example daylength or tide.
- **Endogenous.** These originate within an animal and are under the control of an internal self-sustaining oscillator.

Endogenous rhythms will be maintained even if an external cue is removed, whereas exogenous rhythms lose their periodicity if external cues are eliminated. Biological rhythms are adjustable and can be entrained by a Zeitgeber, an external periodic influence. The Zeitgeber controls the period of a rhythm, and also the phase (the point in the rhythmic cycle) such as the daily onset of activity. This phase control is important in animals because it ensures an individual is in synchrony with its rhythmic environment, it may synchronise the locomotor activity of a predator with its prey for example. Rhythms have a range of different timespans, which can be classified as (after Aschoff, 1981):

- **Ultradian.** The rhythm occurs more than once every 24h, or has a period of less than 20h, for example, tidal rhythms.
- **Circadian.** Circadian rhythms are cycles of around 24h ± 4h.
- **Infradian.** There is less than one cycle per 24h, for example, lunar rhythms or reproductive cycles. Within this category are **circannual** rhythms, which have a period of about one year.

4a.1.3 What cues may regulate these rhythms in fish?

Many studies relating to feeding rhythms in fish have reported a circadian periodicity (Madrid et al., 2001) and it is suggested these rhythms are generally under endogenous control, but can be reset by a geophysical counterpart such as light (Muller, 1978; Spieler and Noeske, 1984; Boujard and Leatherland, 1992*b*; Spieler, 1992; Kadri, 1995). The precise location of the endogenous pacemaker(s) influencing these rhythms is unknown but it is generally assumed to be either the pineal organ or the retina, as both are photosensitive (Tabata, 1992). The pineal organ forms part of the diencephalic brain roof of many vertebrates and there is substantial evidence that it performs a major role in the regulation of circadian rhythms in teleost fish (M^{c}Nulty, 1978; Goudie et al., 1983; Kavaliers, 1984, 1986). Light is widely accepted as the primary Zeitgeber in fish, although the change in light intensity between the light and dark phase is thought to be a more important Zeitgeber than absolute intensity or wavelength in entraining the circadian rhythm (Gibson and Keenleyside, 1966; Boujard and Leatherland, 1992*b*; Kadri, 1995). The daily change in light/dark cycle and seasonal adjustments in photoperiod normally provide the most reliable information on time of day and year (Eriksson and Alanärä, 1992) and aid an animal's adaptation to its environment. Many fish species, including Atlantic salmon, also show seasonal shifts in activity and these shifts may be temperature dependent (Fraser et al., 1993).

Another important factor that governs when food is consumed by fish is appetite. Variation in both feed intake and the motivation to feed can be influenced by gastric emptying time (see Grove et al., 1978). Many studies in salmonids have suggested appetite and its return are closely correlated to stomach fullness (Brett, 1971 in the sockeye salmon, *Oncorhynchus nerka*; Elliott, 1975 in the brown trout, *Salmo trutta*; Grove et al., 1978 in rainbow trout, *O. mykiss*). It may take a considerable amount of time (>24h in adult 1.5kg *O. mykiss*) before fish are motivated to feed after a substantial meal (Ruohonen et al., 1997) and this is dependent upon water temperature, food type and body size. This extended and variable periodicity in the return to appetite may modify the feeding patterns and rhythms of individuals held under high stocking densities, if fish are prevented from feeding to satiation during a meal.

4a.1.4 Variability in appetite and feed intake

In recent years there has been an increasing interest amongst researchers and aquaculturists in feeding rhythms (see Boujard and Leatherland 1992*a,b* for example) and their adaptive significance. For many years aquaculture has relied upon farmers' intuition coupled with feed tables to establish daily rations for fish. Although feed tables are based upon extensive research into fish nutrition, they assume fish will consume food whenever it is offered, irrespective of time of day or feed regime. In fact, many authors have reported that food intake and appetite can fluctuate both within and between days (see for example: MacLeod, 1977; Grove et al., 1978; Juell et al., 1993; Blyth et al., 1993), and can be affected by season (Higgins and Talbot, 1985; Rowe and Thorpe, 1990; Jobling and Baardvik, 1991; Blyth et al., 1993,1999). Various abiotic and biotic factors such as photoperiod, temperature, stocking density and competition (see Alanärä, 1994*b*) may play roles in how variations in appetite manifest themselves in a high-density production cage environment.

An aquaculturist can improve feed management strategies by considering circadian feeding rhythms when implementing feeding techniques (Kadri et al., 1991; Boujard and Leatherland 1992*b*; Heilman and Spieler, 1999). The timing of feed delivery can be associated with increased growth and food utilisation (Spieler 1977, 1990) and should be examined when attempting to improve production efficiency.

4a.1.5 Circadian and circannual feeding rhythms

Many animals are active during the day or night, but do not exhibit uniform activity levels during a 24h period. These rhythms can be related to the time of day when an individual can balance a trade off between food availability and predation risk (Helfman, 1993) and relate to evolutionary adaptations to their environment (Forrester et al., 1994; Thorpe and Cho, 1995). When aquaculture species become domesticated they can lose some of these adaptations, though some general feeding patterns can be retained (Thorpe and Cho, 1995) and may be expressed when fish are fed to satiation (Blyth et al., 1997). Feeding rhythms are widely defined as (Thorpe, 1978):

- **Diurnal.** Feeding take place during hours of daylight.
- **Nocturnal.** An animal feeds during the night.
- **Crepuscular**. Where feeding occurs at dawn and dusk.

It is difficult to strictly classify certain species to any of the above categories as the choice of feeding time can be affected by season (see Fraser et al., 1993, 1995).

Many authors have reported upon the feeding rhythms of salmonids in both the wild and cultured environments (for selected examples of their findings in Atlantic salmon see Table 4a.1).

Previous studies on Atlantic salmon parr have reported a wide range of feeding patterns although these are predominantly diurnal (Hoar, 1942; Pinskii, 1961; Ali, 1964; Higgins and Talbot, 1985; Rawlings, 1994; Kadri et al., 1997*a*). There is also evidence that the timing of feeding can differ with season and juvenile *Salmo salar* may even become nocturnal when temperatures drop below 10°c (Fraser et al., 1993,1995; Fraser and Metcalfe, 1997). This illustrates the variability in findings between small-scale experiments in both the wild and cultured systems. Numerous studies on the feeding activity of adult Atlantic salmon have generally concluded that they exhibit a crepuscular feeding pattern with a large peak in feeding after first light (Kadri et al., 1991, 1997*b*; Blyth et al., 1993,1997,1999; Juell et al., 1994). These studies have been in sea cages under high stocking densities encountered in aquacultural conditions. All of these examples demonstrate that Atlantic salmon do not have a fixed diel feeding rhythm and may not exhibit a propensity to feed whenever food is offered (Kadri et al., 1991). To my knowledge, no studies on the feeding patterns of Atlantic salmon parr have been carried out in large scale production environments. An aquaculturist should be aware of this when implementing feeding practices based upon feed tables.

Other salmonids of commercial importance also exhibit flexible and adaptive feeding patterns both within and between seasons. It has been reported that rainbow trout, *O. mykiss,* have a peak in feeding activity around dawn, followed by a subsidiary peak 8h later during the winter months (Adron et al., 1973; Landless 1976), whereas a later study by Grove et al. (1978), found a nocturnal feeding pattern during winter. Recent studies under laboratory conditions (Boujard and Leatherland 1992*a*; Sánchez-Vázquez and Tabata, 1998) have shown a morning peak in feeding irrespective of length of photoperiod. Arctic char, *Salvelinus alpinus,* can also switch from being nocturnal during the winter to diurnal or crepuscular at other times of the year (Linnér et al., 1990), as can brown trout, *Salmo trutta,* (Eriksson, 1973).

Such a phase shift in feeding is also evident in other fish species such as: the alpine bullhead, *Cottus poecilopus* and bullhead, *Cottus gobio* (Andreasson, 1969,1973); sea bass, *Dicentrarchus labrax*, (Sánchez-Vázquez et al., 1995*a*,*b*,1997,1998); and goldfish, *Carassius auratus* (Sánchez-Vázquez et al., 1996). The brown bullhead, *Ictalurus nebulosus,* also shifts its activity when exposed to low light intensity (Eriksson and Alanärä, 1992). Although the mechanisms involved in dualistic feeding are unknown, it has been suggested by Fraser et al., (1995) that phase shifts are related to predator-prey relationships and to shifts in predation risk.

Feeding intensity can vary with season, with a decrease in feed intake during autumn and an increase in spring (Higgins and Talbot, 1985; Thorpe, 1994; Sæther et al., 1996). These circannual variations may be under the control of an endogenous mechanism. Madrid et al., (2001) have suggested an endogenous circannual clock may be involved, and that this clock can be synchronised by external cues, but there is no direct evidence to support this hypothesis. The autumnal decrease in feeding intensity may be associated with a decrease in feeding motivation as winter approaches (Metcalfe et al., 1986; Simpson et al., 1996; Amundsen et al., 1999). The levels of fat deposits may act as a physiological feedback loop that regulates feeding intensity, with feeding motivation decreasing when an individual has built up sufficient energy reserves (Tveiten et al., 1996). Furthermore, the increase in feeding intensity during spring may be related to the depletion of these energy reserves during winter (Metcalfe and Thorpe, 1992). This circannual variation in feed intensity is often predictable and can be integrated into feed management strategies.

4a. 1.6 What other abiotic factors may affect feed intake?

Fish are poikilothermic animals and temperature influences several processes that relate to feeding intensity (Wurtsbaugh and Davis, 1977; Brett, 1979; Jobling, 1997). An increase in temperature can instigate an increase in feed intake up to a species specific thermal threshold; beyond this threshold feeding responses can be suppressed (Alanärä et al., 2001).

Oxygen depletion, which can occur in cages held in poorly circulating stratified lakes, can affect feed intake (see Thetmeyer et al., 1999). A decrease in the availability of dissolved oxygen is detrimental to feed intake (Randolph and Clemens, 1976 in the channel catfish, *Ictalurus punctatus*). Increased levels of ammonia are also known to suppress feeding activity in the lake trout, *Salvelinus namaycush* (Beamish and Tandler, 1990).

Wave action can influence the feeding activity of fish (Begóut Anras, 1995 in sea bass, *Dicentrarchus labrax*; Juell, 1995 in Atlantic salmon, *Salmo salar*; Mallekh et al., 1998 in turbot *Scophthalmus maximus*). Excessive wind speeds and rainfall may instigate surface avoidance behaviour and cause fish to limit their movements within a cage (Juell, 1995). Freshwater lochs can be affected by strong wind driven currents (C. Noble, pers. obs.) that can carry pellets away from not only the cage, but also underwater sensors in interactive feeding systems; this is a limitation to such systems because it infers the fish are hungry when this may not be the case.

4a.1.7 Behavioural influences upon feed intake

Earlier sections of this chapter have outlined endogenous and environmental variables that may affect feeding variability and the expression of feeding rhythms when fish are held singly or in small groups. In this section I will look at how competition may drive access to the feed resource. When fish are held singly there can be high inter-individual heterogeneity in feeding patterns (Sánchez-Vázquez et al., 1995*a,b* in sea bass, *Dicentrarchus labrax*; Sánchez-Vázquez et al., 1996 in goldfish, *Carassius auratus*). When using self-feeders, where fish regulate feed delivery by pressing a trigger, reward level at each feeder actuation can also modify feeding patterns of fish (Brännäs and Alanärä, 1994 in Arctic charr, *Salvelinus alpinus* and rainbow trout, *O. mykiss*; Gélineau et al., 1998, also *O. mykiss*). Furthermore, intraspecific competition can lead to heterogeneity in feeding patterns within groups. Brännäs and Alanärä (1997) reported that within a group of rainbow trout, *O. mykiss*, the majority of fish exhibited diurnal feeding patterns whereas other fish displayed crepuscular feeding habits. Kadri et al., (1997*b*) also found that the feeding patterns of adult Atlantic salmon, *S. salar*, in tanks were related to the relative size of fish within the population; small fish showed a morning peak in feeding and larger fish showed no consistent daily feeding rhythm. Kadri et al., (1997*a*) also reported that small groups of Atlantic salmon parr refrain from feeding at first light because of aggression; it would interesting to see if this behaviour is displayed under high stocking densities.

4a.1.8 Implications for aquaculture

Aquaculturists are becoming increasingly aware of the benefits of optimising feeding practices. The study of feeding patterns can help a farmer determine when it is best to feed fish in accordance with variations in appetite. This can reduce the potential impacts of underfeeding, which inhibit growth and promote competition, as well as those of overfeeding, which cause food wastage and can lead to increased feed costs and environmental degradation. Inefficient feeding can also decrease the sustainability of an aquaculture venture.

Matching feed delivery to appetite can have a positive effect on growth, nutrient utilisation and the proximate composition of commercially important fish species. Studies on rainbow trout, *O. mykiss*, have shown that feeding time can affect the lipid concentration and protein retention of fish (Boujard et al., 1995; Gélineau et al., 1996) and in the latter study retention was most efficient when fed in phase with appetite rhythms. Furthermore, feeding in phase with appetite can affect the proximate composition and market quality of the fish (Noeske-Hallin et al., 1985; Kerdchuen and Legendre, 1991).

The hypothesis that feeding time affects growth and nutrient utilisation is an attractive one; if possible farmers should attempt to match feed delivery to patterns in appetite. Under certain circumstances, it may not be commercially viable to coincide food delivery with the nocturnal feeding rhythms of fish, as Atlantic salmon only exhibit a feeding efficiency of 35% during the winter nights (Fraser and Metcalfe, 1997) and this may contribute to pellet wastage. In the absence of on-demand feeding systems a farmer could adapt existing techniques by delivering feed differentially across a day in relation to changes in appetite.

4a.1.9 Objectives of the study

In the following experiment I investigated whether large groups of salmon parr exhibited any daily feeding rhythms and how the above factors manifested themselves in a cage environment. The specific questions I addressed during this study were: -

i) How does the pattern of group appetite vary across a day?

ii) Is there any variability in feed intake between days and to what extent can it be explained by environmental variables?

iii) What time of day in relation to sunrise does feeding commence?

The experiment was conducted in a production environment where the objective is to maximise efficiency, product quality and growth. Feed costs are therefore critical and food must not be wasted. It was designed as a full-scale production trial using commercial densities and facilities, but under the constraints of in-house husbandry practices. I hope the detailed nature of the data set can provide information to farmers on how to best distribute the feed resource temporally across a day.

Table 4a.1 Examples of daily feeding rhythms in juvenile Atlantic salmon

Species	Life history stage	Feeding pattern and main findings	Author(s)
Atlantic salmon, *Salmo salar*	fry	Early morning peak in feeding during summer in fry (length 25mm).	Browman and Marcotte (1986)
	parr	Morning peak with subsidiary peak towards evening in summer.	Hoar (1942)
	parr	Diurnal feeders.	Kalleberg (1958)
	parr	Crepuscular feeding activity with an early morning peak.	Pinskii (1961)
	parr	Morning peak in feeding under simulated natural conditions in spring. In continuous light there was no apparent feeding rhythm in spring.	Ali (1964)
	parr	Morning peak in feeding and activity levels.	Hirata (1973)
	parr	Crepuscular feeding pattern in late spring.	Higgins and Talbot (1985)
	parr	Evidence of a nocturnal peak in stomach fullness in late summer, although study site was an arctic river with a short feeding season (May until October) and a temperature range of 0-14°c.	Huru (1986)
	parr	Diurnal feeding with no pattern during summer.	Jorgensen and Jobling (1992)
	parr	Nocturnal feeding patterns during winter when temperature is below 13°c.	Fraser et al. (1993,1995); Fraser and Metcalfe (1997)
	parr	Afternoon peak in stomach fullness in spring and summer. None in autumn and winter.	Rawlings (1994)
	parr	Peak in feeding at the beginning of the day when fish were held under a 12L:12D light regime at 16°c ± 0.2°c.	Paspatis and Boujard (1996)
	parr	Afternoon peak in feeding in spring, no consistent pattern in autumn.	Kadri et al. (1997a)
	parr	No consistent pattern in feeding of 0+ parr during daylight hours in summer. Older 1+ parr exhibited a nocturnal and morning peak in stomach fullness during summer.	Amundsen et al. (1999)

Table 4a.1 cont. Examples of daily feeding rhythms in adult Atlantic salmon

Species	Life history stage	Feeding pattern and main findings	Author(s)
Atlantic salmon, *Salmo salar*	adult	Smolts exhibit crepuscular feeding with morning peak and midday depression in summer.	Hoar (1942)
	adult	Crepuscular feeding pattern during summer when held in sea cages.	Kadri et al. (1991)
	adult	Crepuscular feeding pattern during winter with an early morning peak in fish held in sea cages.	Blyth et al. (1993)
	adult	No diurnal pattern in feeding from autumn until spring.	Smith et al. (1993)
	adult	Morning peak in feeding rhythm when held in sea cages in winter.	Juell et al. (1994)
	adult	Fish held in cages showed morning peak in summer, crepuscular feeding with secondary morning peak during winter, no pattern during spring.	Blyth et al. (1997)
	adult	Feeding patterns of fish in tanks were related to relative size of individuals within the population. Small fish showed morning peak in feeding and larger fish showed no consistent daily feeding rhythm.	Kadri et al. (1997b)
	adult	Fish held in sea cages exhibit a year round crepuscular feeding pattern, with a large morning peak.	Blyth et al. (1999)

4a.2 Materials and methods

4a.2.1 Fish husbandry

Fish used in this experiment were identified in late August 1998 as 1+ upper modal group (UMG) Atlantic salmon that would migrate to sea after one year (Thorpe, 1977). They were from a pooled hatchery stock and held in production cages at a smolt production site in Glenfinnan, (grid ref. NM 887 790) which was owned by Marine Harvest Ltd. Fish were from a high maturing commercial (Lochy x Lochy) stock (native to the West Coast of Scotland). Six groups of approximately 30,000 - 35,000 fish were graded into separate 12x12x4m deep cages (see figure 4a.1 for details), according to densities used in production cages on the rest of the farm. The cages were subject to ambient temperature and photoperiod. These trial cages formed a single cage group, and the trial commenced on the 2nd September 1998, approximately one week after grading (to give the fish sufficient time to recover). The trial ended one week prior to smolt transfer on the 27th March 1999. The exact numbers of fish per cage and also their corresponding stocking densities are shown in figure 4a.1. The figure layout shows the location of the cage group on the loch.

Each cage was fitted with a 50kg centralised feed hopper with a vibrator dispenser attached to a circular spreader (Sterner AS; Aquatess Ltd, Inverness) which spread pellets in a 10m diameter circle. Fish were fed a commercial 2mm extruded pellet, Biomar Ecostart (Biomar UK Ltd., Grangemouth) for the first two periods of the experiment from September until mid February (according to manufacturer guidelines for fish size). For the third period of the experiment (March), the fish were fed a Trouw Royale 2.3mm pellet (Trouw UK Ltd., Northwich) according to manufacturers' guidelines on fish size. Three feed hoppers were controlled by AKVAsmart AQ1 Adaptive Feed Systems (Aquasmart UK Ltd, Inverness), and three hoppers were operated by an Aquatess KC20 Automatic Feed Controller (Aquatess Ltd, Inverness).

The three Aquatess fed groups formed the imposed feeding regime and were fed a fixed ration every 10 minutes from just after dawn until dusk. The hopper dispensed food for about 10 – 15 seconds every feeder actuation. The fish were fed to apparent daily satiation using a combination of in-house feed tables supplemented by feeding a fixed ration by hand at 8.00am and 3.00pm during autumn. In winter fish received a single daily hand-feed at 8.00am. This long day feeding strategy mimicked current husbandry practices for achieving the best growth performance from automatic feeders in salmon parr (D. Mitchell pers. comm.).

The other three groups were fed on-demand from dawn till dusk (as defined by the Royal Observatory, Edinburgh) using an AKVAsmart AQ1 with a feedback loop. This system matched feed delivery to group appetite. In on-demand fed groups discrete meals were observed across a day, but the distinction is that these meals were selected by the groups of fish and were not imposed by a fixed feed management strategy. (Refer to chapter 2 for the exact system set-up and the biological and production significance of the parameters used).

The experiment was broken down into three periods to allow for routine husbandry tasks. The first period lasted from the 2nd September 1998 until the 19th November 1998. After this date fish in each cage were administered an individual injection vaccination for furunculosis. Each population received a 'trimmed grade' where any remaining lower modal group (LMG) fish were removed from the groups along with any obvious precocious parr (sexually mature male parr producing milt). At no time was there any mixing of fish between cages or treatments.

Vaccinating fish for furunculosis has been known to cause suppression in appetite (Midtlyng et al., 1995), so the second part of the experiment did not commence until the 1st December 1998 to allow the fish time to recover. This second stage of the experiment lasted until the 18th February 1999, when it was decided to grade the fish because of large size variations within each group.

Fish within each feeding treatment (fed either to an imposed or on-demand regime) were subjected to a three-way grade with a commercial grader, (Fischtechnik Fredelsloh, Moringen, Germany) according to size (small, medium and large) and returned to the production cages. There was no mixing of fish between treatments to ensure that fish were fed under the same regime before and after handling.

The third period of the experiment ran from the 25th February 1999, until the 27th March 1999, one week prior to smolt transfer. Due to production grading limitations at this latter stage of the experiment, it was difficult to match numbers of fish for every cage. Each on-demand fed group and its corresponding cage under the imposed feeding regime were given approximately the same numbers of fish in each treatment, to attempt to control for this. Due to problems with one on-demand feeder throughout the third period of the experiment, this cage and the corresponding cage under the imposed feeding regime were removed from subsequent analysis (see figure 4a.2 for further details and the experimental set-up).

4a.2.2 Measuring environmental variables

The times of sunrise and sunset for Fort William (Grid ref: NN 108 742) were obtained from the Royal Observatory (Edinburgh, UK). Daylength (hours) was then obtained by working out the difference (hours) between the beginning of civil twilight and the end of civil twilight. The Royal Observatory (Edinburgh, UK) defines these times as the beginning and end of twilight when the outer edge of the sun's disc is six degrees below the horizon. The change in daylength from the previous day (hours) was also recorded (after Smith et al., 1993). Water temperature was measured on the cage group at approximately 8.00am each morning using a mercury thermometer held at a depth of two metres. Water clarity was also measured on the cage group at 8.00am daily using a secchi disc.

The British Atmospheric Data Centre (Chilton, Didcot, UK) kindly provided meteorological and hydrological data, compiled from weather stations in the vicinity of the experimental site. Data on daily rainfall was obtained from the Glenfinnan weather station (Grid ref: NM 898 802), and data on daily wind speed and direction was obtained from the Aonach Mor weather station (Grid ref: NN 189 740).

4a.2.3 Manipulation of the on-demand feeding data

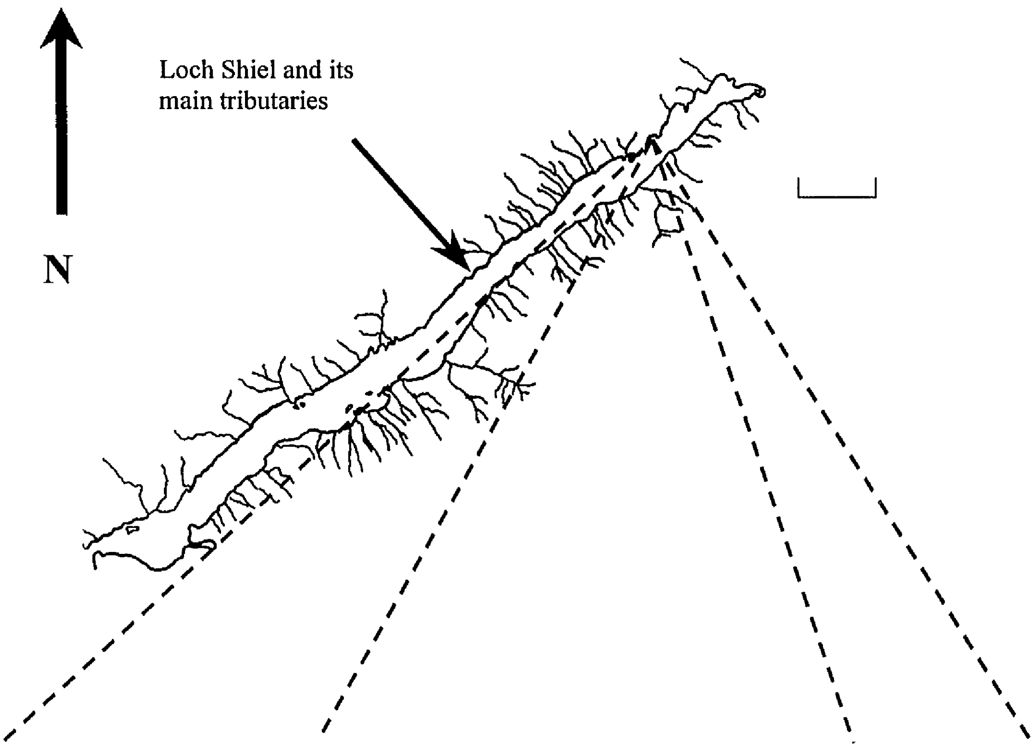
On-demand feeding data was manipulated and transformed using the methods outlined in Chapter 3a. The time feeding commenced and ceased in relation to twilight was also investigated. Previous work by Kadri et al., (1997*a*) found that during autumn and spring, small groups of UMG Atlantic salmon parr delay feeding at first light due to high levels of aggression. In the current experiment I wished to see if this behavioural phenomenon existed in larger groups of fish held in freshwater production cages in autumn.

4a.2.4 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0. Minitab Inc., Philadelphia) or SPSS (v. 10-0. SPSS Inc., Chicago) for MS Windows. A non-parametric ANOVA (Kruskal-Wallis test) was used to test for possible differences in appetite across a day in the on-demand fed treatment. A post hoc multiple comparison test (Siegel and Castellan, 1988) was used to locate any differences observed. Data are presented as median values ± first and third interquartile ranges.

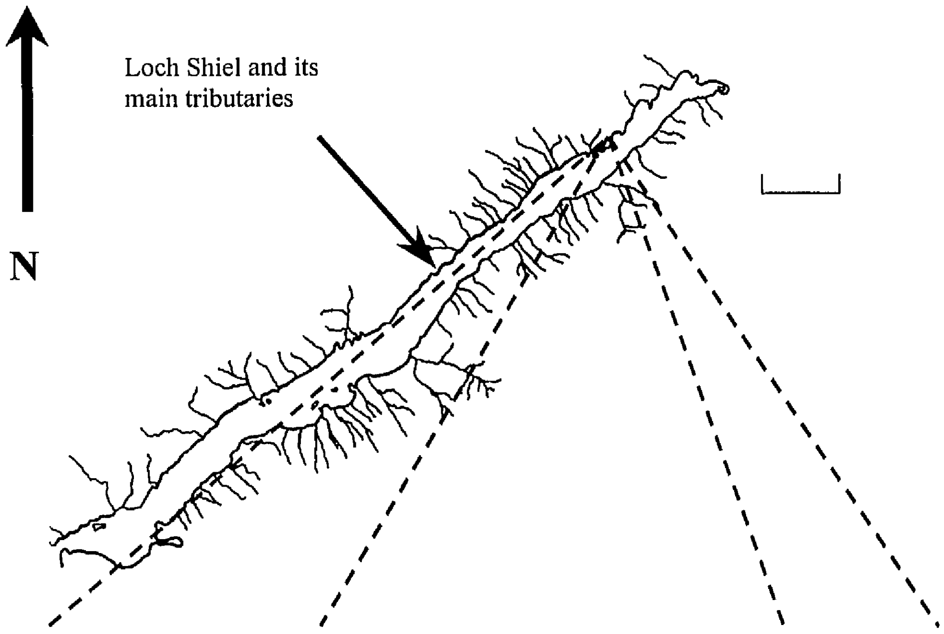
Differences in appetite between days were tested with a Univariate General Linear Model (GLM) model in SPSS. Independent variables were: day, daylength (h), change in daylength from previous day (h), water temperature at 2m depth (^{o}C), water clarity (m), total daily rainfall

(0.1mm d^{-1}), average daily wind speed (knots). All variables were also included in the model with day as an interaction. The dependent variable was daily ration, which was transformed into amount fed as a percentage body weight day^{-1}. This was further transformed using the arcsin square root procedure to satisfy normality and homogeneity of variance requirements. Long-term synchrony in daily food delivery between replicates between October and January was tested for using ANCOVA analysis in SPSS. A significance level of $P < 0.05$ was used for all statistical tests.



Imposed cage: 1 Mean wet weight on 2.9.98 = 23.57g Stocking density = 1.41 kg m^{-3} No. of fish = 34,465	**On-demand cage: 1** Mean wet weight on 2.9.98 = 25.61g Stocking density = 1.45 kg m^{-3} No. of fish = 32,678
On-demand cage: 2 Mean wet weight on 2.9.98 = 20.91g Stocking density = 1.06 kg m^{-3} No. of fish = 29,102	**Imposed cage: 2** Mean wet weight on 2.9.98 = 23.24g Stocking density = 1.15 kg m^{-3} No. of fish =28,607
Imposed cage: 3 Mean wet weight on 3.9.98 = 21.78g Stocking density = 1.15 kg m^{-3} No. of fish = 30,480	**On-demand cage: 3** Mean wet weight on 3.9.98 = 22.41g Stocking density = 1.25 kg m^{-3} No. of fish =32,075

Fig. 4a.1. The location of the experimental production cages on Loch Shiel, showing initial fish numbers, stocking densities and average weights for period one of the experiment. Scale bar = 2 miles.



On-demand cage: 2 Mean wet weight on 25.2.99 = 48.69g Stocking density = 2.53 kg m^{-3} No. of fish = 29,893	**Imposed cage: 2** Mean wet weight on 26.2.99 = 49.12g Stocking density = 2.40 kg m^{-3} No. of fish = 28,176
Imposed cage: 3 Mean wet weight on 25.2.99 = 39.72g Stocking density = 1.09 kg m^{-3} No. of fish = 15,819	**On-demand fed cage: 3** Mean wet weight on 26.2.99 = 40.38g Stocking density = 1.06 kg m^{-3} No. of fish = 15,056

Fig. 4a.2. The location of the experimental production cages on Loch Shiel, showing initial fish numbers, stocking densities and average weights for period three of the experiment. Scale bar = 2 miles.

4a.3 Results

4a.3.1 Diurnal patterns of appetite

On-demand fed Atlantic salmon parr, when held in freshwater production cages, do not exhibit a propensity to feed at all times of the day; each group shows a distinct peak in appetite across the day, although the timing of this peak differs between months and, in some cases, between cages (see Fig. 4a.3and Fig. 4a.4). For this reason I will firstly review each the feeding patterns of each cage independently, and then examine each data set for synchrony between cages.

4a.3.1.1 On-demand fed cage 1

During September this cage of salmon parr exhibited a highly significant morning peak in feed delivery immediately after first light (Kruskal-Wallis $H_{\text{adj}} = 25.54$, d.f. = 9, $P = 0.002$), although this peak was not maintained during October, when there was no significant peak in appetite across the day (Kruskal-Wallis $H_{\text{adj}} = 13.72$, d.f. = 9, $P = 0.133$). Feed delivery increased during the day in November and there was a significant early evening peak in appetite prior to dusk (Kruskal-Wallis $H_{\text{adj}} = 21.44$, d.f. = 9, $P = 0.011$).

In the second period of the experiment, during the winter months of December and January, the salmon held in this cage showed no significant peak in appetite throughout the day (December Kruskal-Wallis $H_{\text{adj}} = 9.84$, d.f. = 9, $P = 0.363$; January Kruskal-Wallis $H_{\text{adj}} = 14.06$, d.f. = 9, $P = 0.12$). During February and March there was a problem with the on-demand feeder on this cage and no data was collected.

4a.3.1.2 On-demand fed cage 2

During September this cage of salmon parr also exhibited a highly significant morning peak in appetite (Kruskal-Wallis H_{adj} = 28.76, d.f. = 9, P = 0.001), and this morning peak was also maintained during October (Kruskal-Wallis H_{adj} = 21.71, d.f. = 9, P = 0.01). In November there was no significant peak in appetite during daylight hours (Kruskal-Wallis H_{adj} = 9.96, d.f. = 9, P = 0.354).

In the second period of the experiment, during the winter months of December, January and February, the salmon held in this cage showed a significant peak in appetite throughout the day although this differed between months. During December, there was a significant peak in appetite around the middle of the day (Kruskal-Wallis H_{adj} = 26.84, d.f. = 9, P = 0.001). In January this peak occurred later in the day, in the sixth segment of the day and there was little feeding after first light (Kruskal-Wallis H_{adj} = 23.63, d.f. = 9, P = 0.005). This peak was also retained during February where it was highly significant (Kruskal-Wallis H_{adj} = 34.44, d.f. = 9, P < 0.001).

During the third period of the experiment (March) in the month prior to smolt transfer the fish began to exhibit the morphological characteristics of smoltification, with a loss of parr markings and the skin became silvery with melanin deposition. During this time the group again began to extend its feeding activity throughout the daylight hours, although there was still a significant late afternoon peak in appetite (Kruskal-Wallis H_{adj} = 21.16, d.f. = 9, P = 0.012).

4a.3.1.3 On-demand fed cage 3

During September this group exhibited a highly significant morning peak in appetite although this was slightly later than first light, during the second segment of the day (Kruskal-Wallis H_{adj} =

37.86, d.f. = 9, $P < 0.001$). This morning peak was also maintained during October (Kruskal-Wallis $H_{adj} = 24.44$, d.f. = 9, $P = 0.004$). In November there was no significant peak in appetite during daylight hours (Kruskal-Wallis $H_{adj} = 8.65$, d.f. = 9, $P = 0.47$).

In the second period of the experiment, during December and January, the salmon held in this cage showed a significant peak in appetite although the timing of this peak differed between months. During February there was a problem with the feeder and not enough data was collected to permit statistical analysis using a Kruskal-Wallis test (Dytham, 1999). In December, there was a highly significant peak in appetite around the middle of the day (Kruskal-Wallis $H_{adj} = 26.46$, d.f. = 9, $P = 0.002$). In January this peak occurred later in the day, in the sixth and seventh segments of the day and there was also little feeding after first light (Kruskal-Wallis $H_{adj} = 35.36$, d.f. = 9, $P < 0.001$). This peak was also retained during February although the sample size did not permit statistical analysis as stated earlier.

During the final period of the experiment (March) the group again began to extend its feeding activity throughout the daylight hours, although there was still a significant late afternoon peak in appetite (Kruskal-Wallis $H_{adj} = 21.49$, d.f. = 9, $P = 0.011$).

4a.3.1.4 Synchrony between cages within the on-demand feeding treatment

There was a high degree of synchrony between two of the three on-demand feeding cages (cages 2 and 3 respectively) in the time fish chose to feed during diurnal hours. Each group showed the same trend in feeding time (although there were slight differences in the exact timing of feeding peaks), and this general synchrony was evident from the beginning until the end of the experiment.

The first on-demand fed cage showed synchrony with the other two during September, although there was no synchrony in feeding patterns in the following months. This may have been an effect of the feeding unit, which experienced a lot of mechanical problems during the trial, or it may be a group effect (see discussion for further details).

4a.3.2 Differences in appetite between days and the effect of environmental variables

Daily feed delivery was analysed in relation to a number of environmental variables using a Univariate General Linear Model in the SPSS statistical package (version 10.0). As in chapter 3a it should be noted that the daily feed delivery variable in each model is not independent as data were taken from each of three on-demand fed cages over an extended time period. Daily variation in feed delivery for each of the on-demand fed cages is plotted with environmental variables (Fig. 4a.5). Univariate GLM analysis showed daylength had a significant effect upon appetite for each of the three cages although the extent of its effect did differ between cages (Table 4a.2a,b,c respectively). The other variables measured did not significantly affect food demand for cages 1 and 3; although windspeed also had a significant effect upon feed delivery for cage 2. The model accounted for 61.7%, 77.9% and 66.5% of daily food demand for on-demand fed cages 1,2, and 3 respectively.

4a.3.3 Effect of cage or population upon daily feed delivery between cages

Although there were differences between cages in the amount of food delivered on any given day (see Fig. 4a.5), I wished to test the hypothesis that over longer periods there was no difference in the amount of food delivered to each cage. To compare the influence of either a feeding unit effect or biological effect upon the pattern of daily feed delivery throughout the experiment, analysis of covariance was used (ANCOVA) with cage as a factor and date (Julian date) and date^2

as covariates (as relationship is non-linear see Fig. 4a.6). The analysis was carried out on data from the beginning of October until the end of January when all on-demand feeders were functioning properly. As expected, there was a significant effect of date and date^2 upon the amount of food delivered (ANCOVA: effect of date, $F_{1,259} = 80.75$ $P < 0.001$; ANCOVA: effect of date^2, $F_{1,259} = 26.47$ $P < 0.001$). There was no significant cage effect on overall feed delivery (ANCOVA: comparison of regression slopes: date, $F_{2,255} = 1.71$ $P = 0.183$, NS; ANCOVA: comparison of regression slopes: date^2, $F_{2,255} = 1.03$ $P = 0.358$, NS; comparison of regression elevations, $F_{2,259} = 1.58$ $P = 0.209$), supporting the hypothesis that fish select similar rations over longer time periods.

4a.3.5. Time feeding begins and ends in relation to twilight

To gain an insight into when fish commenced and ceased feeding in relation to dawn and dusk, the time it took for fish to begin and end a meal in relation to twilight was examined. Feeding in cage held Atlantic salmon parr commenced at the beginning of sunrise and ceased at the end of dusk for the three-month period from September until mid November (Fig 4a.7). This pattern was consistent between cages within the on-demand feeding treatment.

Table 4a.2.a The results of a univariate General Linear Model comparing the daily feed intake of Atlantic salmon parr in on-demand production cage 1, against environmental factors. $R_{2\,adj.} = 0.617$, so the model accounts for 61.7% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Daylength	29.470	42.967	< 0.001
Excluded from model			
Temperature	0.487	0.719	0.405
Rainfall	0.146	0.208	0.653
Windspeed	0.421	0.589	0.451
Turbidity	0.180	0.243	0.628
Change in daylength	0.0085	0.110	0.744

Table 4a.2.b The results of a univariate General Linear Model comparing the daily feed intake of Atlantic salmon parr in on-demand production cage 2, against environmental factors. $R_{2\,adj}$ = 0.779, so the model accounts for 77.9% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Daylength	13.510	16.688	< 0.001
Windspeed	11.83	14.615	< 0.001
Excluded from model			
Change in daylength	0.008	0.089	0.767
Temperature	0.207	0.238	0.629
Turbidity	0.005	0.054	0.818
Rainfall	0.001	0.015	0.904

Table 4a.2.c The results of a univariate General Linear Model comparing the daily feed intake of Atlantic salmon parr in on-demand production cage 3, against environmental factors. $R_{2\,adj.}$ = 0.665, so the model accounts for 66.5% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Daylength	78.721	74.574	< 0.001
Excluded from model			
Windspeed	1.298	1.238	0.273
Turbidity	0.758	0.865	0.359
Change in daylength	0.161	0.179	0.675
Temperature	0.372	0.406	0.529
Rainfall	0.002	0.012	0.912

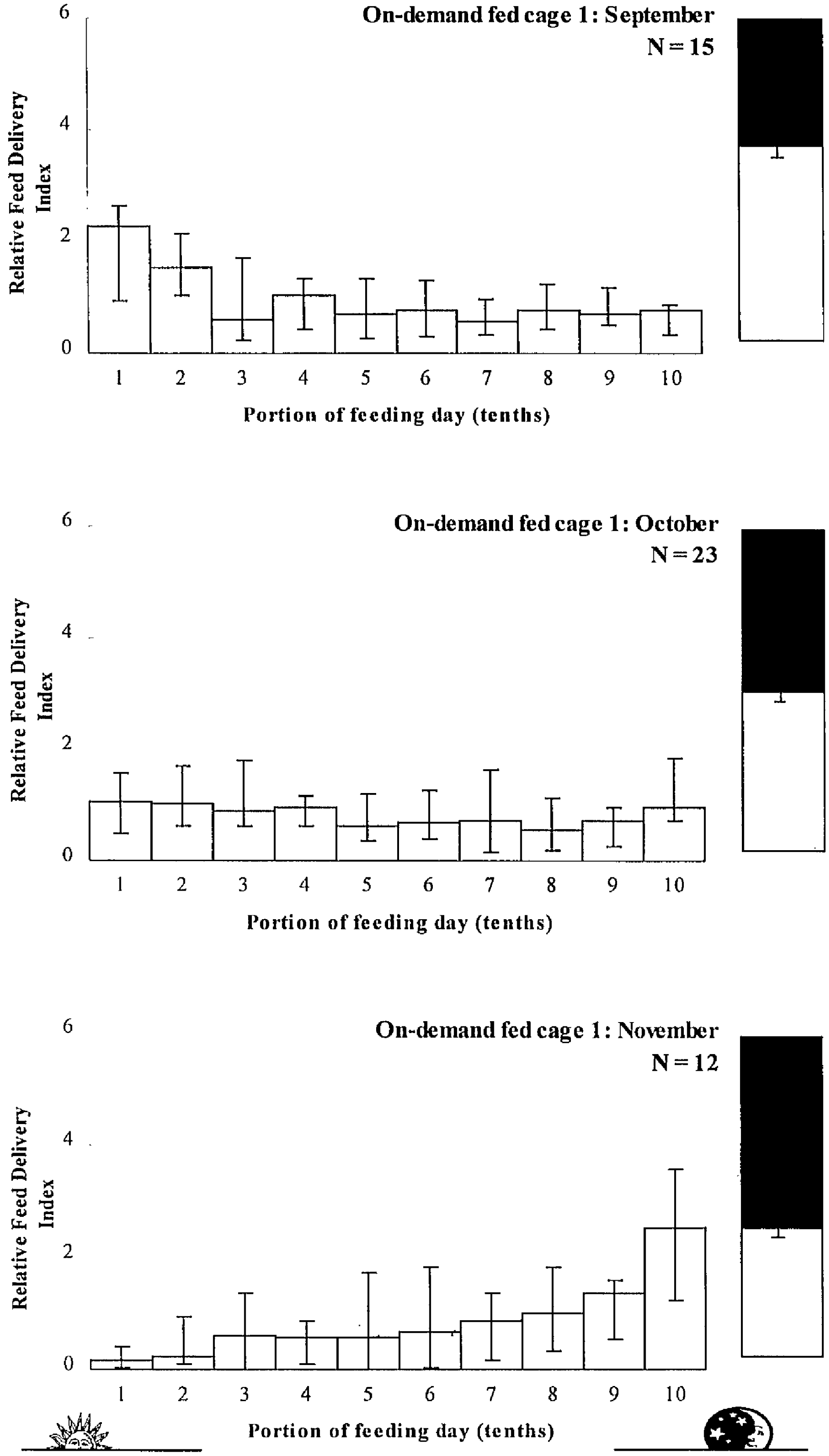


Fig 4a.3 The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean. Images of the sun and moon represent sunrise and sunset. RFDI is defined in the methods section of Chapter 3a.

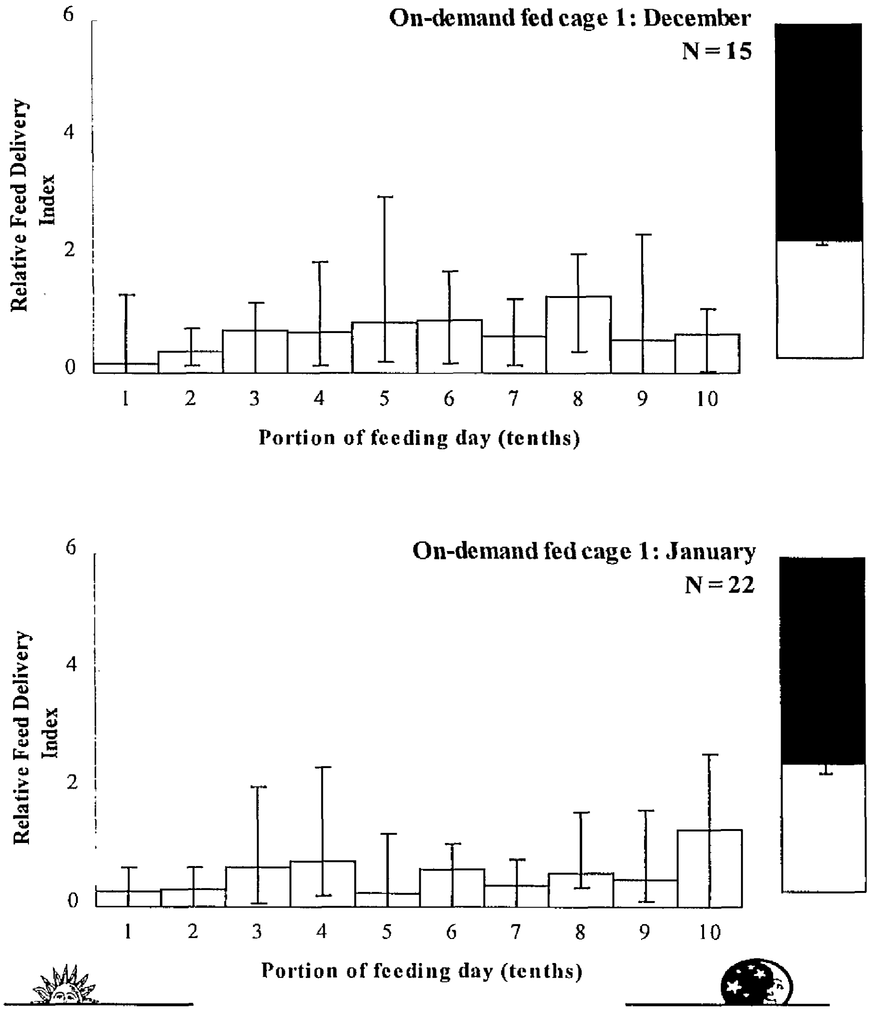


Fig 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean. RFDI is defined in the methods section of Chapter 3a.

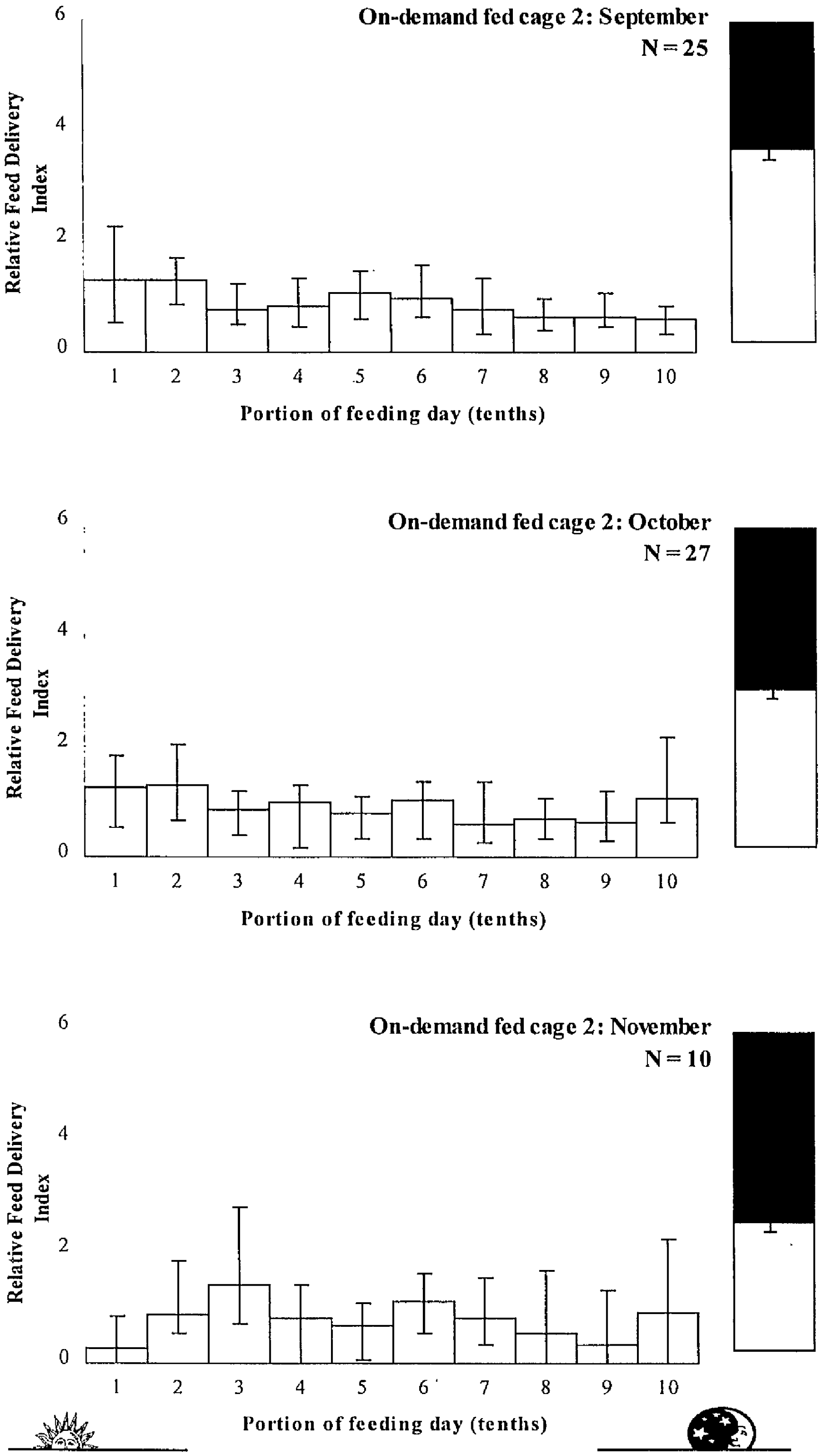


Fig 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

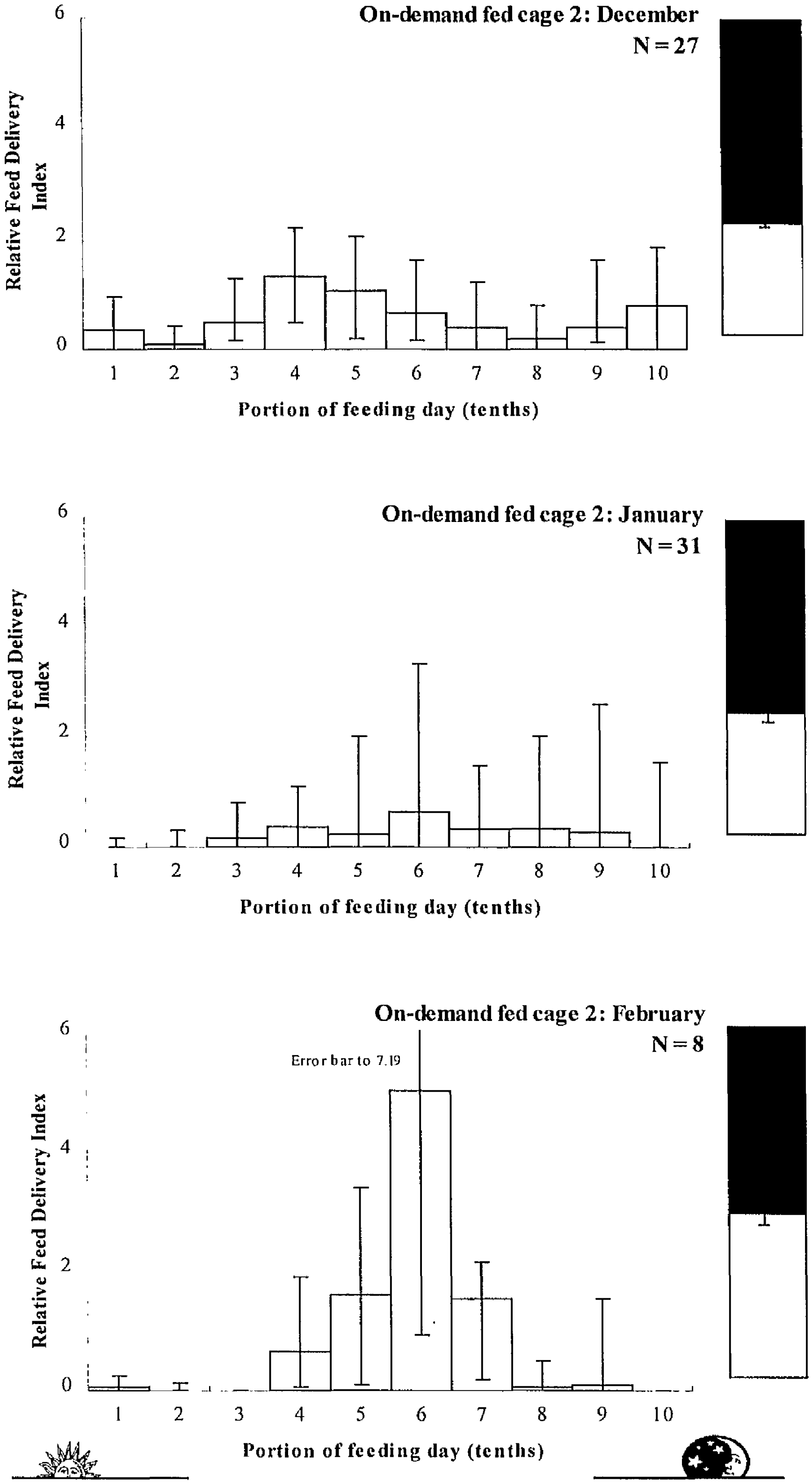


Fig. 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

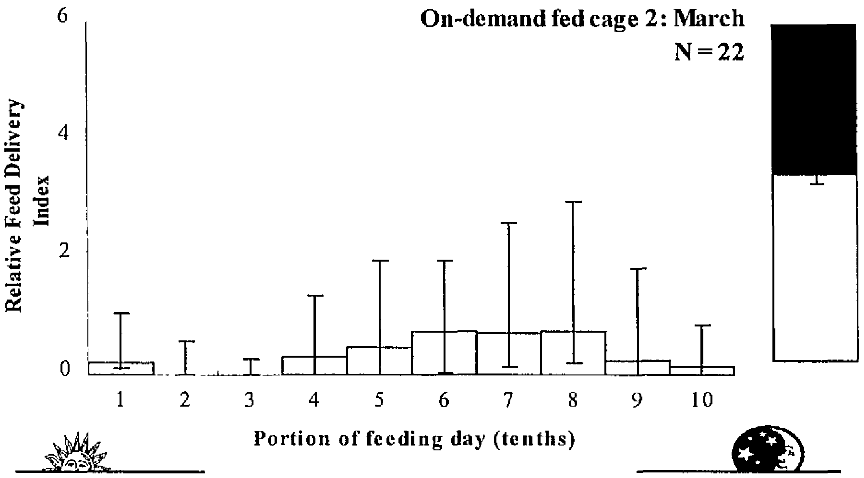


Fig. 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

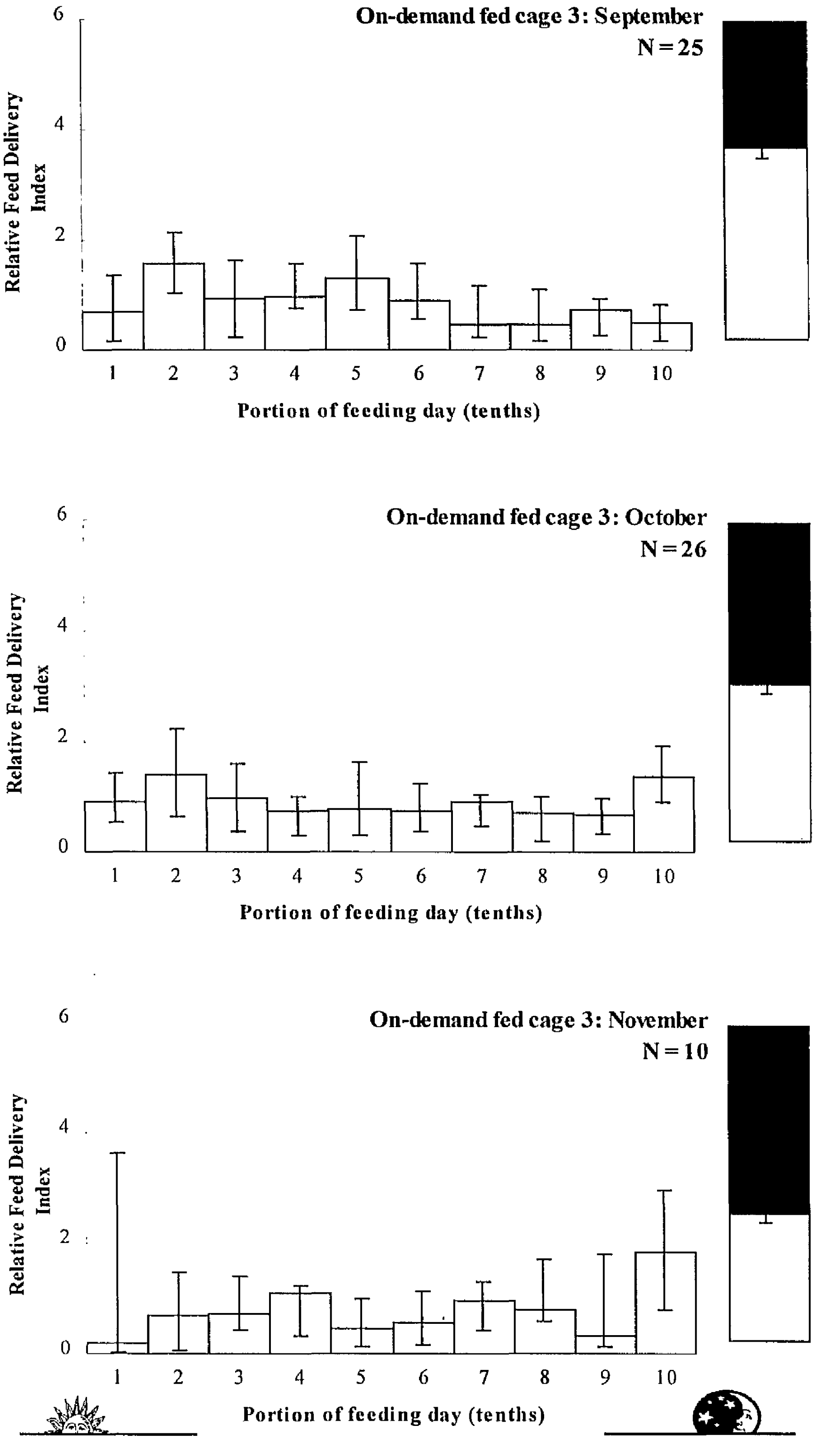


Fig. 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

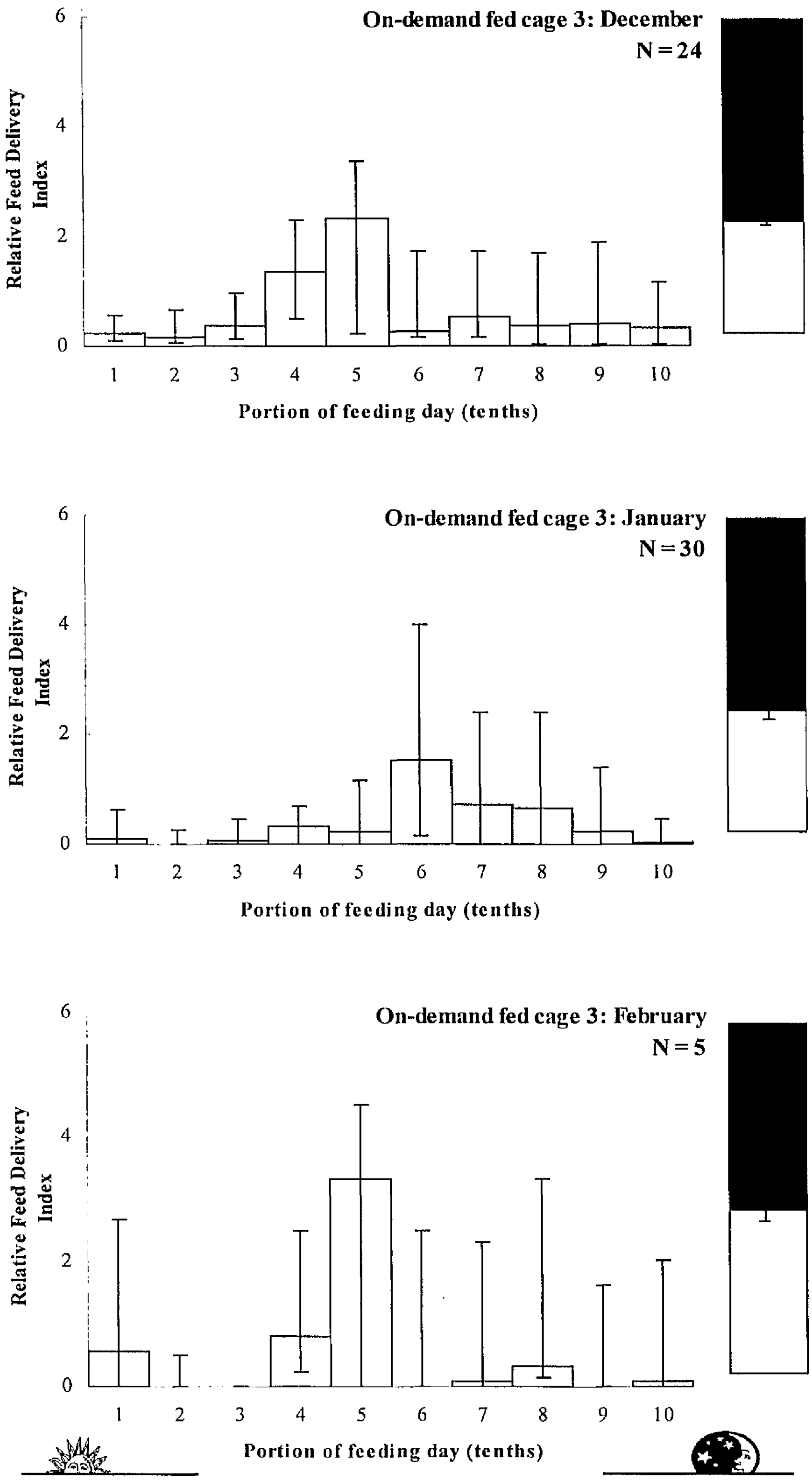


Fig. 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

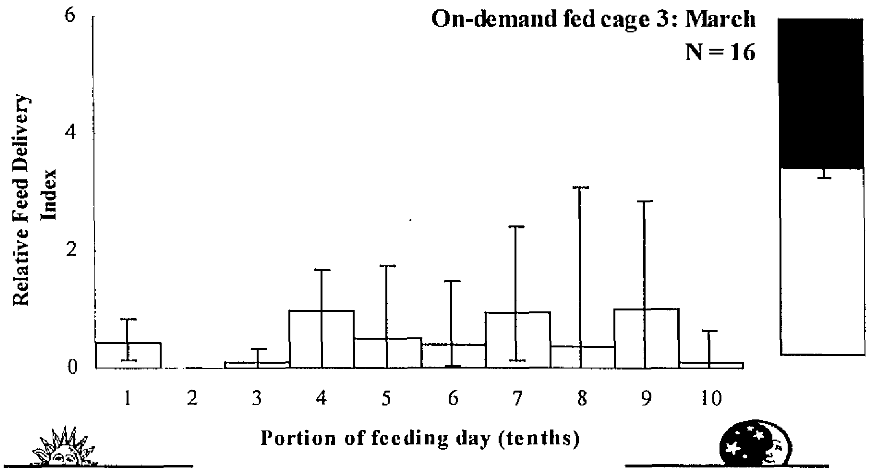
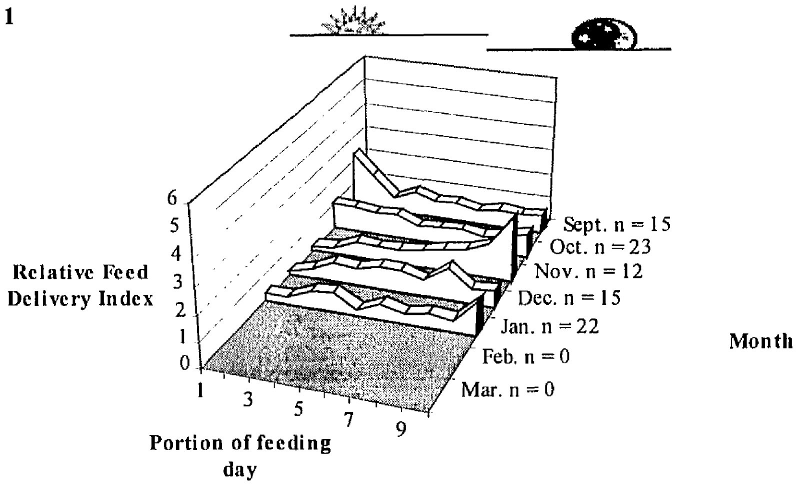
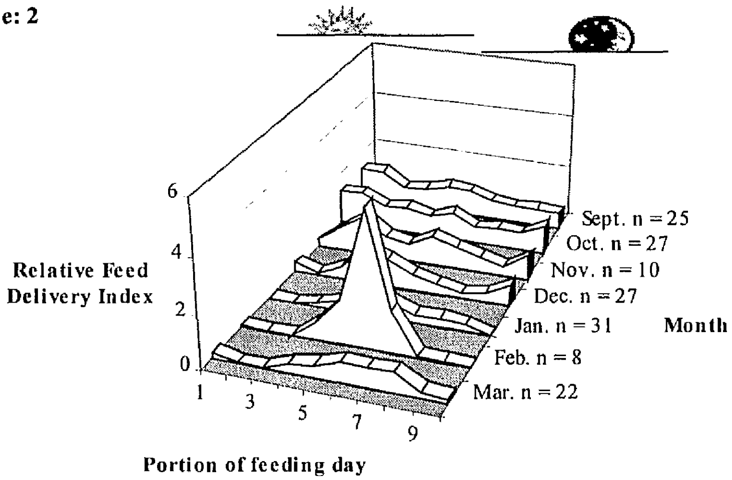


Fig. 4a.3 cont. The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. Error bars indicate interquartile range. N = number of sample days month^{-1}. Chart on right indicates average daylength month^{-1} and its bar indicates standard error of the mean.

On-demand fed cage: 1



On-demand fed cage: 2



On-demand fed cage: 3

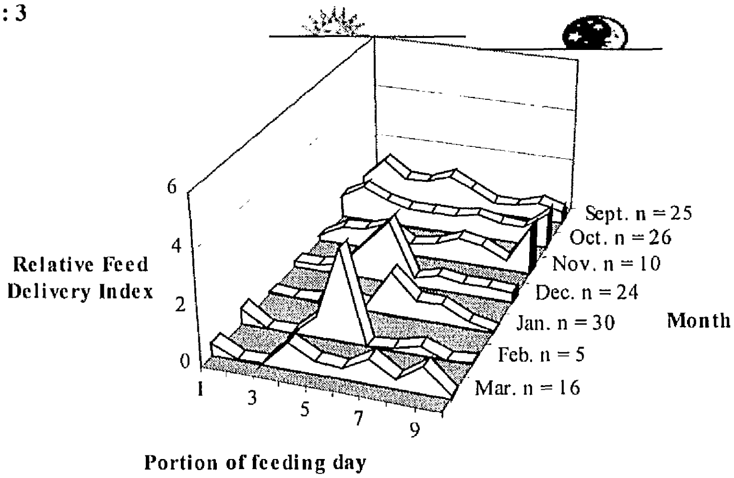


Fig. 4a.4 The relative daily feed delivery of on-demand fed parr in production cages. Feed delivery began at dawn and ceased at dusk. n = number of sample days month^{-1}.

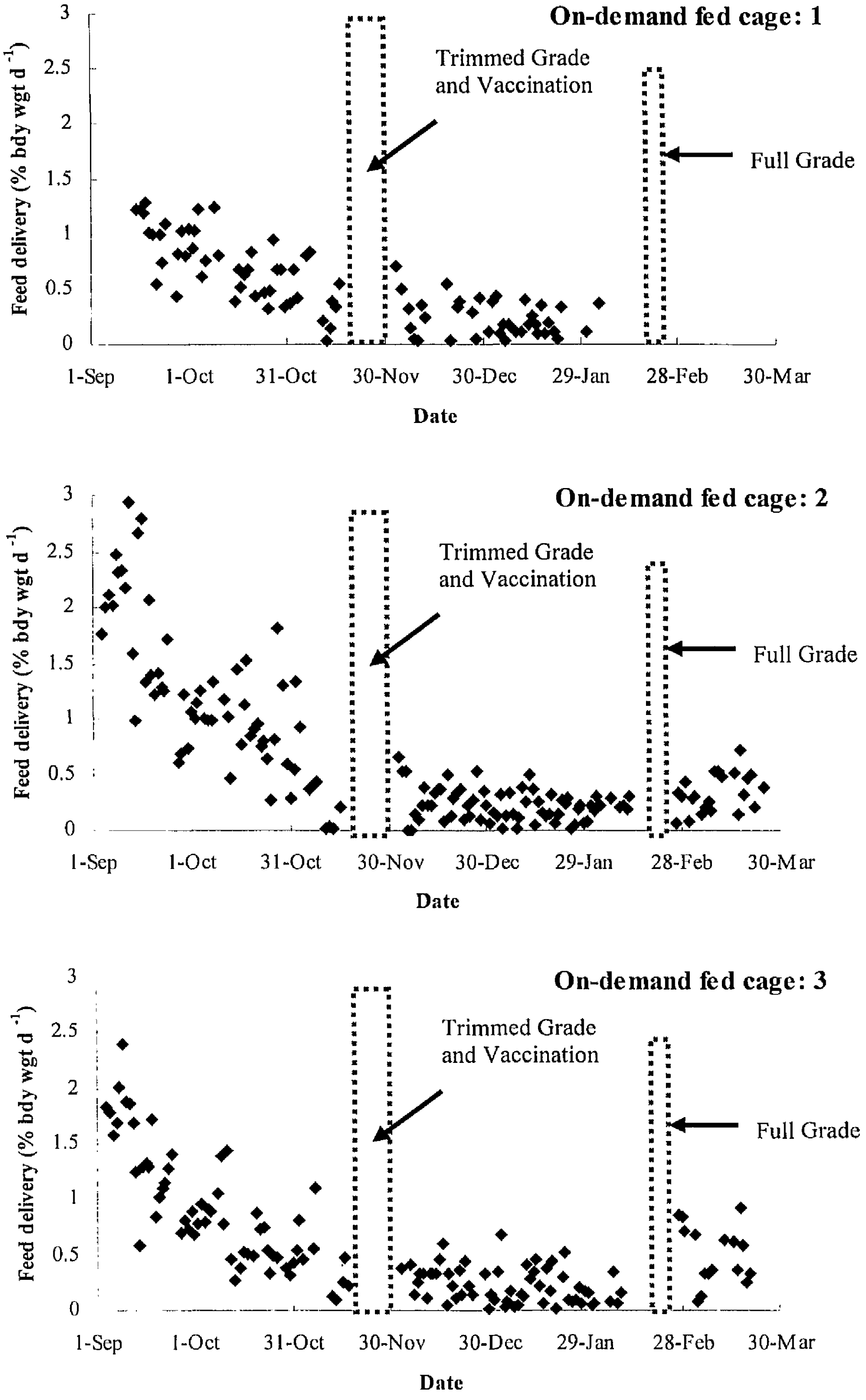


Fig. 4a.5 Daily and seasonal variation in group appetite in three cages of Atlantic salmon parr fed on-demand. Feed delivery is expressed as % body weight day^{-1}. Also shown are vaccinating and grading periods.

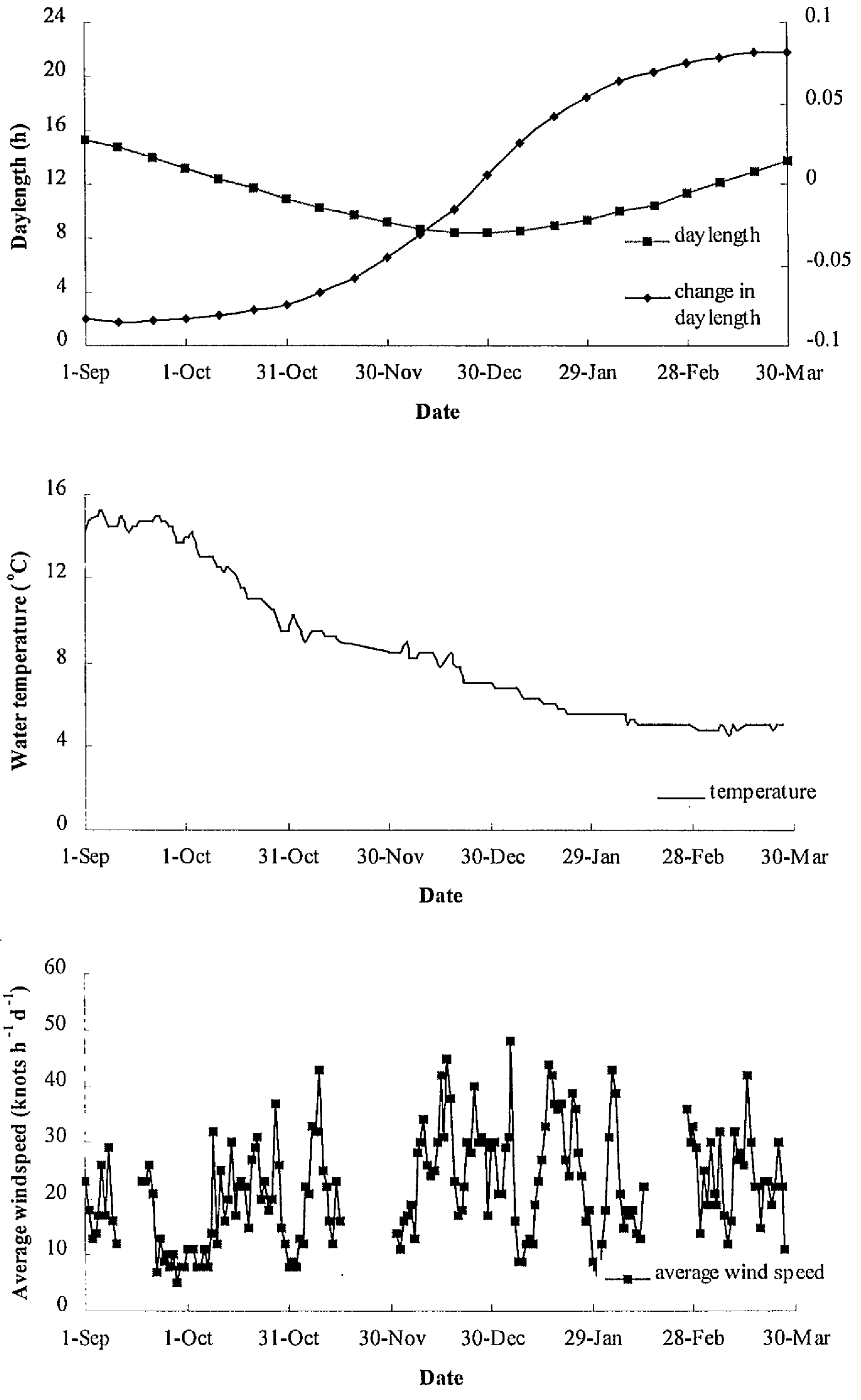


Fig. 4a.5 cont. Daily variation in environmental conditions (daylength, change in daylength and average windspeed hour^{-1} day^{-1})

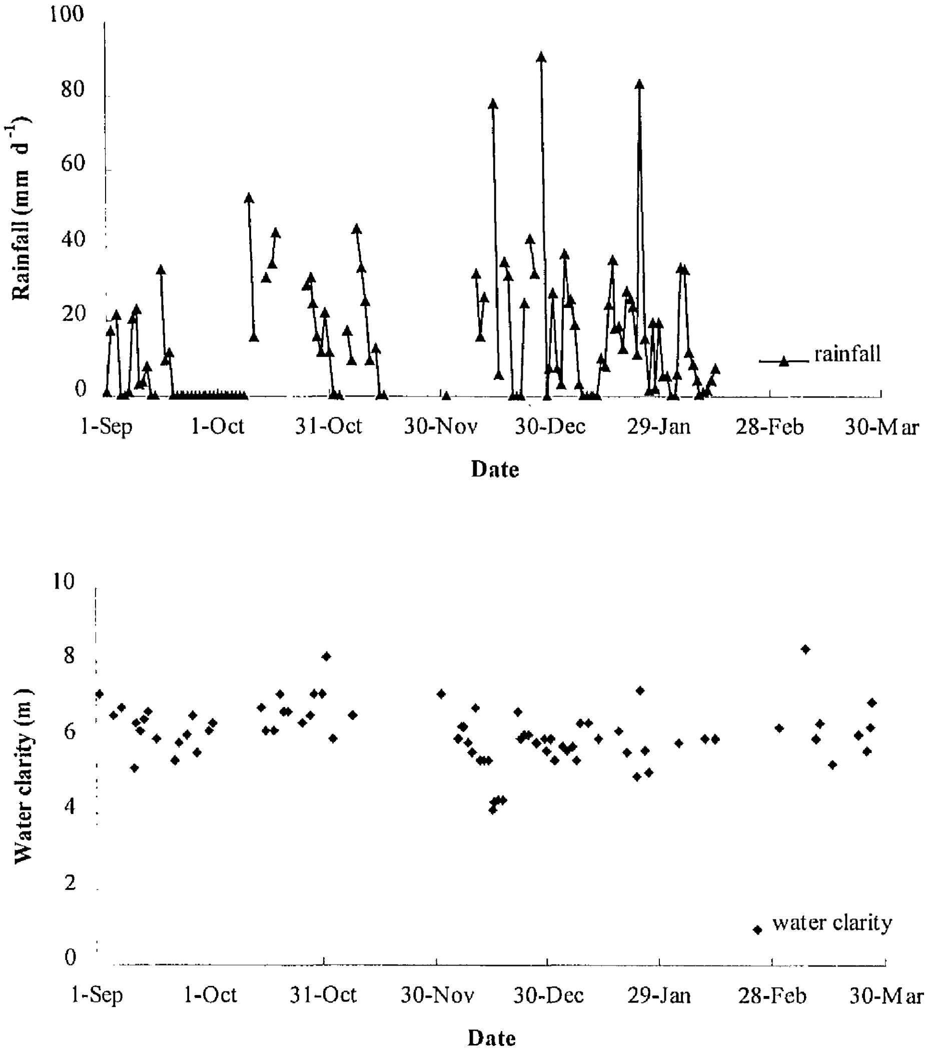


Fig. 4a.5 cont. Daily variation in environmental conditions (rainfall and water clarity)

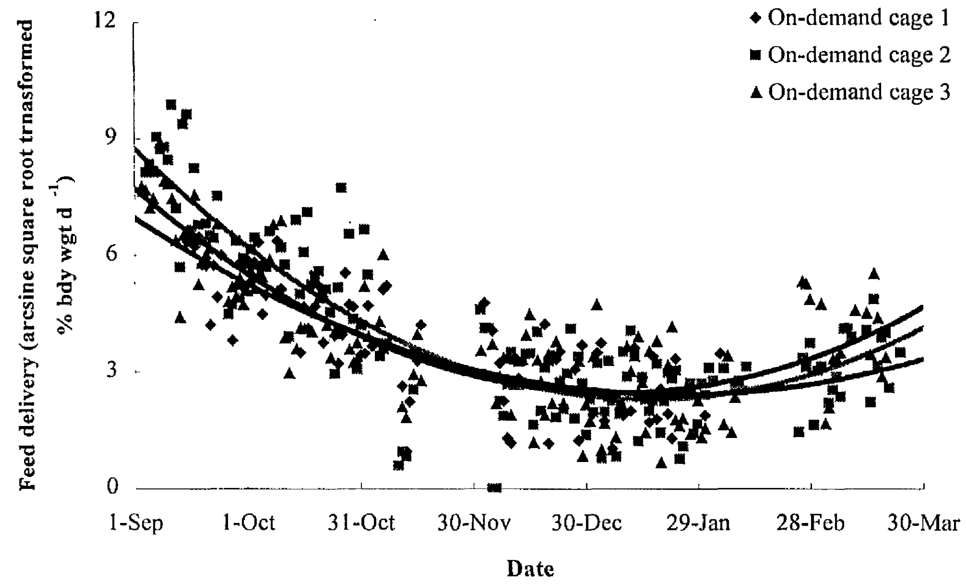


Fig. 4a.6 The differences between the amount of food selected by three groups of Atlantic salmon parr fed on-demand in cages. Data points represent daily feed delivery (arcsine square root transformed % body weight day^{-1}). Also shown are second order polynomial regression lines for each cage.

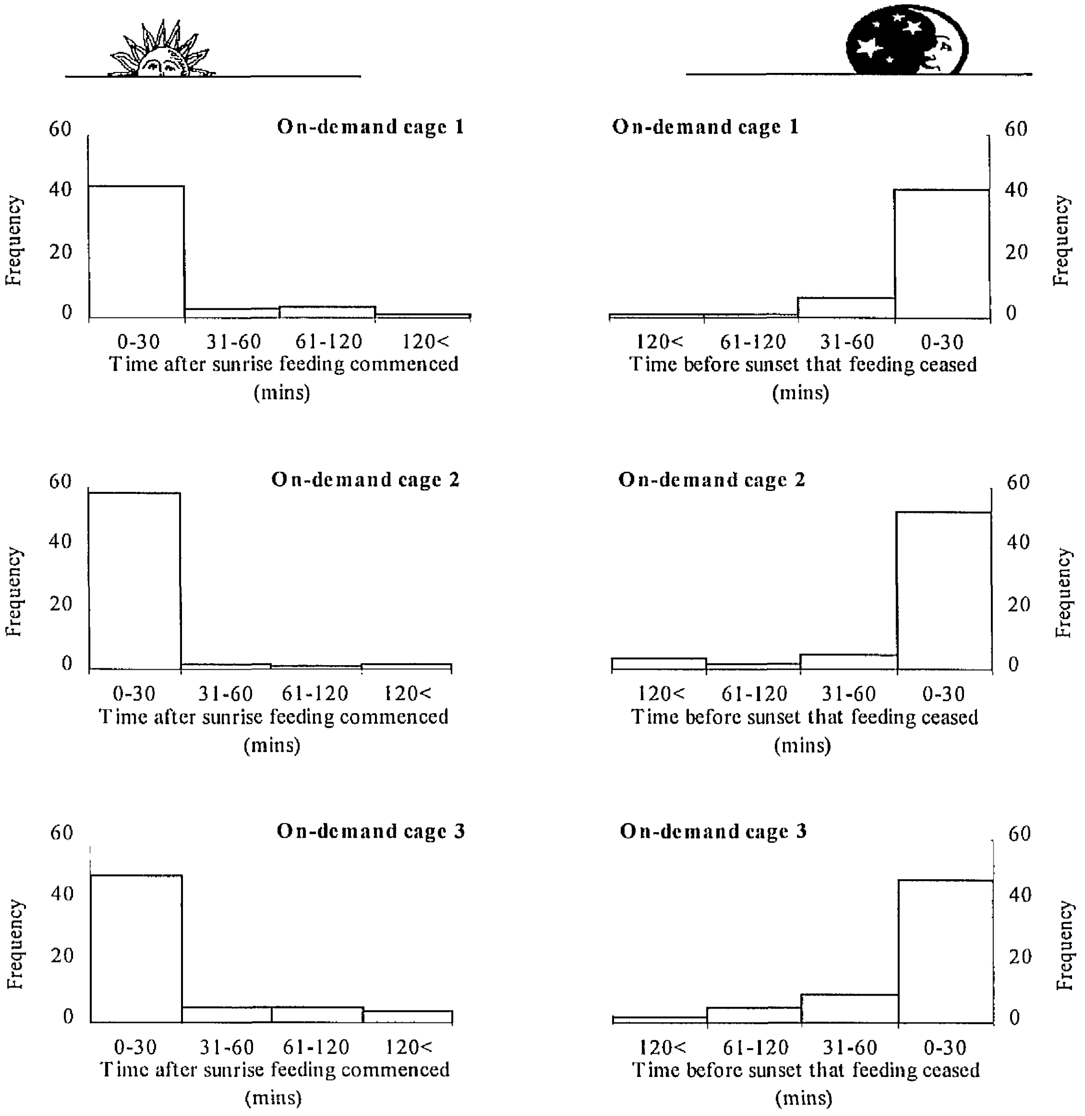


Fig. 4a.7 The effect of the beginning and end of twilight on the time feeding commences and ceases in Atlantic salmon parr fed on-demand. The images of the sun and moon represent sunrise and sunset.

4a.4. Discussion

4a.4.1 Daily and seasonal feeding rhythms

Fish fed on-demand in freshwater production cages exhibit daily rhythms of feeding and these rhythms change as the month's progress. Feeding was not continuous throughout the day and occurred in several meals that varied in their timing and duration. Distinct feeding periods have also been observed in rainbow trout, *O. mykiss* (Boujard and Leatherland, 1992*a*) and the authors attributed them to the presence of a distinct stomach. The motivation to feed can be influenced by gastric emptying time (see Grove et al., 1978) and many studies in salmonids have suggested appetite and its return are closely correlated with stomach fullness (Brett, 1971; Elliott, 1975; Grove et al., 1978).

Atlantic salmon parr exhibit flexible and adaptive feeding patterns that vary with season. During late summer and early autumn there was a significant morning peak in feeding activity in two of the groups fed on-demand. This significant peak in appetite was not retained during November. In December there was a significant peak in feed delivery around midday and this peak shifted to later in the afternoon in January and February (again, for two of the three on-demand fed treatments). In the month prior to smolt transfer (March), this significant peak in feed delivery was retained, although the fish began to extend their feeding through the daylight hours. There were significant differences in feeding patterns between on-demand fed replicates. The third treatment (on-demand fed cage 1) showed a significant peak in feeding after first light during September, but this was not retained during October. In November there was a significant peak in feeding prior to dusk. During December and January the fish did not show a propensity to feed at any specific time of the day. No data were collected after this time because the on-demand feeding unit was damaged by water.

Asynchrony in the activity and feeding patterns of fish held singly or in groups under similar conditions is not uncommon. Variation in on-demand feeding activity between replicate groups has been reported in rainbow trout, *O. mykiss* (Boujard and Leatherland, 1992*a*; Alanärä and Brännäs, 1996) and the Arctic charr, *Salvelinus alpinus* (Brännäs and Alanärä, 1993). Sánchez-Vázquez et al. (1995*a,b*) found sea bass, *Dicentrarchus labrax*, can simultaneously exhibit nocturnal and diurnal feeding patterns when held singly or in groups. The loach, *Noemacheilus barbatulus*, also exhibits asynchronous activity patterns when held in groups (Burdeyron and Buisson, 1982). The basis of this asynchrony may be a group effect, with differing groups preferring to feed at different times of the day because of differences in the levels of competition (Alanärä and Brännäs, 1996). Individuals can exhibit conflicting feeding patterns within a group; Brännäs and Alanärä (1997) reported heterogeneity in the times that individuals choose to feed, and Kadri et al., (1997*b*) found different sized individuals choose to feed at different times of the day within a group of Atlantic salmon post smolts. It may also be an effect of the feeding unit, as the underwater sensor often needed re-calibrating during the trial (C. Noble, pers. obs.).

Other studies on feeding rhythms of Atlantic salmon parr have reported a wide range of findings although these fish are generally diurnal during summer and autumn (see Table 4a.1). In winter, when temperatures drop below 10°c, Atlantic salmon parr can become nocturnal and feed during the night (Fraser et al., 1993, 1995). In this experiment the fish were not offered food at night because feeding efficiency is only 35% of diurnal efficiency (Fraser and Metcalfe, 1997). This low feeding efficiency may contribute to pellet wastage and increase FCR, which is detrimental to the profitability of an aquaculture venture (Thorpe and Cho, 1995). The afternoon peak in feeding during winter observed in this experiment may be a response to the fish being prevented from feeding at their preferred time during the night. Fish possess foraging behaviours that have an adaptive flexibility (Dill, 1983), enabling them to cope with feeding outwith their preferred phase (Eriksson and Alanärä, 1992). Seasonal differences in feeding activity may also be a reflection of natural feeding rhythms in response to predator avoidance, or changes in prey availability (see Amundsen et al., 1999). Further, peaks in feeding activity may depend upon endogenous mechanisms and coincide with times that the physiological utilisation of nutrients is most efficient (Bolliet et al., 2001). If aquaculturists are aware of seasonal changes in feeding patterns they can alter feed delivery to match the appetite requirements of the fish. This can improve growth performance and profits (Spieler, 1977; Boujard et al., 1995; Azzaydi et al., 1999) and can potentially reduce pellet wastage and loss (Thorpe and Cho, 1995).

4a.4.2 Variability in daily feed intake in relation to environmental variables

Many studies have reported daily variability in feed intake when fish are held singly or in groups (Grove et al., 1978; Cui and Wooton, 1988; Jobling and Baardvik, 1991; Blyth et al., 1993) and this variability can manifest itself under stable environmental conditions. Part of this variability may be related to endogenous factors such as gastric emptying time and stomach fullness (Grove et al., 1978), although exogenous abiotic factors may also govern daily feed intake (see Bégout Anras, 1995).

When Atlantic salmon parr are fed on-demand in freshwater cages they exhibit distinct variations in daily feed intake. Daily feed intensity varies with season for all cages within the on-demand treatment. There is a decrease in daily feed intake during autumn, low feed intake during winter and an increase in spring as the fish approach smolt transfer. As fish are ectotherms, they have very low maintenance requirements during winter because of low temperatures (Metcalfe et al., 1998), so this low feed intake is not surprising. Univariate GLM analysis showed daylength was the only consistent significant predictor of daily feed intake, although the extent of its effect did differ between cages. Windspeed was also a significant predictor of daily feed intake for cage 2. The models accounted for around 70% of the daily variability in feed delivery for all treatments. Smith et al., (1993) found daylength and the change in daylength were the only significant predictors of appetite variability in cage-held Atlantic salmon weighing between 2-5kgs. A study in this thesis on cage held post-smolts (Chapter 3a) found daylength was a significant predictor of daily changes in appetite. Furthermore, in pond-held sea bass, *Dicentrarchus labrax*, daylength was one of the main factors that governed daily appetite (Bégout Anras, 1995), whereas photoperiod and temperature regulate the feed intake of sea bass in tanks (Azzaydi et al., 1998).

Daylength is a primary entraining agent governing feeding rhythms (Eriksson and Alanärä, 1992; Boujard and Leatherland 1992*a*,*b*); a short or decreasing photoperiod can suppress feeding activity, whereas an increasing photoperiod can enhance it (Komourdjian et al., 1976; Higgins and Talbot, 1985; Villarreal et al., 1988; Jørgensen and Jobling, 1992). The reduction in feeding during autumn has been observed in several other studies on Atlantic salmon parr and may be associated with a decreased motivation to feed with the onset of winter (Metcalfe et al., 1986; Simpson et al., 1996; Amundsen et al., 1999). Fat deposition may act as a physiological feedback loop governing appetite by decreasing feeding motivation when an individual has accumulated sufficient energy reserves (Tveiten et al., 1996). Higgins and Talbot, (1985) reported similar results to this experiment, with Atlantic salmon parr increasing food consumption and growth during spring when temperatures were still decreasing. The authors suggested that increasing daylength stimulated appetite and growth.

4a.4.3 Synchrony in feed delivery over longer time periods

Atlantic salmon parr can exhibit large fluctuations in appetite between days when held under identical environmental conditions. This may be a group effect due to intrinsic factors within each cage. For example, if fish within an on-demand fed cage are prevented from feeding to full satiation during a day, they consume more when next given an opportunity (see Juell et al., 1994). There may also be daily differences in appetite because of fluctuating stomach fullness and the time required for appetite to return after the gut empties (Grove et al., 1978; Ruohonen et al., 1997). This may modify the feeding patterns and rhythms of individuals held under high stocking densities and account for some of the daily variability between on-demand replicates. Feed management strategies based upon feed tables do not allow for this daily variability, whereas an on-demand system gives a farmer the opportunity to integrate feeding variability into daily farm management. In this experiment I wished to test the hypothesis that fish select similar rations over prolonged periods of time even with this daily fluctuation in appetite. There was no significant cage effect upon overall feed delivery for parr held in freshwater production cages (see Figure 4a.5) between October and January when all on-demand feeders were functioning correctly. Different groups of fish select similar rations over extended periods of time even though there may be clear disparity in the feed intake of on-demand fed groups on any given day; the daily variability in feed intake is absorbed over extended time intervals.

4a.4.4 Timing of feeding in relation to sunrise and sunset

Small groups (n=15) of Atlantic salmon parr have been reported to refrain from feeding at first light in autumn due to high levels of aggression, with competition regulating the timing of feeding (Kadri et al., 1997*a*). Using the timing of feed delivery in relation to sunrise it is possible to evaluate whether this phenomenon exists in large groups held in freshwater production cages. Feeding commenced at sunrise in all the cages within the on-demand fed treatment and suggests there was no suspension of feeding due to aggression. In a production cage the factors that promote aggressive behaviour may breakdown due to favourable resources becoming indefensible under high stocking densities (Kalleberg, 1958; Fenderson and Carpenter, 1971). Aquaculturists should therefore begin feeding salmon parr at first light during the autumnal months when the fish exhibit a significant morning peak in appetite.

4a.4.5 Conclusions

Cage held groups of Atlantic salmon shift their daily feeding patterns with season when fed on-demand. During late summer and autumn fish show an increased propensity to feed at first light, but in winter they show a general inclination to feed in the afternoon. The fish extend their feeding activity throughout the diurnal period in spring as they begin to undergo smoltification. The daily variation in feed delivery between cages within the on-demand fed treatment is absorbed over the majority of the study period and each group of on-demand fed fish selected similar rations. Aquacultural strategies, which integrate this seasonal variability into feed management, may improve growth performance and profits whilst reducing waste by refraining from offering fish food when they are not hungry. Daylength was a significant predictor of daily feed delivery for all three replicates. A farmer should be wary of implementing feed strategies based upon feed tables that state temperature and fish size as crucial factors governing food intake.

Chapter 4b: The effect of feeding regime upon the growth and production of Atlantic salmon parr held in freshwater production cages

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4b.1 Introduction

4b.1.1 Aims and content

The aim of this chapter is to evaluate the effect of feeding regime upon growth performance and production when Atlantic salmon parr are held in production cages. This aim is two-fold; firstly, to evaluate existing imposed feeding regimes using the data from novel on-demand feeding systems and secondly, to provide recommendations for improved feeding practice by utilising the data from the interactive on-demand feeders.

4b.1.2 Feeding regimes

Feed management and feeding regimes regulate feed delivery rate, frequency and duration, ration size and the spatial and temporal distribution of food to fish within a rearing facility (see Talbot, 1993; Talbot et al., 1999). Each of these components can influence a number of production parameters that are fundamentally important to the profitability of a commercial aquaculture venture.

4b.1.3 Feeding rate

The rate of feed delivery can influence competition and pellet wastage within a rearing facility. A high feed delivery rate improves access to feed, reducing competition as food becomes indefensible (Grant, 1993; Ryer and Olla, 1995). It may also increase the potential for food wastage (Ang and Petrell, 1998), as fish may not be able to consume every pellet before these pass out of the rearing system. On-demand interactive feedback systems can detect this excess food, and if not configured correctly, may cause premature cessation of feed delivery even though the fish are still hungry, leading to poor growth and a high feed conversion ratio (Talbot et al., 1999). A low feeding rate can increase competition because the feed resource is more easily defended by better competitors (Thorpe and Huntingford, 1992). A uniform and predictable rate of delivery has also been shown to increase aggression in zebrafish, *Brachydanio rerio*, (Grant and Kramer, 1992).

4b.1.4 Feeding frequency and meal duration

Many studies on feeding frequency in salmonids have shown that between one and four meals per day can be sufficient to achieve optimal growth (Elliott, 1975; Landless, 1976; Grayton and Beamish, 1977; Juell et al., 1994; Thomasson and Fjæra, 1996; Ruohonen et al., 1998). In adult Atlantic salmon, *Salmo salar*, meal duration has been shown to have no effect on growth, body composition, condition factor or feed conversion ratio (Sveier and Lied, 1998). Furthermore, it has been reported that feed regime has little effect on growth performance when fish are fed to satiation (Juell, 1995; Nakagawa et al., 1999). Many fish farms implement feeding regimes that offer small amounts of food over short intervals throughout the day as farmers assume concentrated feeding periods increase pellet wastage (Alanärä et al., 2001). This regime may have a deleterious effect upon growth as frequent feeding may increase swimming activity and energy expenditure (Johansen and Jobling, 1998).

4b.1.5 Spatial and temporal presentation of food

The spatial and temporal distribution of food can also regulate access to a feed resource (Ryer and Olla, 1996*a*). Food may be spatially distributed across a rearing unit in a number of ways ranging from localised clumping to a widespread dispersion depending upon the individual or equipment involved. Increasing the spatial dispersal of food can increase the feeding opportunity of more fish within a group as feed becomes indefensible by stronger competitors (Magnuson, 1962;

M^cCarthy et al., 1992; Grant, 1993; Ryer and Olla, 1996*b*). Increasing feed dispersal can improve feeding opportunities in visual foragers such as Atlantic salmon, as more pellets fall within a fish's reactive distance (Dill, 1978). Increased spatial clumping of the feed resource can potentially increase competition, aggressiveness and food monopolisation with stronger competitors suppressing the feeding response of poorer competitors (Grant, 1993; Grant and Guha, 1993; Metcalfe et al., 1992; Ryer and Olla, 1995, 1996*b*). This disproportionate food acquisition can lead to increased growth variation within the group, which is undesirable in aquaculture as portions of the group are growing at sub-optimal rates (see Magnuson, 1962; Davis and Olla, 1987; Ryer and Olla, 1995).

The way that food is distributed across a day can also determine access to food. Resources that are temporally dispersed are more easily defended, increasing competition and aggression (Grant and Kramer, 1992). A temporally clumped feeding resource is less defensible which may decrease aggression and competition (Davis and Olla, 1987). Furthermore, many species of fish demonstrate daily feeding rhythms in food consumption and appetite (Kadri et al., 1991). Co-ordinating feed delivery with appetite can improve growth, nutrient utilisation and the proximate composition of a number of commercially important fish species. The timing of feed delivery has been reported to affect growth performance and food intake (Noeske et al., 1981; Sundaraj et al., 1982; Noeske and Spieler, 1984; Noeske-Hallin et al., 1985; Kerdchuen and Legendre, 1991; Reddy et al., 1994; Boujard et al., 1995, Chen and Purser, 2001). Feeding time can also affect the protein retention and lipid concentration of rainbow trout, *Oncorhynchus mykiss*, (Boujard et al., 1995; Gélineau et al., 1996) and in the latter study protein retention was best when fed in phase with appetite rhythms. Furthermore, feeding in phase with appetite can affect the proximate composition and market quality of the fish (Noeske-Hallin et al., 1985; Kerdchuen and Legendre, 1991).

4b.1.6 Ration size

Optimal ration level is primarily determined by water temperature and the size of the fish (Brett, 1979), but other factors may also play a subsidiary role in regulating feed intake (see Chapter 4a for examples). Ration size is difficult for a farmer to assess (Ang and Petrell, 1998) as the amount of food required for fish to reach satiation varies between meals (Juell et al., 1994; Ang and Petrell, 1997) and days (MacLeod, 1977; Juell et al., 1993, Blyth et al., 1993). Recent feeding history may also influence hunger levels and feeding activity (Talbot et al., 1984; Juell et al., 1994; Juell, 1995). Overfeeding increases food pellet wastage (Thorpe and Cho, 1995) and feed conversion ratios (Talbot et al., 1999). Wasted food can reduce the quality of water within a rearing facility and can be detrimental to the health of fish (Wedemeyer, 1997). Underfeeding may also increase feed conversion ratio (Cho, 1992), reduce growth and increase competition levels (M^{c}Carthy et al., 1992). Restrictive amounts of food increase competitive ability and aggression (Symons, 1968) and can further accentuate problems of competition if delivered in a spatially localised manner (Ryer and Olla, 1996*b*). Underfeeding may also be deleterious upon growth and growth depensation (Storebakken and Austreng, 1987; Miglavs and Jobling, 1989; M^{c}Carthy et al., 1992) and may affect the proximate body composition of fish within the rearing system (Hung et al., 1993; Shearer, 1994).

4b.1.7. The implications of choice of feeding regime

Feeding regime can affect numerous production parameters of both economic and environmental importance. These can be classified into four main categories:

i) **Growth performance.** Feeding regime can primarily influence fish growth performance, nutrient utilisation and partitioning (Miglavs and Jobling, 1989; Hung et al., 1993; Shearer, 1994).

ii) **Uniformity of growth.** Market forces demand a high quality product of uniform size. Feed management strategies can influence the uniformity of growth within a caged population of fish by either suppressing or stimulating competition which dictates equality of access to a feed resource (Magnuson, 1962; M^{c}Carthy et al., 1992; Thorpe and Huntingford, 1992). If food is dispersed in a temporally or spatially limiting fashion, or if the ration is of insufficient size or duration, it can promote competition between fish held in the rearing system. This has been reported in aquaculture, where 18% of fish held in a sea cage can monopolise half the feed resource (Thorpe et al., 1990). This, as stated in earlier chapters, can lead to growth depensation, which is undesirable for the consumer driven fish farming industry and influences harvesting strategies. The amount of food offered and its presentation to the fish can also affect the condition factor of fish held within rearing systems (Ng et al., 2000).

iii) **Speed of growth.** Feed management strategies can also influence the growth rates of individuals held under commercial densities. Growth rate is affected by whether an individual can gain access to food and if the ration is of a sufficient size. If food is again delivered at an insufficient rate or in insufficient amounts a number of individuals within the population will not gain access to the feed resource and grow at sub-optimal rates (Davis and Olla, 1987; Miglavs and Jobling, 1989; Storebakken et al., 1991; Thorpe and Huntingford, 1992; Jobling 1994, 1995). This is deleterious to profits as the aquaculturist is failing to realise the full growth potential of the fish and their husbandry facilities.

iv) **Cost of growth.** Due to high feed costs, farmers ultimately correlate the productivity of their aquaculture venture with their feed conversion ratio (FCR). If too much food is delivered to the fish, or if it is delivered at times when fish are not hungry pellet wastage and FCR can be increased (Thorpe and Cho, 1995; Talbot et al., 1999). If too little food is delivered when the fish are hungry it can lead to poor growth performance, poor FCR

(Cho, 1992) and high foraging costs whilst the fish attempt to gain access to food in a highly competitive social environment (M^{c}Carthy et al., 1992).

In the competitive fish producing market an aquaculturist may be able to boost profits by improving the efficiency of their current standing investment and husbandry facilities. Reducing pellet wastage can improve farm sustainability (Cho and Bureau, 1998) under current environmental protection regulations and a farmer may even be able to increase the production capacity of each farming unit by reducing environmental degradation per fish (M. Beveridge, pers. comm.).

4b.1.8 Objectives of the experiment

In the following experiment I will examine the effect of feeding regime upon growth performance and the production of Atlantic salmon parr held under commercial rearing densities. I will also evaluate the effectiveness of current feeding techniques by comparing an existing automatic feeding system (that delivers a fixed ration, according to in-house feed tables, at regular intervals throughout a day) with an on-demand interactive feeding system where fish can regulate the timing and duration of feed delivery via their appetite. The comparison will allow a farmer to assess the effects of differing ration size and differences in the temporal presentation of food upon production performance. The questions I will address in this experiment are: -

i) Are there any differences in ration size between that offered by in-house feed tables when compared with an on-demand feeding regime?

ii) How does a feeding regime affect growth performance and growth rate?

iii) How does a feed regime affect the uniformity of growth within a group of cage held fish?

iv) Do feeding strategies affect feeding efficiency?

The experiment was designed as a full-scale production trial, under the constraints of in-house husbandry practices and using commercial stocking densities and rearing facilities, to produce data that are directly applicable to freshwater cage aquaculture. By evaluating existing feeding regimes with data produced by novel on-demand feeding systems I hope to address how feeding strategies may be improved and how a farmer can implement feeding practices that are more economically and environmentally acceptable.

4b.2 Materials and methods

4b.2.1 Fish husbandry

The fish husbandry used in this experiment is outlined in Chapter 4a. Refer to Figs. 4a.1 and 4a.2 for the experimental set-up.

4b.2.2 Sampling methods for morphological studies

Each population of fish was sampled on a monthly basis from late autumn (2nd September 1998) until one week prior to smolting on the 27th March 1999. The timing of these samples will be referred to as T_{A0}, T_{A1}, T_{A2} and T_{A3}; T_{B0}, T_{B1}, T_{B2}; and T_{C0} and T_{C1} respectively (after Jørgensen et al., 1996). This corresponds to both the trial period (either T_A, T_B, or T_C) and the timing of the sample (refer to figure 4b.1). There was a two month interval between the 2nd December 1998 and the 20th January 1999 where the fish were not sampled. During sampling, the entire group of fish were crowded into a small area of the cage and netted out at random. The procedure was carried out using the same equipment and person at each sampling point to reduce sampling error. The number of fish removed was approx. 250 fish per cage. Fish were netted out of the cages and anaesthetised using benzocaine. Excess water was drained off the fish before they were weighed (wet weight to nearest 1.0g), lengthened (fork length to nearest 0.5cm) and assessed for fin injury. Any remaining precocious parr (maturing males producing milt) were removed from the samples and not included in the analyses, as they have differing growth rates to non-maturing parr (Rowe and Thorpe, 1990).

4b.2.3 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0. Minitab Inc., Philadelphia) or SPSS (v. 10-0. SPSS Inc., Chicago) for MS Windows. Data were natural log transformed to satisfy normality and homogeneity of variance requirements. If the data could not be transformed non-parametric statistics were used. The accuracy of the sampling method was evaluated using 95% confidence intervals around each sample mean. Differences in growth between individual cages between treatments were tested for using a non-parametric Kruskal Wallis test. Within group variability in size was calculated using Coefficient of Variation: CV = (S.D. / Mean wet weight (g)) x 100. Where S.D. is standard deviation. Differences between the pooled Coefficient of Variation data for each treatment were tested for using a Mann Whitney test. Condition factor for individual fish was calculated as: $(W / L^3) \times 100$, where W is wet weight (g) and L is fork length (cm). Differences in the mean condition factor of individual imposed and on-demand cages were calculated using a non-parametric Kruskal Wallis test (after Bolger and Connolly, 1989). Differences in median daily ration per growth period were calculated for each individual imposed and on-demand cage using a Mann Whitney test. To account for the large number of tests ($n = 24$ for median weight and condition factor respectively; $n = 15$ for ration) a sequential Bonferroni calculation was used to adjust significance levels (see Zar, 1999). Specific Growth Rate (SGR, a function of % body weight per day) was calculated using [(ln Final weight (g) – ln Initial weight (g))/ Time in days] x 100. Where ln is natural log. Feed Conversion Ratio (FCR) was calculated using the formula: Amount Fed (kg)/ Wet weight gain (kg), for each growth period. Differences in SGR and FCR per growth period for all replicates within each treatment were tested for using a Mann Whitney test. Data are presented as means ± S.E. unless stated in the legend. Growth and conditional factor data, although based upon non-parametric statistics, are presented as means ± S.E. in line with current practice at Marine Harvest Ltd, the industrial CASE partner (allowing farmers to direct compare this data set with existing findings). A significance level of $P < 0.05$ was used for all statistical tests.

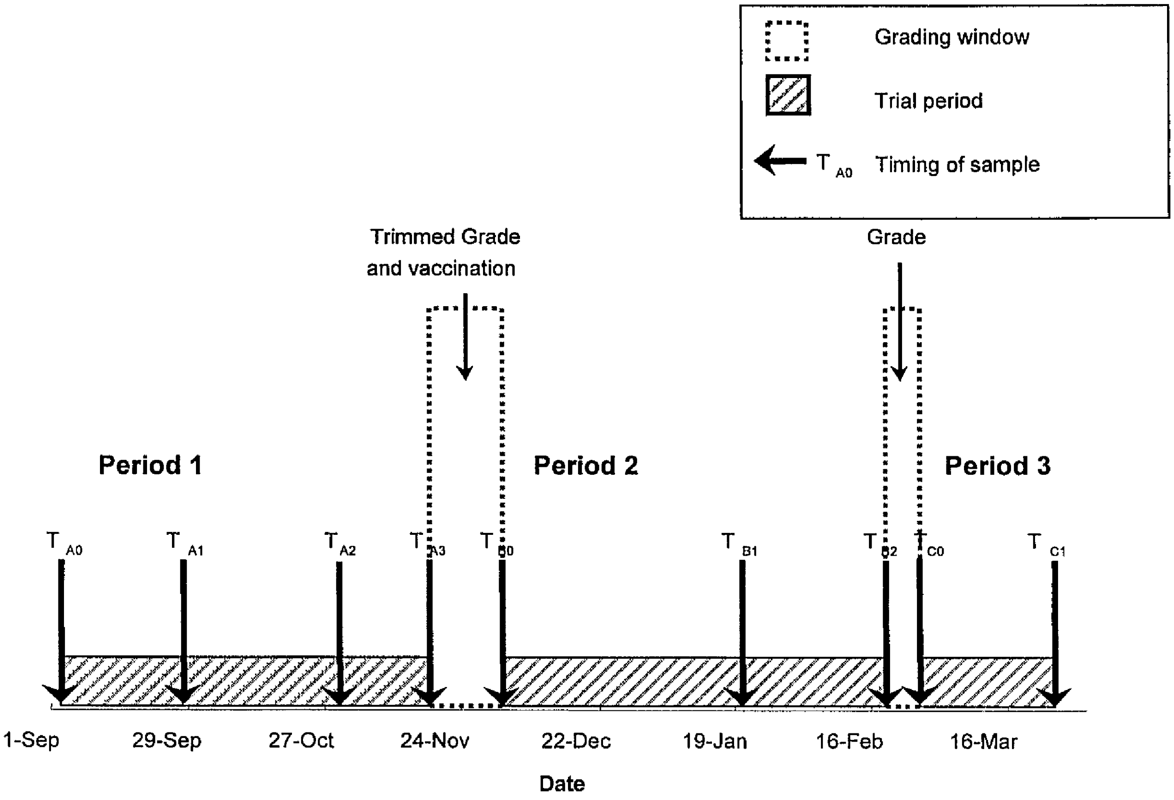

Fig. 4b.1 The timing of sample weights for growth and morphological studies. The experiment was broken down into three periods to allow for routine grading and vaccination.

4b.3 Results

4b.3.1 Sampling error and post-hoc corrections

At sampling point T_{B2} it appeared that there was a reduction in mean weight in comparison to the previous period in cage 3, under the imposed feeding regime. This was very surprising, as the fish were fed to an established in-house feeding regime, which under current farming conditions usually achieved the best growth performance from automated feeding systems (D. Mitchell, pers. comm.). Before making any inferences based upon this data an observer should evaluate the sampling method to see if it the obtained sample mean is representative and close to the real population mean. There was a large size variation (wet weight) in the cages (as the fish had only received a minor trimmed grade during the prior six months of the experiment) and the sample mean was not a precise reflection of the true population mean (Zar, 1999), even with a large sample size of 250 fish. The data can be refined to remove any outliers that can possibly skew the data. This was done by removing any data point that was outwith ± 3 standard deviations of the original sample mean, and is a method commonly used by aquaculture researchers (V. Crampton, pers. comm.). A revised sample mean (based upon 99% of the size variability within the group) was then calculated. The calculation of a confidence interval for this refined sample mean allows an observer to evaluate its precision (Zar, 1999) and 95% confidence intervals were calculated for each corresponding imposed and on-demand treatment cage (see Fig. 4b.2). A commercial freshwater farming outlet is satisfied with a sampling precision of± 2 grams around the sample mean, before deciding whether to size grade the fish (D. Mitchell, pers. comm). For sample periods T_{B1} and T_{B2} (see * on fig. 4b.2) 95% confidence intervals were outwith ± 2 grams of the refined sample mean (and these intervals overlapped the previous sampling point and treatment); any inferences drawn from this growth data should be approached with caution, in spite of the post-hoc data correction. This lack of sampling precision may also account for the misleading results observed in SGR and FCR calculations (see later in section). The data point from cage 3 under the imposed regime (which appeared to have lost growth in comparison with the previous period) was omitted from the SGR and FCR calculations.

4b.3.2 Effect of feeding regime on growth (weight gain)

In general, the choice of feeding regime did not have a significant effect upon median weight gain, but there were differences between corresponding imposed and on-demand cages during a minor number of growth periods (see Fig. 4b.2). Two of the three on-demand fed replicates grew significantly less than the fish under the imposed regime during growth period T_{B1}, although these results should be treated with caution due to sampling error (see earlier). Statistical analysis was carried out on individual corresponding imposed and on-demand cages, although the data between replicates was pooled for graphical representation to aid clarity (Fig. 4b.3). For statistical analyses comparing growth between corresponding treatment cages refer to Table 4b.1.

There was no significant difference in median weight between the first pair of treatment cages for the duration of the experiment. For the second pair of treatment cages there was a significant difference in median weight, with on-demand fed fish being significantly smaller at the start of the trial, T_{A0}, although this difference was not retained until T_{B1}. At T_{B1} there was a significant difference between treatments, where on-demand fed fish were significantly smaller. After this point there were no further significant differences. In the third pair of treatment cages the only significant difference in weight was at T_{B1}, where fish fed on-demand were significantly smaller (median weight).

4b.3.3 Effect of feeding regime on growth heterogeneity

There was no significant difference in the size heterogeneity of fish in either the imposed or on-demand fed treatment for the duration of the experiment (see Table 4b.2 for results of statistical analyses). At the beginning of the experiment the size variation of on-demand fed fish decreased after one month of the feeding treatment in comparison with that of the imposed regime (Fig. 4b.4), but the sample size at this growth point (n = 2) was prohibitive for statistical investigation.

4b.3.4 Effect of feeding regime on mean condition factor between treatments

Statistical analysis was carried out on individual corresponding imposed and on-demand cages (see Table 4b.1) although the data between replicates was pooled for graphical representation to aid clarity (Fig. 4b.5). Condition factor decreased with time during winter and spring as the fish approached smoltification.

During September and October there was no significant difference in the condition factor of fish held in the first pair of treatment cages. There was a significant difference in condition factor by mid November, T_{A3}, with fish fed on-demand exhibiting significantly better C.F. After the trimmed grade at the start of period T_{B0}, there was a significant difference between treatments for the duration of this second experimental period, T_{B0}, T_{B1} and T_{B2} respectively; with fish fed on-demand exhibiting significantly better condition factor.

In the second pair of treatment cages, there was no significant difference in condition until T_{B0}, where fish fed on-demand had a better C.F. After T_{B1}, there was a significant difference in median C.F. between treatments (fish under the imposed feeding conditions had a better C.F.) and this trend was retained until the end of the trial.

In the third pair of treatment cages there was a significant difference in condition factor at growth period T_{A3}, where fish under the imposed regime had a significantly better condition factor than those fed on-demand. After the trimmed grade at the start of period T_{B0}, there was again no significant difference in mean C.F. between treatments and this trend was retained during January. At the end of this experimental period there was a significant difference between treatments, with fish fed on-demand having a significantly better C.F. After the full size grade at the start of period T_{C0}, fish fed on-demand again exhibited significantly better C.F. although this difference was not retained at the end of the experiment, T_{C1}.

4b.3.5 Ration differences per growth period between feeding regimes

Table 4b.1 shows the results of Mann Whitney analyses to compare the rations between feeding regimes per growth period. Again, ration size differed significantly between feeding regime but this differed with both the growth period and treatment replicate (see Fig. 4b.6). There was no significant difference in ration between the first pair of treatment cages for the duration of the experiment. There were significant differences between regimes for the second pair of treatment cages at periods T_{A1} and T_{B2} where fish fed to the imposed regime received significantly more food. Fish fed to the imposed regime again received significantly more food in relation to those fed on-demand for the third pair of treatment cages at period T_{A1}.

4b.3.6 Effect of feeding regime upon FCR and SGR

There were no significant differences between treatments for FCR data for the periods of the experiment that permitted statistical analysis (see Table 4b.2). This was initially surprising, as there were marked differences in FCR between treatments within growth periods (see Fig. 4b.7). This may have been due heterogeneity in results between replicates, which indicates the experimental design (low number of replicates due to production constraints) was not very powerful (Jobling, 1995). This may not provide the means to demonstrate significant effects of treatment (Searcy-Bernal, 1994; Gardeur et al., 2001). The first striking incidence of poor FCR in the imposed treatment was caused by poor growth of one replicate between T_{A2} and T_{A3}. The poor

FCR noted in the imposed regime at the later period $T_{B1} - T_{B2}$ should be treated with caution due to sampling problems (see earlier). In general, the feeding efficiency of the on-demand treatment (although not significantly different) was better than fish fed to an imposed regime. There were no significant differences in SGR between treatments for the duration of the experiment (see Table 4b.2 and Fig. 4b.8).

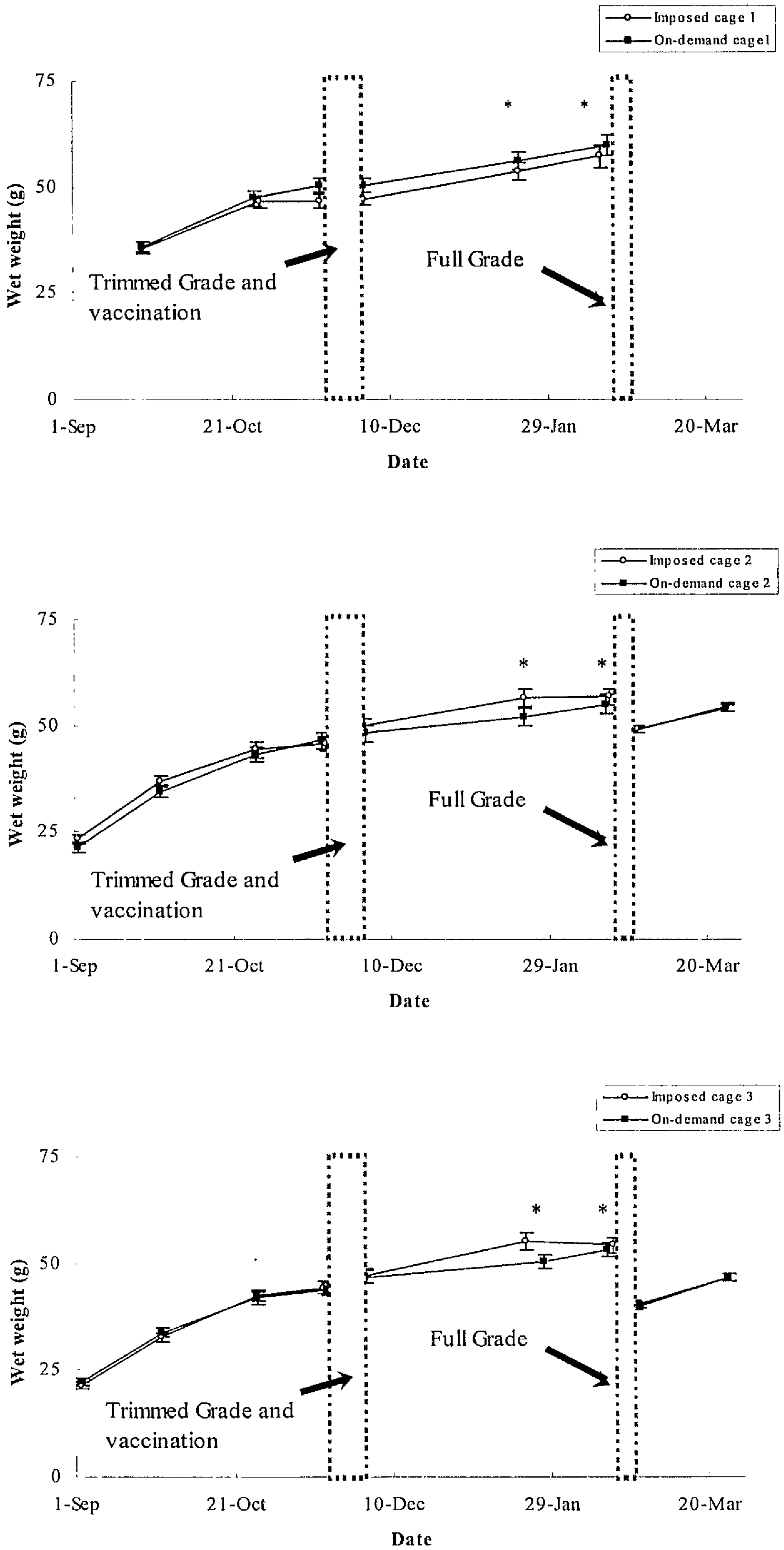

Fig. 4b.2 The effect of feeding regime upon the growth of parr in production cages. Growth is expressed as mean wet weight (grams) and error bars indicate 95% confidence intervals. * indicates sampling periods where confidence intervals were outwith ± 2 grams of sample mean.

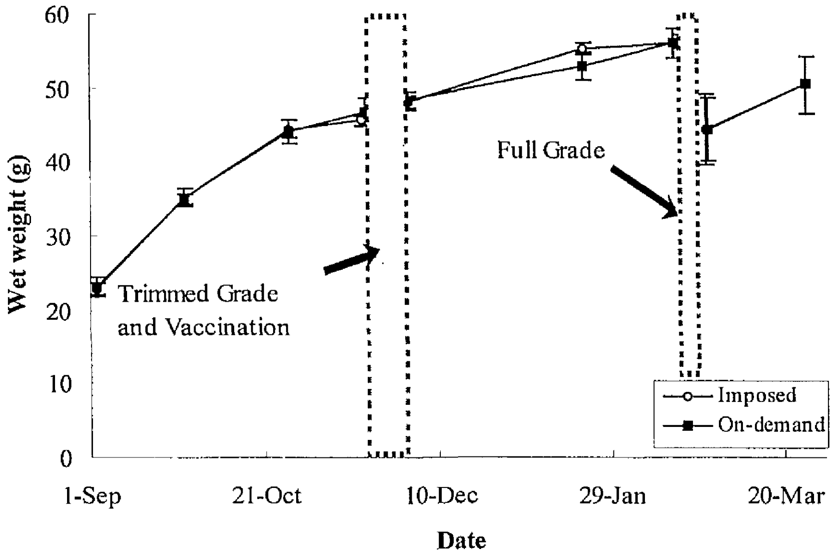

Fig. 4b.3 The effect of feeding regime upon the growth of parr in production cages. Growth is expressed as mean wet weight (grams) and error bars indicate standard error of the mean. Data from replicates within treatments are combined to aid clarity.

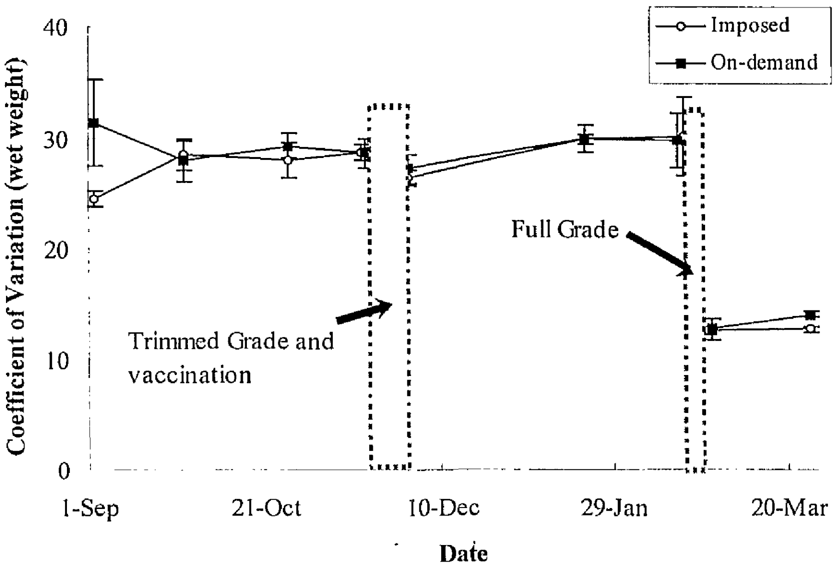

Fig. 4b.4 The effect of feeding regime upon the size variation of parr in production cages. Size variation is expressed as coefficient of variation (wet weight) and error bars indicate standard error of the mean. Data from replicates within treatments are combined to aid clarity.

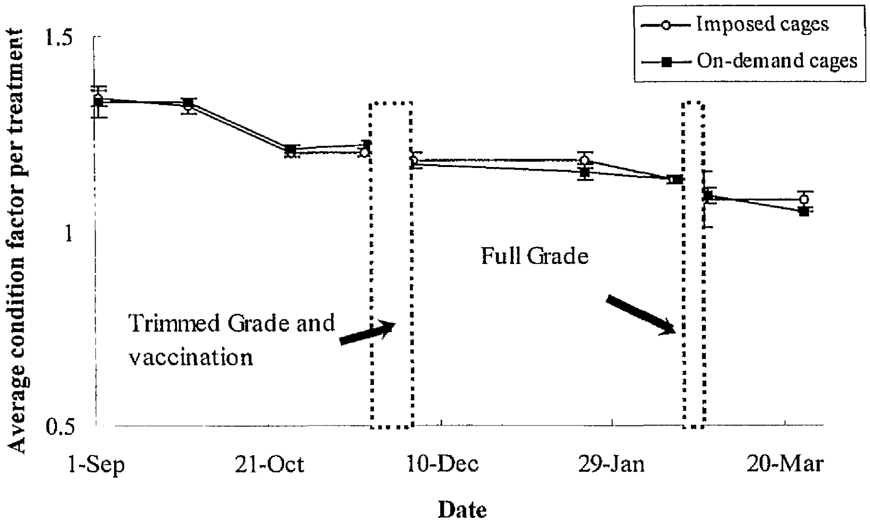

Fig. 4b.5 The effect of feeding regime upon the condition factor of parr in production cages. Data from replicates within treatments are combined to aid clarity and error bars indicate standard error of the mean.

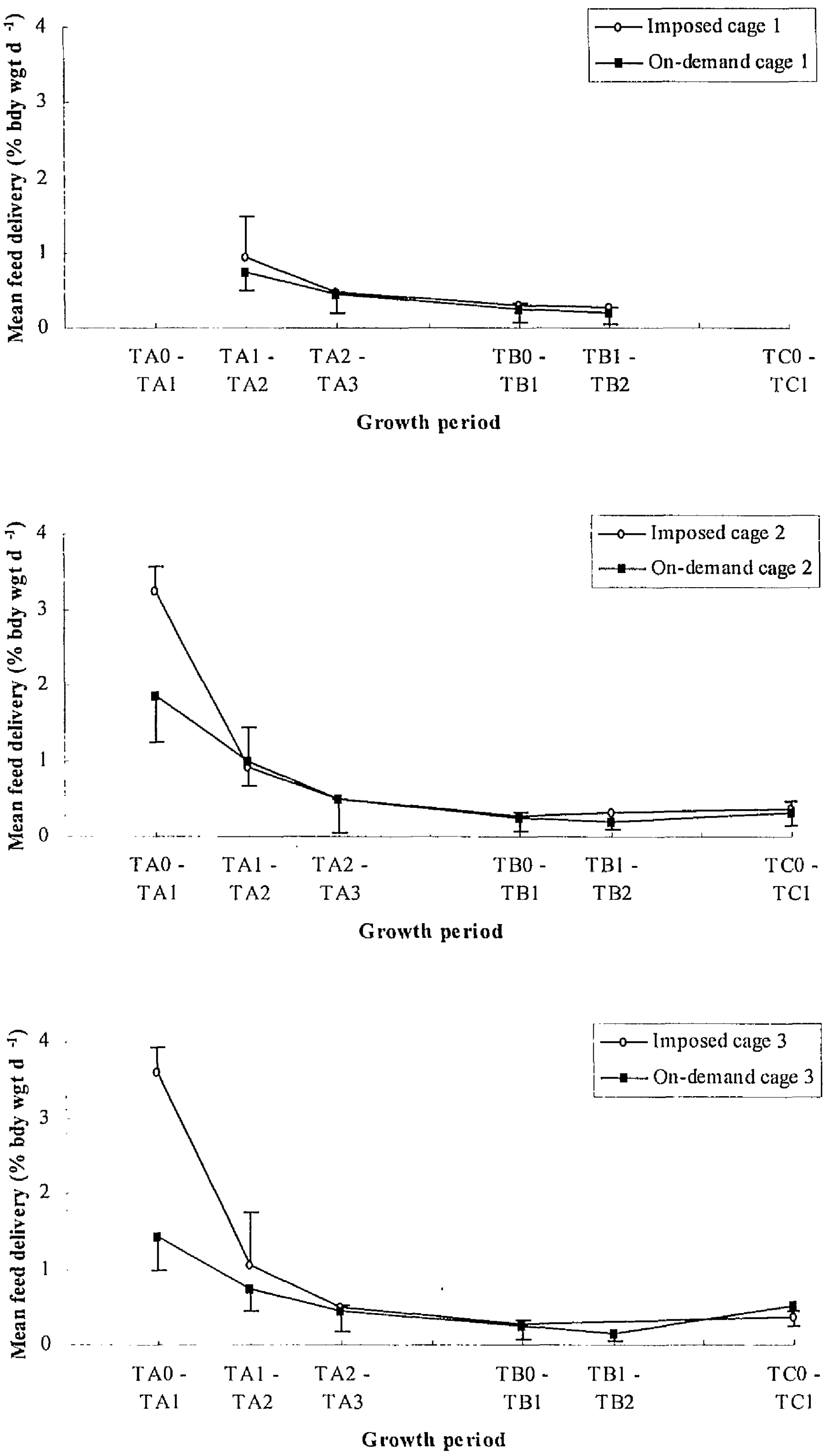

Fig. 4b.6 The differences in feed delivery between the on-demand and imposed feeding regimes. Data points represent mean daily fed delivery per growth period and error bars indicate standard deviation (the + or - error bar is omitted to aid clarity for each treatment). Imposed data point TB1-TB2 is omitted on cage 3 due to an error in the data.

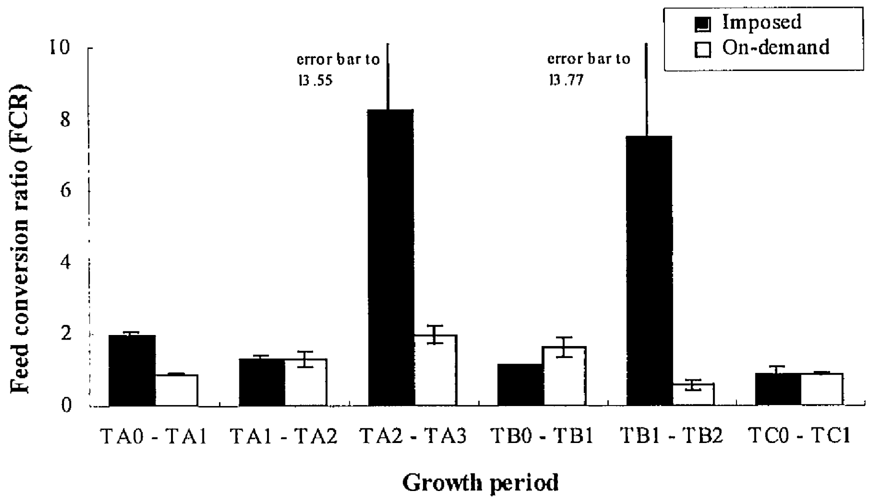

Fig. 4b.7 The effect of feeding regime upon the feed conversion ratio (FCR) of parr held in production cages. Data points represent mean FCR (all replicates combined within each treatment) for each growth period. Error bars indicate standard error of the mean. Refer to text for formula to calculate FCR.

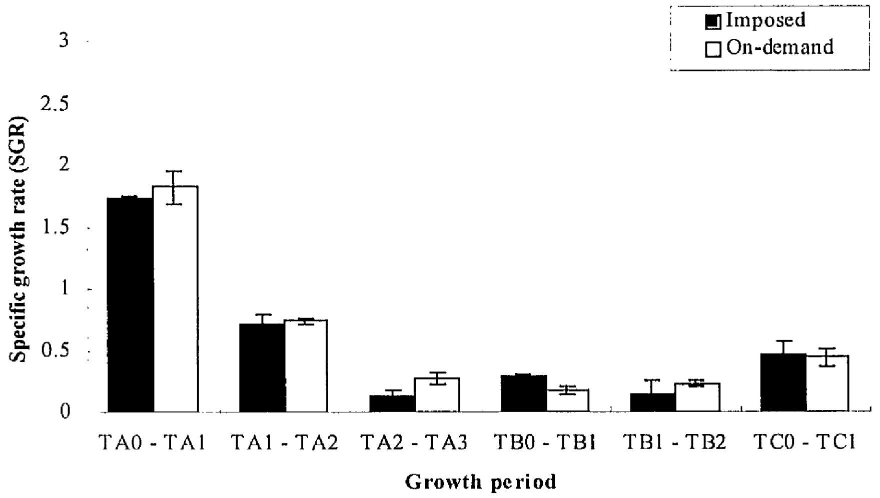

Fig. 4b.8 The effect of feeding regime upon the Specific Growth Rate (SGR) of parr held in production cages. Data points represent mean SGR (all replicates combined within each treatment) for each growth period. Error bars indicate standard error of the mean. Refer to text for formula to calculate SGR.

Table 4b.1. Results of Kruskal-Wallis analyses for comparing weight and condition factor between the corresponding individual treatment cages. Also shown are the results of Mann Whitney tests to compare mean daily feed delivery between treatments per growth period. All significant ration results denote periods where fish in the on-demand treatment selected a significantly smaller daily ration thanthose fed to an imposed regime. Significance levels were adjusted using a sequential Bonferroni calculation and * denotes a non-significant result.

Cage treatment	Growth period	Significant differences between replicates in:		
		Mean weight	Condition factor	Ration
On-demand and Imposed cages: 1	T_{A0}			
	T_{A1}	No ($H_{adj} = 0.53$, d.f. = 1, $P = 0.468$)	No ($H_{adj} = 2.38$, d.f. = 1, $P = 0.123$)	
	T_{A2}	No ($H_{adj} = 1.53$, d.f. = 1, $P = 0.217$)	No ($H_{adj} = 3.01$, d.f. = 1, $P = 0.083$)	No ($W = 1230$ $P = 0.347$)
	T_{A3}	No ($H_{adj} = 6.61$, d.f. = 1, $P = 0.010$)*	Yes ($H_{adj} = 9.50$, d.f. = 1, $P = 0.002$ - on-demand bigger)	No ($W = 359$ $P = 0.367$)
	T_{B0}	No ($H_{adj} = 6.32$, d.f. = 1, $P = 0.012$)*	Yes ($H_{adj} = 9.62$, d.f. = 1, $P = 0.002$ - on-demand bigger)	
	T_{B1}	No ($H_{adj} = 3.47$, d.f. = 1, $P = 0.062$)	Yes ($H_{adj} = 11.52$, d.f. = 1, $P = 0.001$ - on-demand bigger)	No ($W = 2320$ $P = 0.410$)
	T_{B2}	No ($H_{adj} = 2.95$, d.f. = 1, $P = 0.086$)	No ($H_{adj} = 5.09$, d.f. = 1, $P = 0.024$)*	No ($W = 429$ $P = 0.502$)
On-demand and Imposed cages: 2	T_{A0}	Yes ($H_{adj} = 10.92$, d.f. = 1, $P = 0.001$ - imposed larger)	No ($H_{adj} = 0.03$, d.f. = 1, $P = 0.858$)	
	T_{A1}	No ($H_{adj} = 5.35$, d.f. = 1, $P = 0.021$)*	No ($H_{adj} = 0.04$, d.f. = 1, $P = 0.835$)	Yes ($W = 963$ $P < 0.001$)
	T_{A2}	No ($H_{adj} = 0.65$, d.f. = 1, $P = 0.421$)	No ($H_{adj} = 2.05$, d.f. = 1, $P = 0.152$)	No ($W = 773$ $P = 0.103$)
	T_{A3}	No ($H_{adj} = 0.18$, d.f. = 1, $P = 0.670$)	No ($H_{adj} = 8.23$, d.f. = 1, $P = 0.004$)*	No ($W = 429$ $P = 0.266$)
	T_{B0}	No ($H_{adj} = 3.29$, d.f. = 1, $P = 0.070$)	Yes ($H_{adj} = 27.68$, d.f. = 1, $P < 0.001$ - on-demand bigger)	
	T_{B1}	Yes ($H_{adj} = 11.43$, d.f. = 1, $P = 0.001$ - imposed larger)	Yes ($H_{adj} = 68.29$, d.f. = 1, $P < 0.001$ - imposed bigger)	No ($W = 2772$ $P = 0.079$)
	T_{B2}	No ($H_{adj} = 2.66$, d.f. = 1, $P = 0.103$)	Yes ($H_{adj} = 25.17$, d.f. = 1, $P < 0.001$ - imposed bigger)	Yes ($W = 938$ $P < 0.001$)
	T_{C0}	No ($H_{adj} = 0.37$, d.f. = 1, $P = 0.542$)	Yes ($H_{adj} = 47.00$, d.f. = 1, $P < 0.001$ - imposed bigger)	
	T_{C1}	No ($H_{adj} = 0.09$, d.f. = 1, $P = 0.763$)	Yes ($H_{adj} = 13.96$, d.f. = 1, $P < 0.001$ - imposed bigger)	No ($W = 788$ $P = 0.524$)
On-demand and Imposed cages: 3	T_{A0}	No ($H_{adj} = 1.30$, d.f. = 1, $P = 0.254$)	No ($H_{adj} = 5.16$, d.f. = 1, $P = 0.023$)*	
	T_{A1}	No ($H_{adj} = 1.05$, d.f. = 1, $P = 0.306$)	No ($H_{adj} = 1.19$, d.f. = 1, $P = 0.276$)	Yes ($W = 875$ $P < 0.001$)
	T_{A2}	No ($H_{adj} = 0.57$, d.f. = 1, $P = 0.450$)	No ($H_{adj} = 2.76$, d.f. = 1, $P = 0.097$)	No ($W = 1004$ $P = 0.012$)*
	T_{A3}	No ($H_{adj} = 0.61$, d.f. = 1, $P = 0.433$)	Yes ($H_{adj} = 17.30$, d.f. = 1, $P < 0.001$ - imposed bigger)	No ($W = 444$ $P = 0.104$)
	T_{B0}	No ($H_{adj} = 0.05$, d.f. = 1, $P = 0.817$)	No ($H_{adj} = 0.64$, d.f. = 1, $P = 0.423$)	
	T_{B1}	Yes ($H_{adj} = 13.97$, d.f. = 1, $P < 0.001$- imposed larger)	No ($H_{adj} = 2.88$, d.f. = 1, $P = 0.090$)	No ($W = 2838$ $P = 0.208$)
	T_{B2}	No ($H_{adj} = 0.69$, d.f. = 1, $P = 0.406$)	Yes ($H_{adj} = 11.18$, d.f. = 1, $P = 0.001$ - on-demand bigger)	
	T_{C0}	No ($H_{adj} = 2.84$, d.f. = 1, $P = 0.092$)	Yes ($H_{adj} = 78.87$, d.f. = 1, $P < 0.001$ - on-demand bigger)	
	T_{C1}	No ($H_{adj} = 0.05$, d.f. = 1, $P = 0.825$)	No ($H_{adj} = 0.00$, d.f. = 1, $P = 0.989$)	No ($W = 615$ $P = 0.039$)*

Table 4b.2 Results of Mann Whitney analyses for comparing growth uniformity, FCR and SGR between treatments per growth period. N = 3 in all cases. Data is omitted from T_{A0}, T_{C0}, and T_{C1} because N = 2 and is prohibitive for statistical analysis. Each data point represents data from the previous growth period.

Cage treatment	**Growth period**	**Significant differences between replicates in:**		
		Growth uniformity	**FCR**	**SGR**
All On-demand and Imposed cages	T_{A0}			
	T_{A1}	No ($W = 10.0$ $P = 1.000$)		
	T_{A2}	No ($W = 8.0$ $P = 0.383$)	No ($W = 11.0$ $P = 1.000$)	No ($W = 10.0$ $P = 1.000$)
	T_{A3}	No ($W = 10.0$ $P = 1.000$)	No ($W = 15.0$ $P = 0.081$)	No ($W = 6.5$ $P = 0.127$)
	T_{B0}	No ($W = 9.0$ $P = 0.663$)		
	T_{B1}	No ($W = 12.0$ $P = 0.663$)	No ($W = 8.0$ $P = 0.383$)	No ($W = 15.0$ $P = 0.081$)
	T_{B2}	No ($W = 11.0$ $P = 1.000$)	No ($W = 9.0$ $P = 0.145$)	No ($W = 5.0$ $P = 0.773$)
	T_{C0}			
	T_{C1}			

4b.4 Discussion

4b.4.1 Effect of feeding regime upon growth

Both ration size and method of food presentation can affect growth performance and uniformity of growth. As stated in earlier chapters, an aquaculturist can enhance the growth of many commercial species if food is delivered in accordance with changes in appetite.

In general, the results of this current experiment show that there was an increase in growth (weight gain) for both treatment replicates, up until the full grade at the end of February. After the grade there was an increase in growth until the fish were transferred to seawater. Any differences in growth between treatments were at period T_{B1}, where fish fed by the imposed regime in two of the three replicates were significantly larger than those fed on-demand. There were problems associated with the sampling method at this time and the results should be interpreted with caution (the sample mean may not be an accurate reflection of the true population mean). Indeed, at period T_{B2}, it appeared that fish in one of the imposed regime cages suffered a weight decrease in comparison to the previous period, which was very surprising; the imposed feeding regime had consistently achieved the best growth performance on this farm for many years (D. Mitchell, pers. comm). The results of this present study generally indicate that fish fed on-demand did not grow better than their corresponding cages under the imposed feeding regime, which were fed a pre-determined fixed ration according to tables. This suggests that the on-demand feeding regime can match the growth performance of the existing fixed ration feeding regime. It does not appear that the fixed ration strategy is detrimental to growth even though fish exhibit increased swimming activity and energy expenditure (see Chapter 4c). This is in accordance with other studies on Atlantic salmon, where it has been suggested that growth performance is not overtly affected by feed regime if fish are fed to satiation (Juell et al., 1994; Juell, 1995).

4b.4.2 Effect of feeding regime upon growth uniformity and condition factor

In an aquacultural environment, growth depensation and food monopolisation by stronger competitors can be influenced by two variables: group size and access to food supply (Li and Brocksen, 1977; Jobling and Baardvik, 1994; Alanärä and Brännäs, 1996). As resources become restrictive, competition increases (Symons et al., 1968; Grant, 1993; Jobling and Koskela, 1996), which can enhance intraspecific differences in access to food (Thorpe et al., 1990), leading to growth depensation (Jobling, 1995) - an unattractive component of salmonid culture that a farmer wishes to control.

In the present experiment, growth depensation remained relatively constant for the duration of the experiment (until the grading event in February) after an increase in growth heterogeneity at the start of the trial in the imposed regime, accompanied by a corresponding reduction in the size heterogeneity of on-demand fed fish. There were no significant differences in growth heterogeneity between treatments for the period of the trial that permitted statistical analysis, T_{A1} – T_{B2}. This suggests that there was no difference in access to food between treatments, and that increased competition levels amongst fish under the imposed regime (see Chapter 4c) did not significantly affect growth depensation. A possible explanation is that behavioural interactions may not have a critical influence on the regulation of feed intake in the large groups of fish encountered in cage culture; high intra-specific variability in metabolic and growth rate may be more important than competition in governing growth heterogeneity in freshwater cages. These results are in accordance with other studies that have reported that feed regime does not affect size heterogeneity (Koskela et al., 1997; Azzaydi et al., 1998; Johansen and Jobling, 1998; Sveier and Lied, 1998). In addition to the suggestion by previous authors, that feed regime does not affect growth performance if fish are fed to satiation (Juell et al., 1994; Juell, 1995), this experiment shows that if daily ration is closely matched to daily feed requirements, feed regime does not significantly affect size heterogeneity in freshwater cages.

Reports upon the condition factor of fish are based upon analysis of length/weight data on individual fish, and infer that heavier fish are in better condition (Bolger and Connolly, 1989). In the current study, condition factor is used as a non-intrusive indicator of changing gross nutritional balance (De Silva, 1985), both within and between feeding treatments. Condition factor decreased from autumn to spring in both feeding treatments (see Fig. 4b.5). There was no general trend in significant differences in condition factor between treatments and growth period (see table 4b.1). In the first pair of treatment cages, fish fed on-demand had a better condition factor from period $T_{A3} - T_{B1}$. In the second pair of cages fish fed on-demand had better condition factor at period T_{B0}, after this point the imposed fed fish had a better C.F. In the third pair of treatment cages fish fed to the imposed regime had a better condition factor at the end of the first period of the trial. At the end of the second trial period and the beginning of the third, fish fed on-demand had a significantly better condition factor. The lack of any obvious trend in the effect of feeding treatment upon gross nutritional balance may suggest that there is no clear relationship between feeding regime and condition factor when fish are fed to satiation. This is in agreement with another study on Atlantic salmon, where meal duration has no effect on growth, body composition and condition factor of adult fish, if they are fed to satiation (Sveier and Lied, 1998). Cui et al. (1997) have also reported that feed regime does not affect the condition factor of white sturgeon, *Acipenser transmontanus.*

4b.4.3 Ration differences between feeding regimes

If an aquaculturist can evaluate differences in ration size between an on-demand feeding regime (where it is assumed that daily ration is an accurate estimate of daily feed requirements) and an existing imposed regime, it is possible to investigate the accuracy of existing techniques that are used to determine satiation. The results of the current experiment suggest the existing feed regime (based upon a combination of feed tables and hand-feeding) can accurately match daily feed requirements during winter and spring, in comparison with an on-demand feeding regime (see Fig. 4b.6 and Table 4b.1). During late summer/autumn the imposed regime fed significantly more food in relation to the on-demand feeding regime. An alternative interpretation of these results could be that on-demand fed fish were underfed at the start of the trial, although it can be surmised that there was less pellet wastage in the on-demand feeding regime, as the food restriction did not have a negative effect upon growth. Previous studies have reported that monitoring surface feeding activity as an index of satiation can be inaccurate in cage culture (Ang and Petrell, 1997; Talbot et al., 1999), so discordance between treatments for certain periods of the year is not surprising. Supplying a ration that closely matches daily feed requirement is a primary tenet of aquaculture; existing in-house feed management strategies appear to achieve this aim for the majority of the growing period before smoltification.

4b.4.4 Effect of feeding regime upon FCR and SGR

In an increasingly competitive fish producing market, farmers can improve production efficiency by improving feed management. If fish are overfed this can increase pellet wastage and FCR (Thorpe and Cho, 1995; Talbot et al, 1999), which is uneconomical and can potentially lead to environmental degradation (Cho and Bureau, 1998). Feed conversion ratios (FCR) were generally lower in fish fed on-demand (see Fig. 4b.7), although this difference was not significant (Table 4b.2). The erroneous results in FCR of the fish fed to an imposed regime at period T_{A2} – T_{A3}, was caused by a poor growth return in one of the cages under the imposed treatment, which caused the fish to be overfed. The high FCR of fish fed under the imposed regime at period T_{B1} – T_{B2}, should be interpreted with caution, as there were problems with the sampling method around this time. The on-demand feeding regime can reduce pellet wastage as the system terminates feed delivery when the fish are satiated, improving FCR (Thorpe and Cho, 1995). On-demand feeders can also restrict feed delivery to the times when fish are hungry, again reducing FCR. This has been reported in other commercial species, such as sea bass, *Dicentrarchus labrax* (Azzaydi et al., 1999; Paspatis et al., 1999) which have better FCR when fed on-demand using self-feeders.

Growth rate (as defined by SGR) varied with season in both feeding treatments: growth rate decreased during autumn and winter, before increasing again during spring prior to seawater transfer. Similar findings have been reported in other studies on salmon parr (Higgins and Talbot, 1985; Villarreal et al., 1988) and the decrease in growth rate has been associated with decreased feeding motivation during winter (Metcalfe et al., 1986; Simpson et al., 1996). Differences in growth rate between treatments, irrespective of time of year, can be affected by ration size and access to food (Storebakken et al., 1991; Thorpe and Huntingford, 1992; Jobling 1994, 1995). There was no significant difference between treatments in specific growth rate (SGR) in the current experiment. This suggests that fish under the imposed feeding regime are achieving optimal growth along with those fed on-demand, and the fish are not being underfed. This is in accordance with other studies which found feed regime had no significant effect upon growth rate (Jørgensen and Jobling, 1992; Sveier and Lied, 1998), although Paspatis et al. (2000) reported that sea bass fed with self feeders had a better growth rate than those fed with automatic feeders in tanks. Under existing feeding regimes in freshwater cages, an aquaculturist is not failing to realise the full growth potential of the fish under ambient rearing conditions in freshwater cages.

4b.4.5 Conclusions

In a pair-wise comparison of the performance of on-demand feeding regimes and an existing in-house fixed ration strategy, there was generally little difference in growth or uniformity of growth between feeding treatments. A long-day automated feeding strategy, based upon in-house feed tables and supplemented by hand feeding, can match the growth performance of a feeding management strategy that feeds fish on-demand. Existing feed management strategies overfeed fish in late summer, which can be uneconomical and potentially detrimental to the loch environment. This overfeeding was deleterious upon feeding efficiency, although the differences observed in this experiment were not statistically different.

Chapter 4c: How feeding regime can affect social interactions between Atlantic salmon parr in freshwater production cages

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4c.1 Introduction

4c.1.1 Aims and content

In the final section of this chapter I will discuss the behaviour of Atlantic salmon parr held under commercial aquaculture conditions in relation to methods of food presentation. I will further discuss the form and context of some behavioural interactions between conspecifics and the problems these can cause in an aquaculture environment, comparing my results with those of others studies in this field. The aim of the experiment described in this chapter was to examine how a feed regime can regulate the levels of aggression in cage aquaculture and how feed management can address this.

4c.1.2 Aggression

Aggression can be defined as 'specialised and directed behaviour in which an individual potentially inflicts damage upon another individual' (see, for example, Huntingford and Turner, 1987). It is the most active form of interference competition; which is an activity where advantages are derived indirectly from direct costs to a competitor (Krebs and Davies, 1997). Aggression is widespread in the animal kingdom (in crustaceans: Huber and Kravitz, 1995; Pavey and Fielder, 1996; and in ungulates: Clutton-Brock et al., 1979; Barrette and Vandal, 1990) and common in teleost fish (see for example, Chellappa et al., 1989). It takes the form of displays as well as attacks and it is often over resources such as food, shelter or mates; it can also manifest itself in dominance behaviour or territoriality, or a combination of both (Huntingford et al., 1993). Territoriality is often an indirect result of competition for such resources, as an animal will defend a territory because the area is a rich source of food (Parker, 1974).

4c.1.3 Aggressive behaviour of Atlantic salmon

Whilst in their freshwater phase, wild Atlantic salmon are known to be aggressive and territorial (Kalleberg, 1958; Keenleyside and Yamamoto, 1962; Wankowski and Thorpe, 1979; Wankowski, 1981). Aggressive interactions and subsequent injury are also common in aquaculture systems (Abbott and Dill, 1985; Turnbull et al., 1998; MacLean et al., 2000). The aggressive behaviour of Atlantic salmon is either low intensity in the form of displays, or high intensity entailing chases, nips or bites (see Table 4c.1). There is growing evidence that injuries inflicted upon the fins are primarily caused by aggressive behaviour, and this damage is generally termed fin rot, fin erosion or fin damage. It entails pale, nodular thickening with erosion of the distal part of the fin, followed by epidermal hyperplasia and a fibrocellular response (Turnbull et al., 1998).

A known attack site in salmonids is the dorsal fin and whilst other fins may be damaged during aggressive interactions, the dorsal fin incurs more damage than other areas of the body (Abbott and Dill, 1985; Christiansen et al., 1992; Turnbull et al., 1998). Fin damage is widely regarded as a symptom of unsuitable rearing conditions (Alanärä and Brännäs, 1996). Its presence can be used as an indicator of the level of aggression within culture systems and of the existence of alternative competitive strategies between individuals for resources (MacLean et al., 2000). The prevalence of fin damage has also been used to assess the strength of social hierarchies (Christiansen and Jobling, 1990). Furthermore, the occurrence of fin damage may permit an observer to distinguish whether an individual is from farmed or wild origin (Craik et al., 1987; Bosakowski and Wagner, 1994). In this study fin injury will be used as an indicator of levels of aggression and also as an indirect index of competition for food.

4c.1.4 Competition

In any population the resource gain by any given individual will depend upon its ability to compete, and aggression can arise over competition for food (Kadri et al., 1997*a*). Competition is an important stimulus in the origin and maintenance of dominance hierarchies, which tend to be stable in salmonids (Metcalfe et al., 1989), and can remain intact at high stocking densities (Thorpe et al., 1990; Alanärä and Brännäs, 1996). Highly competitive individuals can aggressively exclude less competitive individuals from a resource, which leads to resource monopolisation (see for example: Ryer and Olla, 1998 in teleosts; or Schnebel and Griswold, 1983 in ungulates). This form of competitive exclusion exists in salmon parr, where individuals have been observed to veer away from food when faced with a competitor (Metcalfe, 1989; Huntingford et al., 1993). Kadri et al. (1996) and Huntingford et al. (1993) hypothesised that the impaired feeding of subordinates was caused by intimidation from more dominant conspecifics. Fish may become highly competitive as hunger levels escalate (Nicieza and Metcalfe, 1997) and competition for food can be energetically costly; an individual's net rate of energy intake is dependent upon the time and energy expended whilst searching for, attacking and handling a food item (Godin and Rangeley, 1989).

Increased swimming activity leads to an increase in energy expenditure (Niimi and Beamish, 1974) and swimming costs are suggested to increase with manoeuvre complexity (Boisclair and Tang, 1993). Swimming activity that includes variable turning angles and acceleration rates is more energetically costly than swimming in a straight line (Krohn and Boisclair, 1994; Tang and Boisclair, 1995). Accelerated and spontaneous swimming can be exhibited during feeding activity. It is difficult to directly evaluate the energetic costs of feeding activity in production cages, but it can be inferred that during a feed attempt an individual expends more energy as its swimming manoeuvres become more variable and complex. Using this hypothesis I can explore whether swimming manoeuvres vary between individuals fed to different feeding regimes.

Furthermore, it can be suggested that the complexity of a swimming manoeuvre may reflect feeding motivation and therefore competition levels.

4c.1.5 Implications of aggressive behaviour and competition

Aggressive behaviour is energetically costly for both the aggressor and recipient. It entails swimming costs when initiating an attack or escape response (Metcalfe, 1986; Noakes and Grant, 1992) and when a recipient is displaced from a feeding site it can be potentially costly (Metcalfe, 1986). During conflict initiation both participants can also miss a feeding opportunity (Ruzzante and Doyle, 1991). Furthermore, recipients of aggressive interactions have the added costs of tissue repair and increased metabolism (Nicieza and Metcalfe, 1999) and it has been suggested by the same authors that the costs of aggressive behaviour may be an intrinsic constituent in the energy budgets of juvenile salmon. As stated earlier, aggressive behaviour can cause fin injury, and this can lead to secondary bacterial infections by pathogens such *as Aeromonas salmonicida*, which causes furunculosis (Turnbull et al., 1996). It may also reduce the market appeal of whole fish, and avoidance of the condition is therefore desirable.

Fish are exposed to a number of stressors in the wild and in culture systems, and stress has been recognised as an inevitable component of finfish aquaculture (Pottinger and Pickering, 1997). Aquacultural stressors can be classified into two categories (see Barton and Iwama, 1991). These are acute stressors, where the effect is severe but short-lived (such as grading, handling, short-term crowding and transport) or chronic stressors, where the effect is less severe but prolonged (including poor water quality, long-term crowding, exposure to pathogens, and behavioural stressors). Furthermore, fish may become stressed due to social interactions and aggression from conspecifics (Wedemeyer, 1997). These stressors can instigate physiological responses that lead to osmoregulatory disturbances (Randall et al., 1972), cause increases in brain serotonin levels (Winberg and Nilsson, 1993; Winberg et al., 1993) and elevate the production of stress hormones

(Pottinger and Pickering, 1992). These responses can be deleterious upon growth (Pickering, 1993), appetite and food conversion efficiency (Jobling and Wandsvik, 1983; Abbot and Dill, 1989) and immuno-competence (Peters et al., 1988; Salonius and Iwama, 1993).

Aside from causing physical injury and elevating the stress responses of the individuals involved, aggressive behaviour can be linked to competition. Poor food dispersal can stimulate competition, which causes uneven distribution of food within a population and can subsequently affect growth rate. When food is unevenly dispersed in both time and space, an individual can restrict its movement to a small region of high resource density (Grant, 1993). Unequal access to food can promote growth depensation, where there is an increase in the variance of size distribution with time due to differences in individual growth rate (see Kadri et al., 1996). Growth depensation may be driven by genetic or environmental differences related to interference competition between individuals for food (Jobling, 1993), or a combination of both. Farmers have a commercial interest in reducing the implications of aggression as competitively excluded fish are growing at sub-optimal rates, which is undesirable when wishing to maximise growth and produce fish of a more uniform size (Noakes and Grant, 1992).

4c.1.6 Methods to reduce aggression in aquaculture

In the somewhat homogenous environment of aquaculture systems, food supply and stocking density can be regarded as primary factors governing social interactions within the rearing system (Alanärä, 1994*b*). To make an aquaculture venture cost effective the farmer must try to maximise stocking density without sacrificing growth potential from overcrowding, so decreasing stocking density may not be a viable option. In fact, Brown's (1964) economic defendability theory implies an animal should defend a feeding site only when energetic benefits outweigh the costs of defence (time, energy, or potential for injury). This theory can be applied to studies on fish behaviour, as it has been suggested that aggression levels decrease with increasing stocking density, where repeated attacks and defence of a feeding territory cannot be maintained by dominant individuals (Kalleberg, 1958; Keenleyside and Yamamoto, 1962). Browns' hypothesis has also been applied in aquaculture (Hecht and Uys, 1997), where methods to increase the costs and reduce the benefits of aggressive interactions are becoming more widespread.

To increase the costs of aggression it may be beneficial to increase current speed within the culture system, because an animal will have to expend more energy when initiating attacks (Noakes and Grant, 1992). This method has been shown to reduce aggression in salmonids held under aquacultural conditions (Christiansen and Jobling, 1990; Adams et al., 1995) but may have limited application in production cage culture. Increasing feed dispersal (in both time and space) can lead to a food resource becoming indefensible, reducing the benefits of being aggressive (Grant, 1993; Ryer and Olla, 1996*b*).

With recent developments in feeding systems, one factor that can be freely manipulated by the farmer is feed management. Ration size, food presentation and food dispersal can govern satiation levels, which in turn may affect social interactions amongst conspecifics and subsequent stress levels. The amount eaten by a population can vary both within and between days (see Landless, 1976; Boujard and Leatherland, 1992*b*; Alanärä, 1994*b* for examples) and it is difficult to judge satiation levels when feeding a population using current techniques, such as a cessation in surface activity (Paspatis et al., 1999). If ration size is incorrect it can lead to underfeeding, which stimulates competition, or overfeeding, which promotes food wastage and can lead to increased feed costs and environmental deterioration; a further hindrance to feed management.

On-demand feeding systems, where fish can regulate both the timing and size of feed delivery via their appetite, may offset or circumnavigate some of these problems, and may integrate this feeding variability into feed management. They may allow the farmer to produce fish of more uniform size, as it has been inferred that the size heterogeneity of a population can decrease when fish have increased access to a food resource (McCarthy et al., 1992). With the following experiment I wish to compare the effects of a current feeding technique upon social interactions within production cages (using automated feeding systems, supplying pre-set amounts of food at prescribed intervals during the day according to feed tables) against a commercially available on-demand feeding system (as outlined in Chapter 2).

4c.1.7 Objectives of the study

The questions I will address in this chapter are: -

i) How does a feeding regime affect the social environment of Atlantic salmon parr within a cage?

ii) Do feeding strategies have any impact on the levels of injury in farmed fish?

iii) Which size categories of fish are most affected by fin damage?

iv) What implication does this have for production?

The experiment was conducted in a production environment whose end point is maximising efficiency, product quality and growth. Feed costs are therefore critical and food must not be wasted. It was designed as a full-scale production trial using commercial densities and facilities, but under the constraints of in-house husbandry practices. By utilising this approach I will address how we can improve feeding strategies and produce data for more acceptable feeding practice. I also hope the results can give an insight into what is happening within a production cage in relation to methods of food presentation.

Table 4c.1. The primary components of fights between juvenile Atlantic salmon conspecifics. Outlining the behavioural responses of the individuals involved.

Type of behaviour	Definition
Display	Low intensity form of aggression. The primary components are lateral and frontal displays. • Lateral orientation with a conspecific. Display consists of tail beating (a flexing of the vertebral column towards opponent) with erect fins and flaring of the operculae. • In the wild, a territory holder often exhibits frontal displays. Fish performing the display usually orientates towards the conspecific with its head, flaring its operculae (Keenleyside and Yamamoto, 1962). All or part of these repertoires may be exhibited during display behaviour (Noakes, 1980).
Chase	A succession of directed attacks at a conspecific causing it to flee. The aggressor approaches its opponent with its mouth open and attempts to nip the caudal fin of the retreating fish. The recipient retracts its fins whilst fleeing (Keenleyside and Yamamoto, 1962).
Nip	High intensity overt aggression. Consists of a fish biting a conspecific after an attack. Nipping is generally directed against the fins or opercular region (Keenleyside and Yamamoto, 1962). Fish can grab a fin and hold it for up to a second before release, although this is rare (Turnbull et al., 1998), and nipping often results in fin damage. During escalated fighting attacks may be reciprocated (Turnbull et al., 1998).

4c.2 Materials and methods

4c.2.1 Fish husbandry

The fish husbandry used in this experiment is outlined in Chapter 4a. Refer to Figs. 4a.1 and 4a.2 for the experimental set-up.

4c.2.2 Sampling methods for morphological studies

Refer to Chapter 4b and Fig. 4b.1 for sampling methodology. The number of individuals with dorsal fin damage was noted at the start of the trial and monitored monthly thereafter. Dorsal fin damage was scored into one of the following five categories: moderate fraying, severe fraying, moderate splitting, severe splitting, and fin folding (refer to Table 4c.2). Only moderate splitting and severe splitting were used for subsequent analysis as these are acute injuries; the other forms are chronic injuries that are beginning to heal (Turnbull, 1992) and may be artefacts of the husbandry experienced by the fish before the trial commenced.

The size of individuals (wet weight) affected by fin injury was assessed after each sample. The condition was related to the size range within each cage group, rather than using the absolute size of the fish affected by dorsal fin injury: a 25g fish may be the largest in a cage in September, but may be one of the smaller ones in November. Percentiles divided each monthly weight-frequency distribution at 20, 40, 60 and 80, splitting the distributions into five categories (1 – 20 containing the smallest fish with the population, 81- 100 containing the largest) and the presence or absence of fin injury per category was then noted.

4c.2.3 Observations of social behaviour

An underwater camera (Model RS410, Rovscan UK Ltd, Perth) positioned 3m below the surface and 1m from the cage wall was used to record the behaviour of fish before, during and after a meal. The camera was connected to a video recorder (either a Sony Video Walkman or a 12v Panasonic 1070 video recorder), which was housed in a waterproof container.

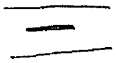
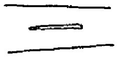
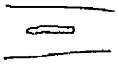
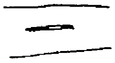
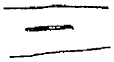
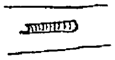
Where possible, each of the remaining two cages from each treatment were filmed for three consecutive days during a two week period prior to smolt transfer. Filming took place only on calm days, which meant there were only five filming replicates of each treatment, as there were strong wind driven water currents for two of the filming days. The fish under the imposed feeding regime were filmed at approximately 13.00 hours and the on-demand fed fish were filmed during their closest meal to that time. From the video recordings, the number of aggressive interactions were counted in the three minutes prior to and following a meal. When an aggressive interaction was observed the video analysis was paused and the number of fish on screen was counted. In the imposed regime the feeding bout lasted approximately one minute. As the fish in the on-demand fed groups selected the duration of a meal, the length of feeding bout was variable and the entire feeding episode was analysed. Data from both imposed and on-demand cages were transformed into the number of aggressive interactions fish^{-1} minute^{-1} to correct for this. Aggressive behaviour was initially defined as being a display, a chase or a nip. It was difficult to define frontal or lateral displays due to fish numbers, so these were removed from the analysis. If there were any overt interactions the attack site on the assaulted individual was noted.

Swimming speed and turning angle data were analysed in accordance with the methods used in Chapter 3b, and using Fig. 4c.1. During analysis the behaviour of fish both before and after pellet capture was noted. Feed attempts were classified only where they could be easily observed from the video analysis, and only when they were successful. It was noted that fish approached pellets in a straight and direct line, and the turn was executed after pellet capture.

4c.2.4 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0. Minitab Inc., Philadelphia) or SPSS (v. 10-0. SPSS Inc., Chicago) for MS Windows. Differences in the monthly incidence of fin injury between treatments were tested for using 2 x 2 Chi^2 tests (χ^2). A 2 x 5 Chi^2 test (χ^2) was used to test for differences between the size classes of fish affected by morphological injury within treatments. Any differences in swimming speeds around meal times, both within and between treatments, was tested for using a repeated measures ANOVA. Differences in the choice of turning angle during pellet capture were tested for using a non-parametric Mann Whitney test. Parametric data is presented as means ± S.E, whereas non-parametric data is presented as medians ± first and third interquartile ranges. A significance level of $P < 0.05$ was used for all statistical tests. Further statistical testing, to locate differences between the number of aggressive interactions around meal times both within and between treatments, was prohibitive as there were only five filmed replicates for each treatment (non-parametric Kruskal-Wallis tests require a minimum sample size of six, Dytham, 1999).

Table 4c.2 Template used to classify five categories of dorsal fin damage exhibited by juvenile Atlantic salmon. NB. Fin rays omitted from drawings of damaged fins to aid clarity (after Turnbull, 1992; MacLean et al., 2000)

Fin damage category	Appearance		Description
Normal	i) 	ii) 	Uninjured dorsal fin with fin rays intact. i) Lateral view. No evidence of nodularity or tissue loss. ii) Dorsal view. No evidence of thickening.
Moderate splitting	i) 	ii) 	Acute injury. Tissue loss, fin no *less* than 60% its normal size. i) Damage and splitting along the distal edge of the fin. ii) Mild thickening along distal edge of fin.
Severe splitting	i) 	ii) 	Acute injury. Tissue loss, fin no *more* than 60% its original size. i) Extreme splitting along distal edge of the fin, evidence of tissue loss. ii) Severe nodular thickening along distal edge of fin.
Moderate fraying	i) 	ii) 	Chronic injury, partially healed. Up to two clefts between fin rays, terminating towards the base of the fin. No tissue loss or nodular thickening
Severe fraying	i) 	ii) 	Chronic injury, partially healed. A minimum of three clefts per fin. No tissue loss or nodular thickening.
Folded fin	i) 	ii) 	Healed fin, showing distorted re-growth. Fin twisted laterally following defective fin ray regeneration. No evidence of splitting or nodular thickening along distal edge of fin.

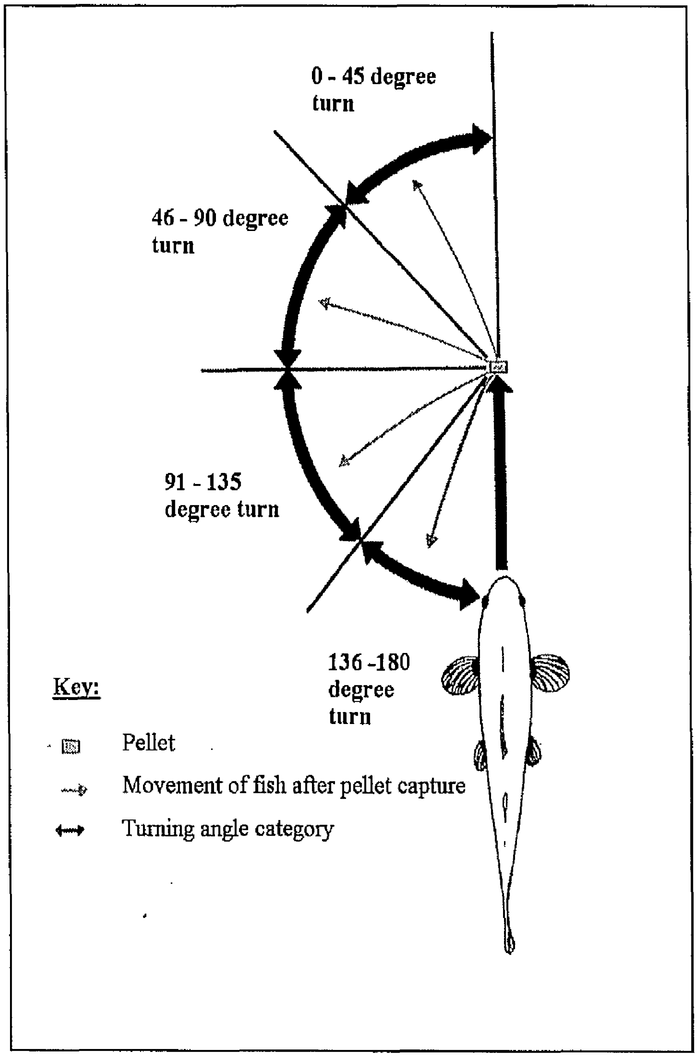


Fig. 4c.1 Classification of degree of turn executed during a feed attempt in parr. The turn was executed after pellet capture.

4c.3 Results

4c.3.1 Aggressive interactions within the sea cages.

Overt aggressive behaviour in the form of chases or nips was rarely observed in either treatment. Any overt aggression resulted in the aggressor attacking the opercular region, pectoral fin or lower segment of the caudal fin. No reciprocal acts of aggression were observed in either treatment. Aggressive interactions during feeding were less frequent in the fish fed on-demand (refer to Fig. 4c.2). No aggressive interactions were observed before and after feeding for both treatment groups. These results suggest on-demand feeding reduces the incidence and frequency of aggression per capita around meal times. There were also other behavioural differences between groups of fish held under different feeding regimes.

4c.3.2 Swimming speeds and turning angle

To examine the hypothesis that feed regime can affect swimming speeds (presumed to reflect hunger levels), the swimming activity of fish was compared before, during and after a meal for each treatment using repeated measures analysis of variance. There was a quadratic relationship between swimming speed and time around a meal (repeated measures ANOVA: effect of time $F_{2,97} = 31.93$, $P = 0.005$; quadratic term of polynomial contrasts: $F_{1,98} = 64.31$, $P < 0.001$). This relationship was not consistent between feeding regimes (interaction between time around a meal and regime: $F_{2,97} = 19.15$, $P < 0.001$). There was a significant effect of feed regime upon swimming speed (effect of regime: $F_{1,98} = 4.18$, $P = 0.044$), see also Fig.4c.3.

Interestingly, when examining turning angle it was noted that turns are executed post pellet capture, whereas post-smolts in cages execute turns before pellet capture (see Chapter 3b). The results in this study suggest that fish fed to an imposed regime incur increased costs during pellet capture, with turning angles being significantly greater following pellet capture than in on-demand fed fish (imposed median = 2, on-demand median = 1, Mann Whitney $W = 6652$, $P < 0.001$); the median turn executed by fish under the imposed regime was 46-90°, whereas the median turn for on-demand fed fish was 0-45° (Fig. 4c.4).

4c.3.3 Fin injury

To examine the effect of feed regime on fin damage (moderate splitting; no fish were affected by severe splitting) the trial was divided into three phases due to grading events. No fish were affected by fin damage at the beginning of the trial in late summer (sample T_{A0}) irrespective of feed regime (Fig. 4c.5). After three months (T_{A3}) there was an increase in fin damage before the trimmed grade. During the second phase of the experiment (T_{B0}, T_{B1}, T_{B2}) and fin damage increased throughout the winter months, peaking at the beginning of the third phase (T_{C0}), one month prior to smolt transfer. Fish fed on-demand exhibited significantly lower incidences of fin damage from the end of October until the end of February (T_{A3} - T_{B2}; for Chi^2 analyses see Table 4c.3). Fin damage was higher in fish fed on-demand after grading at the beginning of the third phase (T_{C0}), of the experiment but this difference was not significant prior to smolt transfer (T_{C1}) when it appears the fish recovered from fin splitting.

Fish that showed evidence of dorsal fin damage were split into five size categories (wet weight) using percentiles. There was no effect of fish size on the prevalence of fin damage during the first phase of the experiment during late summer-autumn (see Fig. 4c.6). After the trimmed grade in December (T_{B0}) the small fish held under the imposed regime had less fin damage, whereas there was no effect of size in fish fed on-demand. During February (T_{B2}) smaller fish had the highest levels of fin injury in the imposed feeding regime. There was no effect of fish size on the prevalence of fin injury in fish fed on-demand until February (T_{B2}) when the largest fish showed least fin injury. After the second grade (T_{C0}) there was no size effect for either treatment. In both feeding regimes the smallest fish showed the highest incidence of fin injury one week prior to smolt transfer (T_{C1}; see Table 4c.4 for all Chi^2 analyses). This experiment indicates that during certain months of the year there is an association between feed regime and the size of fish affected by fin injury, as well as its prevalence.

Table 4c.3 Comparisons by χ^2 test of frequencies of fin damage between feeding treatments in groups of Atlantic salmon parr at different sampling periods. Data were pooled for all three replicates within each treatment. χ^2 values were regarded as invalid and are omitted if cross-tabulation yielded expected frequencies of less than five in one or more cells. d.f. = 1 in all cases.

Growth period	*n*	χ^2	***P***
T_{A1}	1491	0.812	0.367
T_{A2}	1453	5.277	0.022 - fish fed on-demand have less fin damage
T_{A3}	1482	3.888	0.049 - fish fed on-demand have less fin damage
T_{B0}	1482	3.966	0.046 - fish fed on-demand have less fin damage
T_{B1}	1480	30.845	<0.001- fish fed on-demand have less fin damage
T_{B2}	1474	25.486	<0.001 - fish fed on-demand have less fin damage
T_{C0}	1240	7.788	0.005 - imposed fed fish have less fin damage
T_{C1}	996	0.012	0.911

Table 4c.4 Comparisons by χ^2 test of frequencies of fin damage between groups of Atlantic salmon parr divided by percentiles at 20, 40, 60 and 80 of wet weight between feeding treatments at different sampling periods. Data were pooled for all three replicates within each treatment. χ^2 values were regarded as invalid and are omitted if cross-tabulation yielded expected frequencies of less than five in one or more cells. d.f. = 4 in all cases.

Treatment	Growth period	*n*	χ^2	*P*
Imposed	T_{A2}	743	4.044	0.400
	T_{B0}	739	12.578	0.014 - smallest have least fin damage
	T_{B1}	740	2.873	0.579
	T_{B2}	736	27.639	< 0.001 - smallest have greatest damage
	T_{C0}	632	7.443	0.114
	T_{C1}	508	18.805	0.001 - smallest have greatest fin damage
On-demand	T_{B0}	743	5.736	0.220
	T_{B1}	735	3.269	0.514
	T_{B2}	738	12.27	0.015 - largest fish have least fin damage
	T_{C0}	620	0.621	0.961
	T_{C1}	499	23.965	< 0.001 - smallest two categories of fish have greatest fin damage

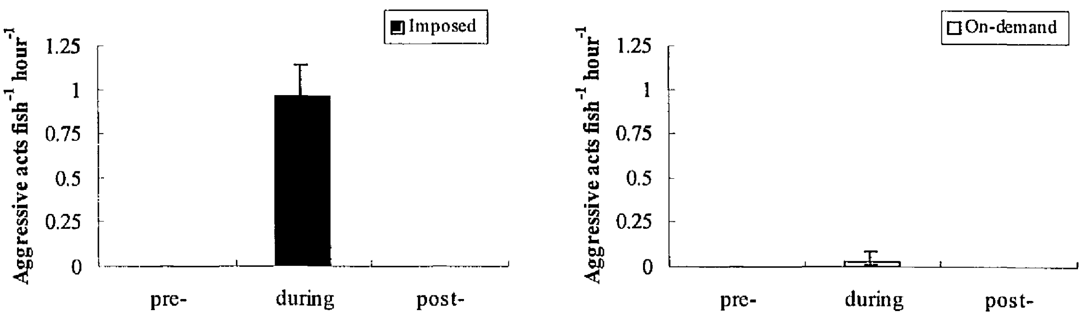


Fig. 4c.2 The effect of feed regime upon aggressive interactions (median values) around meal times between parr held in freshwater production cages. Error bars indicate interquartile range.

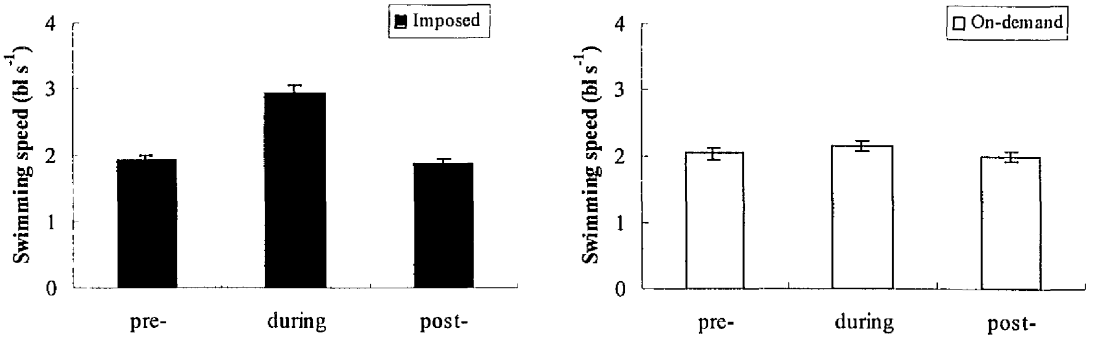


Fig. 4c.3 The effect of feeding regime upon mean swimming speeds of parr around meal times. Error bars indicate standard error of the mean.

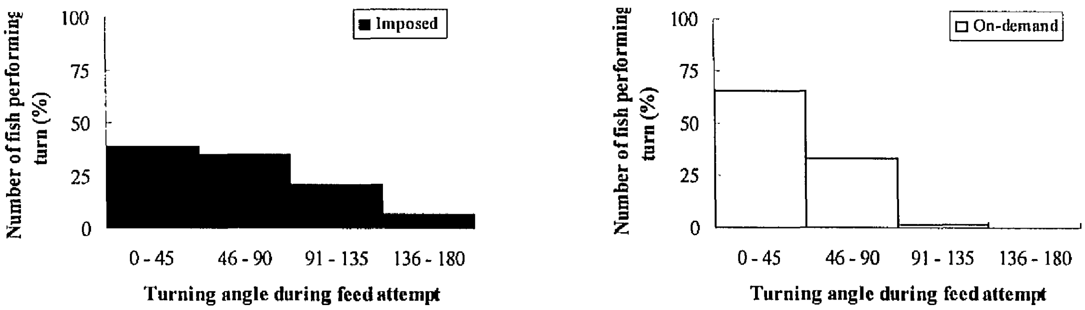


Fig. 4c.4 The relationship between feeding regime and turning angle executed during feed attempts in parr. Note that the turn was executed after pellet capture (see text).

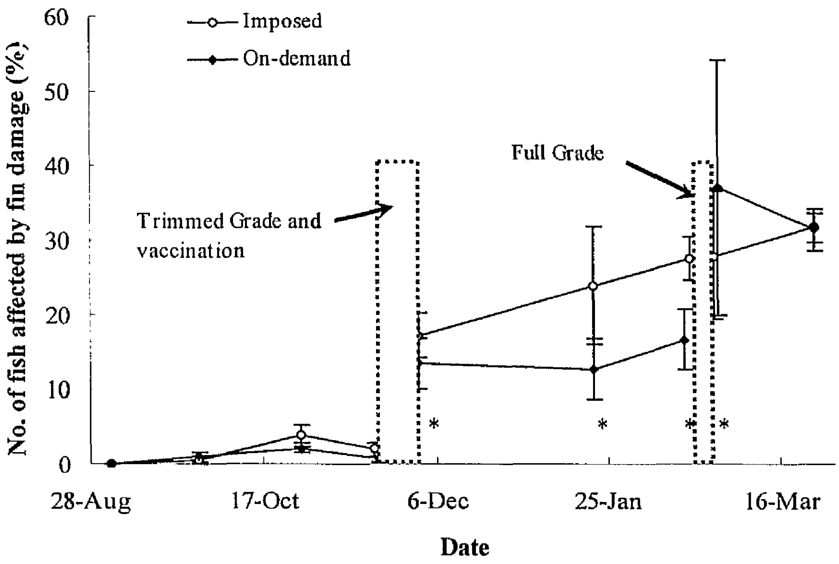


Fig. 4c.5 Mean changes in the prevalence of dorsal fin splitting in parr held in production cages. Error bars represent standard error of the mean. * indicates significant differences at $P < 0.05$.

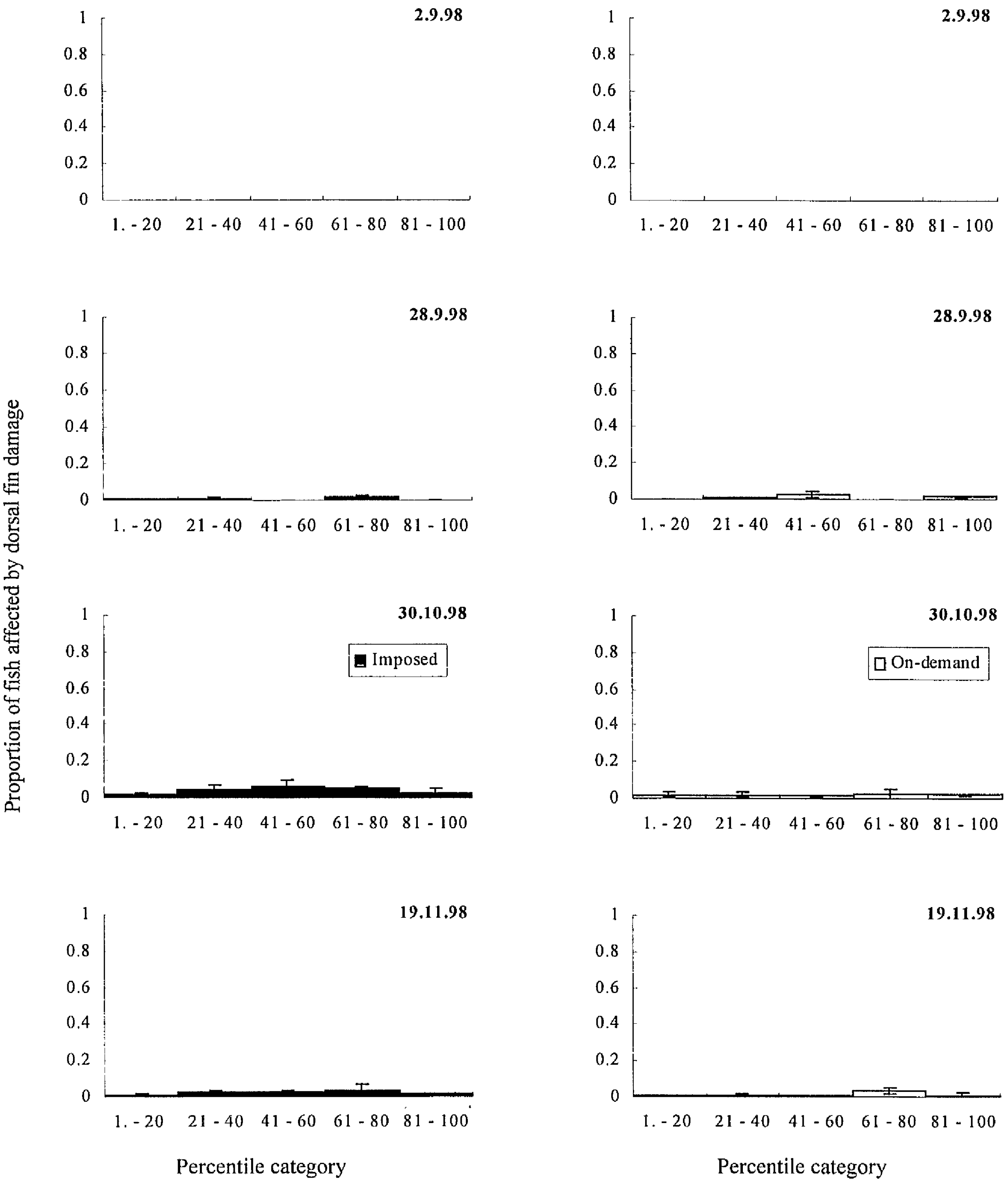


Fig. 4c.6 The effect of feeding regime upon the prevalence of fin damage according to size category in parr. Fish that showed evidence of fin damage were split into five relative size categories using percentiles at 20, 40, 60 and 80 (0-20 being the smallest fish, 81-100 being the largest). Error bars represent standard error of the mean.

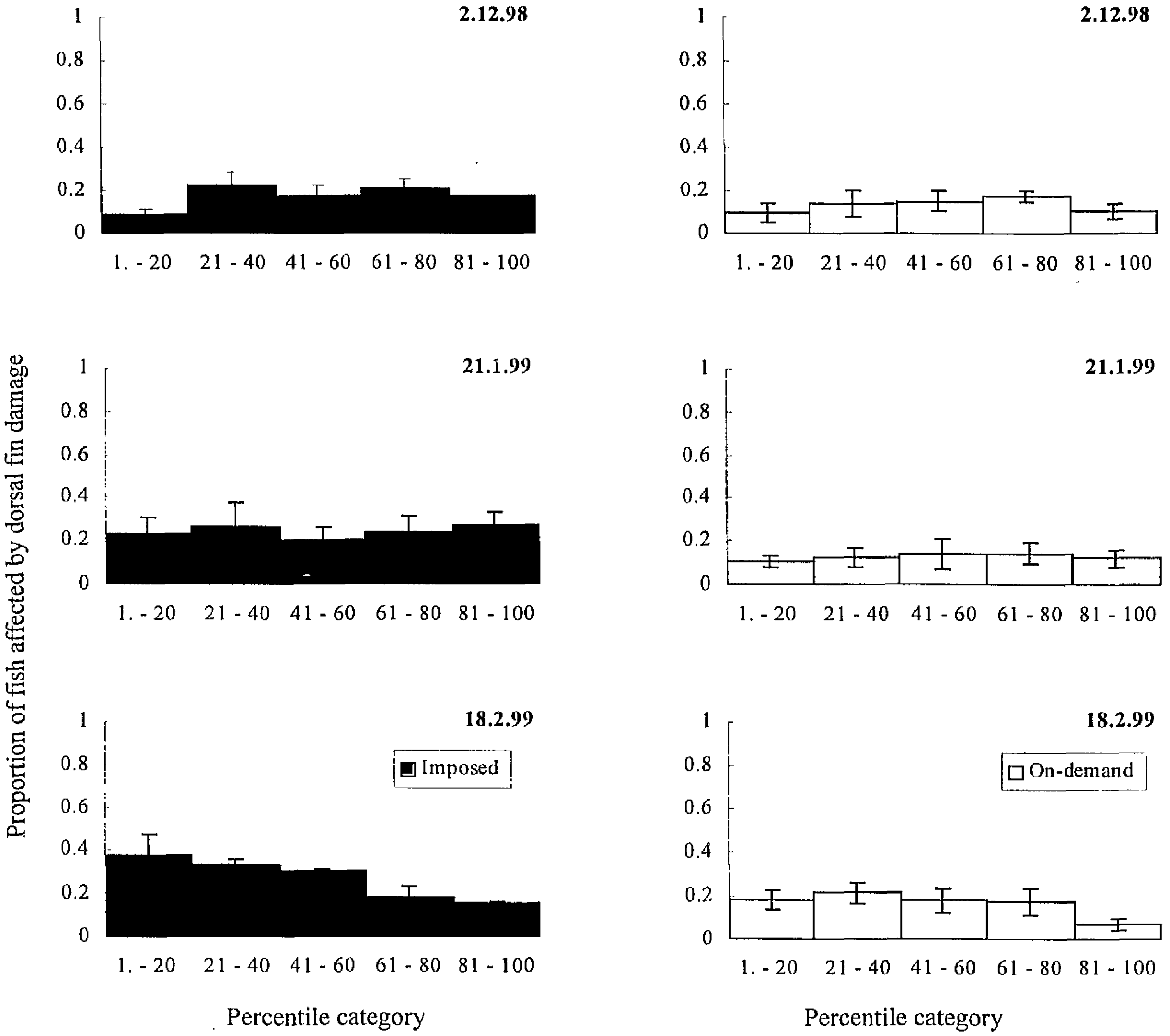


Fig. 4c.6 cont. The effect of feeding regime upon the prevalence of fin damage according to size category in parr. Fish that showed evidence of fin damage were split into five relative size categories using percentiles at 20, 40, 60 and 80 (0-20 being the smallest fish, 81-100 being the largest). Error bars represent standard error of the mean.

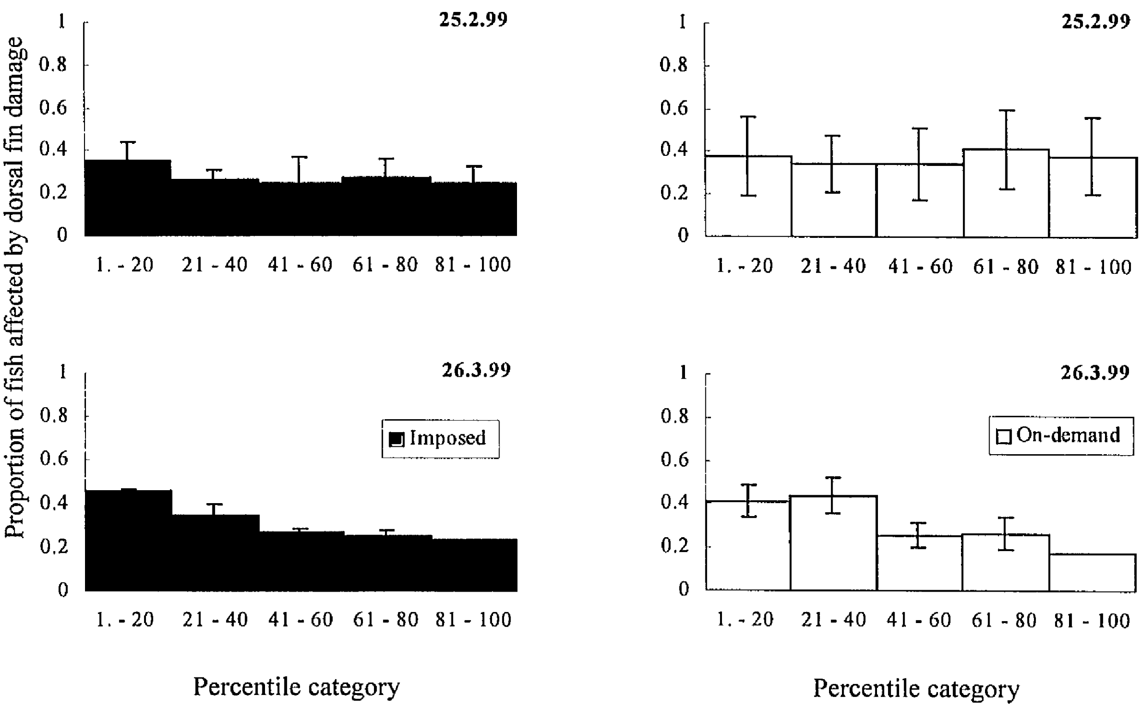


Fig. 4c.6 cont. The effect of feeding regime upon the prevalence of fin damage according to size category in parr. Fish that showed evidence of fin damage were split into five relative size categories using percentiles at 20, 40, 60 and 80 (0-20 being the smallest fish, 81-100 being the largest). Error bars represent standard error of the mean.

4c.4 Discussion

4c.4.1 Effect of feeding regime upon aggression

Aggression is a recognised component of salmonid culture (Abbott and Dill, 1985; Turnbull et al., 1996) and there is increasing commercial interest in developing husbandry practices that reduce its occurrence and impact. The results of this experiment indicate that there is a significant association between feed regime and the social environment within production cages of Atlantic salmon parr as well as smolts (see Chapter 3b). Using direct behavioural observations we have demonstrated that during the month prior to smolt transfer there was a low level of overt aggression in the cages. Certain authors (for example Kalleberg, 1958) have suggested an abatement of aggressive behaviour may be associated with the smoltification process where juvenile salmon begin to form non-aggressive schools. However, more recent work by Gibson (1983) and Damsgård and Arnesen (1998) found parr and smolts exhibit similar agonistic behaviour, which led the latter authors to suggest there is a poor relationship between smolting and the transformation from territoriality to schooling. Furthermore, facultative schoolers, such as juvenile salmon, may exhibit acute aggressive behaviour when competing for a food resource (Ryer and Olla 1995, 1996*b*; Olla et al., 1998). This is in agreement with our findings, as any overt aggression was only observed during a meal. On the basis of these results we suggest low levels of aggression can be attributed to current husbandry methods. More importantly, individuals fed on-demand are less aggressive than those fed to an imposed regime as shown in figure 4c.2. This is not entirely unexpected: if a group of fish can dictate the timing and size of feed deliveries, ration can be accurately matched to appetite, reducing hunger levels. Hunger has been shown to increase competition in juvenile Atlantic salmon (Nicieza and Metcalfe, 1997) and competition stimulates aggression (Kadri et al., 1997*a*). The higher incidence of aggression during meals under the imposed regime may be a component of the feeding technique; feed was delivered in a predictable and uniform manner, which can increase aggression (Grant and

Kramer, 1992). Spontaneous activity associated with aggressive interactions can escalate metabolic rate (Brett, 1964), which in turn may be detrimental to food utilisation and feed conversion ratio (Fagerlund et al, 1981).

4c.4.2 Effect of feeding regime upon swimming speed and manoeuvre complexity

Using swimming speed and manoeuvre complexity as a proxy for energy expenditure, these results suggest fish expend less energy during feeding bouts when fed on-demand. There were significant differences in the feeding response of fish fed under each treatment. On-demand fed fish showed no significant change in swimming activity in response to feed delivery, whereas fish fed according to tables exhibited a significant increase in swimming activity, which corresponds to feeder activation. If a fish is hungry it can increase its pellet encounter rate by increasing swimming (or foraging) activity. This is supported by a study on juvenile turbot,*Scophthalmus maximus*, where food delivery increases swimming activity. Further, the range of turning angles executed by individuals during feed attempts also differed between treatments. During a feed attempt fish executed these turns after pellet capture and this turn was more acute in fish held under the imposed regime. This may have been part of an individual's feeding strategy to reduce the risk of injury, by quickly darting out of the feeding area after a successful feed attempt. Petterson et al. (1996) has suggested rainbow trout, *Oncorhynchus mykiss*, may reduce the risk of sustaining injury from conspecifics by sneaking into the area of feed delivery.

Increased spontaneous swimming activity can be detrimental to growth performance in many salmonids (Niimi and Beamish, 1974; Farrell et al., 1997; Gregory and Wood, 1999) and is an undesirable component of cage culture. Increased competition may be a consequence of elevated hunger levels (see Brännäs and Alanärä, 1994) in fish fed to an imposed regime, where there can be asynchrony between ration size and appetite. Atlantic salmon parr become more competitive under conditions of food restriction (Nicieza and Metcalfe, 1997) and reduced ration can increase swimming activity (Huse and Skiftesvik, 1985; Hammer, 1997). In this study we suggest differences in the feeding response of individuals between treatments may reflect contrasting competition levels and feeding motivation under each feeding regime.

4c.4.3 Levels of injury

Previous studies have demonstrated that fin injury is predominantly caused by aggression (Abbott and Dill, 1985; Turnbull, 1992; Turnbull et al., 1998). The severity of acute fin damage (splitting) observed throughout this trial was generally low, with no fish showing more than 40% erosion of the dorsal fin. During September there was a low incidence of fin injury irrespective of feed regime. From October until the middle of February there was a significant difference in the incidence of fin damage between treatments, with the on-demand feeding regime reducing the prevalence of fin injury. After the second size grade in February (where fish were handled during the parr-smolt transformation) there was asynchrony between the remaining two on-demand feeding replicates, and it was noted that one cage had a high percentage of individuals exhibiting fin injury (see figure 4c.5). Fish fed on-demand showed a significant increase in the prevalence of fin injury in comparison with fish under the imposed regime at this sampling point. The following month this effect receded and there was no significant difference in the incidence of fin damage between treatments. These results suggest an on-demand feeding regime can improve fin condition by reducing the incidence of overt aggression relative to an imposed regime. Restrictive rations can increase the prevalence of fin damage in salmonids (Storebakken and Austreng, 1987; Dåmsgard et al., 1997) and other species (Shelverton and Carter, 1998), but the daily ration requirements were closely matched between feeding regimes for the majority of the experiment (see Chapter 4b). A previous study in the cutthroat trout, *Oncorhynchus clarki*, found that feeding on-demand did not reduce fin erosion relative to hand fed fish in raceways (Wagner, 1995) and the author suggested the hand feeding regime created similar social conditions to the on-demand feeding regime. However, the fish were fed with an operant on-demand feeder, and these systems can promote competition around the trigger mechanism (Landless, 1976). The results of the present study suggest that competition plays a greater role in governing access to feed in fish fed predetermined rations when compared to those fed on-demand.

It would be expected that some fish show evidence of dorsal fin damage during the late summer as juvenile salmon become aggressive within days of emerging from the redd (Kalleberg, 1958; Elliott, 1990). During summer the majority of fish have exceeded the 70mm length threshold where attacks become directed at the dorsal region rather than the frontal area (Kalleberg, 1958), so an observer would expect to note some examples of fin injury. This was not the case. It has been previously suggested that the occurrence of fin damage is dependent upon the frequency of overt interactions and temperature (Turnbull et al., 1998). At low temperatures the hyperplastic response of epithelium is more conspicuous (Roberts and Bullock, 1976) and the rate of healing is suppressed (Turnbull, 1992). High temperatures during summer may mean the healing response is rapid and inconspicuous, meaning an observer cannot distinguish between recent and older damage to fins using the naked eye.

There was a general increase in the occurrence of fin damage following the trimmed grade and vaccination in autumn for both treatments and this was maintained throughout the winter in fish under the imposed regime. During autumn the effect of grading on fin injury seems much larger than the effect of feeding regime. Previous studies have used temporal variation in the prevalence of fin injury as an indicator of changes in aggression levels (MacLean et al., 2000) or to assess the intensity of social hierarchies (Christiansen and Jobling, 1990). This experiment was subject to ambient temperatures, which fluctuate markedly with season (see chapter 4a). As decreased temperatures during the winter may be in some part responsible for increases in fin injury (Turnbull, 1992), I do not wish to use its prevalence as an indicator of changes in aggression levels with time (MacLean et al., 2000). In this study it was only used to assess the impacts of feeding regime upon the prevalence of overt aggression between treatments. Feeding regime can influence the size of fish affected by fin erosion. During late winter the smallest fish held under the imposed regime showed a significantly higher prevalence of fin injury than other fish within the treatment. There was no evidence of such an effect in fish fed on-demand during the same period. Furthermore, in February the largest fish in the on-demand fed treatment sustained significantly less fin damage than other fish within the group. Prior to smolting there was a clear relationship between fish size and the incidence of fin damage, with the smallest fish in each treatment sustaining significantly greater fin damage than other members of the group (which is in agreement with findings in Chapter 3b on the size of fish affected by fin damage). This suggests that feeding regime can also affect the prevalence of fin injury *within* a treatment in addition to between treatments. There are a number of hypotheses that either individually or in combination may explain the differences in the amount of fin damage exhibited by different sized fish.

What may be responsible for the smaller fish exhibiting greater fin injury during winter when fed predetermined rations? Many studies have suggested that restrictive rations can increase competition and aggression (Symons, 1968; Thorpe and Huntingford, 1992; M^{c}Carthy et al., 1992). Under these conditions socially subordinate fish receive increased aggression from conspecifics (Abbott and Dill, 1989; Gregory and Griffith, 1996; Dåmsgard et al., 1997; Moutou et al., 1998), which can in turn affect fin damage if this aggression is overt. In this present study, ration was closely matched between regimes for the majority of the experiment, as stated earlier. Under on-demand feeding conditions, competition may be less influential in governing access to a feed resource, which decreases the incidence of overt aggression (see earlier in chapter) and associated fin damage.

Why do the smallest fish under each regime show significantly greater incidences of fin damage prior to smolt transfer? Most salmonids undergo various physiological, behavioural and morphological changes prior to smolt migration (Hutchison and Iwata, 1998) in preparation for life in the sea. There is a natural increase in plasma cortisol levels during smoltification (Young, 1986) and the energy reserves of fish decrease (Woo et al., 1978; Folmar and Dickoff, 1980; Virtanen and Forsman, 1987). The rise in plasma cortisol is somewhat responsible for the mobilisation of energy reserves during smolting (Sheridan, 1994). Behavioural stressors can also cause an increase in plasma cortisol (Ejike and Schreck, 1980; Pottinger and Pickering, 1992), which may be more severe in smaller subordinate fish (Ejike and Schreck, 1980). The stress response may also be more severe in pre-smolts in comparison with parr (Barton et al., 1985; Carey and M^{c}Cormick, 1998), which may be partly due to the natural increase in plasma cortisol during smoltification. A combination of smolting and social subordination may mean smaller fish have higher levels of cortisol relative to other fish within the group, which can have a deleterious effect on fin condition (Gregory and Wood, 1999). A further hypothesis could be that the smallest fish have less energy available for tissue maintenance relative to other members of the group, due partly to depletion of energy reserves during smolting (Virtanen and Forsman, 1987) and direct effects of starvation if they are competitively excluded from a feed resource (see Storebakken and Austreng, 1987).

Furthermore, smaller subordinate fish may have increased hunger levels and may be more willing to accept a risk of injury to gain access to food (Gotceitas and Godin, 1991). Highly competitive conspecifics can aggressively exclude smaller individuals from a feeding resource and salmon parr have been observed to refrain from feeding and abort feed attempts when faced with competitors (Metcalfe, 1989; Huntingford et al., 1993). If this is the case why was the condition not more widespread during the earlier months of the experiment? The greater incidence of fin damage in smaller fish under the imposed regime during late winter tends to support this hypothesis, and on-demand feeding may suppress its occurrence until just prior to smolt transfer.

Other studies on fin damage in salmonids have reported that small fish exhibit the greatest incidence of fin injury (Abbott and Dill, 1989; Gregory and Griffith, 1996; Dåmsgard et al., 1997; Moutou et al., 1998). Studies on Atlantic salmon parr in aquacultural conditions have reported different findings to this experiment. Turnbull et al., (1998) found no relationship between the prevalence of fin damage and fish size but the authors do not report the time of year or the smolt status of the fish involved in the experiment. MacLean et al. (2000) reported that when fin damage first appeared in July it affected the largest fish amongst parr held in tanks under an accelerated smoltification strategy. Prior to smolt transfer there was no relationship between fish size and the incidence of fin injury. The experimental conditions in their trial were different from the present study as fish received food from a localised food source and were not size graded during the course of the experiment. This may account for the disagreement between our results (MacLean pers. comm.). These studies show that in Atlantic salmon parr fin damage is dynamic; it can affect different sized individuals at various times of the year and may differ with rearing system and feeding technique.

4c.4.4 Conclusions

Feeding regimes can govern levels of competition within a group. Using the hypothesis that energetic expenditure increases with manoeuvre complexity (Krohn and Boisclair, 1994), my results suggest that fish under an imposed regime incur escalated costs during feeding. On-demand feeding systems can reduce the incidence of competition within large scale rearing facilities. An on-demand feeder can reduce competition for food and the prevalence of injuries caused by aggressive interactions in cages, which in turn may improve welfare considerations and the market appeal of whole fish for the consumer. They can also influence which fish are affected by fin injury within commercial scale groups of fish.

Chapter 5: The use of photoperiod manipulation in aquaculture

5.1 Introduction

5.1.1 Aims and content

In the final section of this thesis I will outline some recent developments in manipulating daylength to produce out-of-season smolts and regulate the timing of reproduction. I will discuss how photoperiod manipulation can impact upon production parameters and farm practices before describing a two fold experiment on the effect of photoperiod manipulation upon the feeding patterns and production performance of Atlantic salmon parr.

5.1.2 Photoperiod manipulation

Traditional smolt production has involved Atlantic salmon smolts being transferred to seawater during a restricted spring period. This has led to seasonal fluctuations in the availability of market sized fish, which can be in direct conflict with market demands; salmon markets increasingly require continuity in the supply of fish of uniform size and quality. The salmon producer would also like a year round availability of consistently sized fish to boost stability in the industries pricing structure (Ritson, 1994). This can be achieved in part using photoperiod manipulation.

Certain events in the life cycle of a fish, for example migration or reproduction, have a circannual periodicity synchronised by photoperiod (Sumpter, 1990; Bromage et al., 1993). Photoperiod acts as a proximal cue to these circannual rhythms because of its stability and predictability (Brett, 1979). Manipulating the length of the photoperiod by artificially increasing or decreasing daylength can influence the timing of migration or reproduction provided the intensity of artificial light is above a certain threshold (Oppedal et al., 1997). It is relatively straightforward to increase daylength by using artificial lighting in cage culture, but decreasing daylength can be more difficult in the open cage environment.

5.1.3 Photoperiod manipulation in aquaculture

Photoperiod manipulation is extensively used in aquaculture for controlling maturation in adult fish (Bromage et al., 1993; Blythe et al., 1994) and a farmer can use this to increase the capacity to produce eggs at any time of the year for use in fingerling production. Artificial light regimes can also have a pronounced effect on the growth of adult salmonids (Stefansson et al., 1991; Oppedal et al., 1997) and can also reduce the prevalence of early maturing adult fish (Porter et al., 1999). After one sea winter a proportion of adult Atlantic salmon begin to mature as grilse. Grilse are undesirable as flesh quality begins to deteriorate before maturation. In the following experiments photoperiod manipulation was used to produce out-of-season smolts (smolts produced outwith spring) in freshwater production (Clarke, 1989; Duston and Saunders, 1990; Thrush et al., 1994; Berge et al., 1995; Duncan and Bromage, 1998; Duncan et al., 1998).

5.1.4 Applications of photoperiod manipulation in smolt production

Juvenile salmonids have a flexible life history and can spend between one and seven years in freshwater before making the migration to sea (Thorpe et al., 1992). During the first summer in freshwater potential migrating and resident individuals differ in their size trajectories, with migrating fish undergoing smoltification the following spring (Thorpe, 1977). These fish are upper modal group (UMG) parr and in aquaculture are termed S1 fish. It has been suggested that these individuals adopt a fast growth strategy (Metcalfe et al., 1988) which is desirable for the aquaculture industry as residence time in freshwater is minimised and the fish require less time and cost to produce.

Using an artificial light regime to simulate longer spring days after a short winter period a farmer can advance this circannual rhythm of migration. These long days allow a fish to develop full hypo-osmoregulatory capacity and undergo smoltification before spring. Freshwater smolt producers use photoperiod manipulation to produce out-of-season smolts along with their S1 fish (in 1999 and 2000 around 60% of Marine Harvest smolt production was out-of-season, D. Mitchell, pers. comm.). A farmer can now produce out of season smolts as early as October and these are termed S1/2 fish. Marine Harvest Ltd. generally produces two runs of out-of-season smolts in November/December and January/February and these are referred to as PP1 and PP2 fish respectively.

5.1.5 What are the potential advantages of producing out-of-season smolts?

The production of out-of-season smolts can offer several potential benefits to the fish farmer. A capacity to increase the year round availability of smolts can provide more consistent levels of salmon production and market accessibility throughout the year. This is desirable for both the salmon producer and consumer as it stabilises the availability of salmon and also the pricing structure. Additionally, it shortens the growth cycle and rearing time, reducing rearing requirements and accelerating the time it takes for the farmer to get the fish to market. Thrush et al. (1994) demonstrated that smolts display better growth after early seawater transfer and this growth advantage is retained throughout the production cycle. It also shortens the fish's residence time in freshwater where growth is less marked than in seawater. The S1/2 fish smolting in November are transferred to sea before winter in order to avoid poor growth and inefficient feeding in freshwater.

5.1.6 Potential disadvantages?

Unsuitable lighting conditions can suppress the hypo-osmoregulatory capacity of out-of-season smolts, inhibiting smoltification and can potentially lead to high mortalities at seawater transfer (Berge et al., 1995), low growth (Björnsson et al., 1988) and osmotic disturbances (Stagg et al., 1989). Exposure to continuous light may affect the behaviour of the fish in freshwater. Under ambient light conditions adult Atlantic salmon in cages normally shoal during the day, but these schools breakdown at dusk when the fish rise towards the surface (Juell, 1995). An artificial photoperiod regime may interfere with these activity rhythms and extend the period of active schooling. The increased energy expenditure associated with elevated swimming may have a detrimental effect on the growth performance of the fish (Oppedal et al., 1997, 1999).

Chapter 5a: The effect of biological and environmental variables on the feeding rhythms and daily feed intake of Atlantic salmon parr in production cages under an artificial photoperiod regime

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5a.1 Introduction

5a.1.1 Aims and content

The aim of this chapter was to evaluate the effects of continuous lighting (as part of an accelerated smoltification strategy producing out-of-season S1/2 fish) upon the feeding patterns of cage-held Atlantic salmon parr fed by on-demand feeding systems, to assess whether it would be commercially viable to feed fish during the artificially illuminated nocturnal period. I will also investigate the importance of light as a Zeitgeber entraining endogenous feeding rhythms.

5a.1.2 Effect of light upon rhythms of feeding

As stated in earlier chapters (3a and 4a), light is considered to be the primary Zeitgeber that synchronises endogenous feeding rhythms in fish (Muller, 1978; Spieler and Noeske, 1984; Boujard and Leatherland, 1992*b*; Spieler, 1992; Kadri, 1995). Daily light/dark cycles and seasonal changes in the length of the photoperiod provide accurate information about the time of day or year (Eriksson and Alanärä, 1992), although the change in intensity between the light and dark phase (on a daily basis) is considered the primary entraining agent (Gibson and Keenleyside, 1966; Boujard and Leatherland, 1992; Kadri, 1995). The endogenous or exogenous character of a rhythm is revealed under constant environmental conditions. If the periodicity of the rhythm is lost, it is under the control of an external Zeitgeber and is exogenous in nature. If it is retained with a free-running period close to 24hr then it is under endogenous control (Boujard and Leatherland, 1992*b*). The periodicity of the endogenous rhythm usually shifts a little from the cycle it has been entrained to whilst free running and if it persists without weakening it is defined as a self sustaining oscillator (Madrid et al., 2001). A number of commercially important species retain circadian feeding and activity rhythms under constant conditions: burbot, *Lota lota*, (Kavaliers, 1980); pink salmon, *Oncorhynchus gorbuscha*, (Godin, 1981); sea bass, *Dicentrarchus labrax*, (Sánchez-Vázquez et al., 1995*b*); goldfish, *Carassius auratus* (Sánchez-Vázquez et al., 1996); and rainbow trout, *Oncorhynchus mykiss* (Sánchez-Vázquez and Tabata, 1998). In Atlantic salmon, *Salmo salar*, free-running rhythms have not been observed (Richardson and M^{c}Cleave, 1974), or are temporary and last only two or three days (Ali, 1964).

Other exogenous environmental variables aside from light can influence feed intake and these include: temperature (Brett, 1979; Alanärä, 1992), oxygen depletion (Brett, 1979; Thetmeyer et al., 1999), wind speed and rainfall (Bégout and Lagardère, 1993; Juell, 1995) and turbidity (Berg and Northcote, 1985; Ang and Petrell, 1997; Mallekh et al., 1998). Intraspecific competition can also influence timing of feed intake by governing access to a feed resource. Kadri et al. (1997*a*) reported that salmon parr do not feed at first light during autumn/spring due to a high incidence of aggressive encounters.

Previous studies (see Table 4a.1) on the feeding rhythms of salmonids report upon a wide range of patterns although these are generally diurnal (Hoar, 1942; Pinskii, 1961; Ali, 1964; Kadri et al, 1997*a*). Many authors have also reported that long or increasing photoperiods promote feeding activity, whereas shorter photoperiods can decrease feeding activity and motivation (Komourdjian et al., 1976; Higgins and Talbot, 1985; Jørgensen and Jobling, 1992). Under the extended lighting conditions of photoperiod manipulation it would be interesting to see if parr extend their feeding activity throughout the 24hr. This can be investigated in a cage culture environment using on-demand feeders.

Interactive on-demand feeding systems offer a biologist the opportunity to investigate the feeding patterns of fish held under a 24hr light regime by efficiently feeding fish to near satiation, whilst monitoring feed wastage. This can provide information that can help a farmer improve existing feed practices and techniques as the fish approach smoltification. It would also be beneficial for the farmer to attempt to match the feed delivery of existing feeding systems to appetite, as it is possible to determine when food is eaten and when it is wasted.

5a.1.3 Objectives of the experiment

This experiment evaluated the effects of photoperiod manipulation upon the feeding rhythms of cage held Atlantic salmon parr using interactive on-demand feeding systems. It was conducted as part of growth trial, using a control treatment (where fish were fed pre-determined fixed rations) as part of an imposed in-house feeding regime. The questions addressed were:

i) How does the pattern of feed intake vary across an ambient day prior to photoperiod manipulation?

ii) Does feeding occur during the illuminated nocturnal period?

iii) How does a 24hr light regime in winter affect feed intake?

iv) Is there any variability in the daily amount of food consumed and can this variability be interpreted by changes in environmental variables?

v) How do feeding patterns in S1/2 fish compare with those of S1 fish at the same time of year?

The experiment was designed using production densities and facilities and was under the constraints of in-house husbandry practices, to provide information that was directly applicable to freshwater smolt production.

5a.2 Materials and methods

5a.2.1 Fish husbandry

Fish used in this experiment that were identified in early August 1999 as 1+ upper modal group (UMG) Atlantic salmon that migrate to sea after one year (Thorpe, 1977). They were from a pooled hatchery stock and held in 12x12x4m deep production cages at a smolt production site in Glenfinnan, (grid ref. NM 887 790) which was owned by Marine Harvest Ltd. Due to the large numbers of fish involved in the experiment I had to use fish that were from two distinct stocks, and there was no mixing of these stocks during the trial. Four cages of fish were from a medium maturing stock (treatment replicates two and three) and two were from a low maturing stock (treatment replicate one). Stocks were balanced between treatments. Six groups of around 60,000 fish were graded into separate cages (see figure 5a.1 for details), according to production densities used in cages on the rest of the farm. These trial cages were on one cage group, and the trial commenced on the 23rd August 1999. It ended approximately one week prior to smolt transfer on the 8th November 1999. Fish were transferred to seawater cages during the week commencing the 15th November 1999. Fish were subjected to a three way grade with a commercial grader, (Fischtechnik Fredelsloh, Moringen, Germany) according to size (small, medium, large) and returned to the production cages three weeks prior to the start of the trial, and were not graded again for the duration of the experiment. The exact numbers of fish per cage and their corresponding densities are shown in figure 5a.1.

The cages were subject to ambient temperature and photoperiod until the 16th October. After this point the fish were held under a 24hr light regime until smolt transfer as part of an accelerated smolt production strategy. The fish were subject to artificial light during hours of ambient darkness, and these lights were turned on at approximately 16.30hrs each evening and switched off at approximately 8.00hrs each morning. Artificial light was provided via two 400 watt submerged lights (Aquabeam UK, Grantham) held at a depth of two metres. Each light was suspended across the centre of the cage and held approximately three metres from the corners of the cage wall.

The set-up of the feed regimes was similar to that of Chapter 4. Each cage was fitted with a 50kg centralised feed hopper with a vibrator dispenser attached to a circular spreader (Sterner AS; Aquatess Ltd, Inverness) which spread pellets in a 10m diameter circle. Fish were fed a commercial 2mm extruded pellet, Biomar Ecostart, (Biomar UK Ltd, Grangemouth) until the last month of the trial. For the last month of the experiment, the fish were fed a Trouw Royale 2.3mm pellet (Trouw UK Ltd, Northwich) according to manufacturer's guidelines on fish size. Three feed hoppers were controlled by AKVAsmart AQ1 Adaptive Feed Systems (AKVAsmart UK Ltd, Inverness), and three hoppers were operated by an Aquatess KC20 Automatic Feed System (Aquatess Ltd, Inverness).

The three Aquatess fed groups were fed a fixed ration every 10 minutes from just after dawn until dusk. The hopper dispensed food for about 10 – 15 seconds every feeder actuation. The fish were fed to apparent daily satiation using a combination of in-house feed tables supplemented by daily hand feeding at 8.00am and 3.00pm, using cessation in surface activity as an indicator of satiation. This long day feeding strategy mimics current husbandry practices for achieving the best growth performance from automatic feeders in salmon parr (D. Mitchell pers. comm.). From the 16th October 1999, when the fish were subject to a 24hr artificial light regime the daily ration was extended throughout the 24hr light period.

The other three groups were fed on-demand from dawn till dusk (as defined by the Royal Observatory, Edinburgh) using an AKVAsmart AQ1 with a feedback loop. This system matches feed delivery to group appetite. In on-demand fed groups discrete meals are observed across a day, but the distinction is that these meals are selected by the group of fish and are not imposed by a fixed feed management strategy. When the fish were subjected to 24hr light from the 16th October the fish were given the opportunity to feed from 00.01 until 23.59 each day. (Refer to chapter 2 for the exact system set-up and the biological and production significance of the parameters used).

5a.2.2 Measuring environmental variables

The times of sunrise and sunset were calculated using the methodology of Chapter 4a. The change in daylength from the previous day (hours) was also recorded (after Smith et al., 1993). Water temperature was measured on the cage group at approximately 8.00am each morning using a mercury thermometer held at a depth of two metres. Water clarity was also measured on the cage group at 8.00am daily using a secchi disc. The British Atmospheric Data Centre (Chilton, Didcot, UK) kindly provided meteorological and hydrological data, compiled from weather stations in the vicinity of the experimental site. Data on daily wind speed and direction was obtained from the Aonach Mor weather station (Grid ref: NN 189 740) and there was no data available from the Glenfinnan weather station on rainfall. Current speed was also measured on the cage group but the data obtained was of insufficient quality to be used in further analysis.

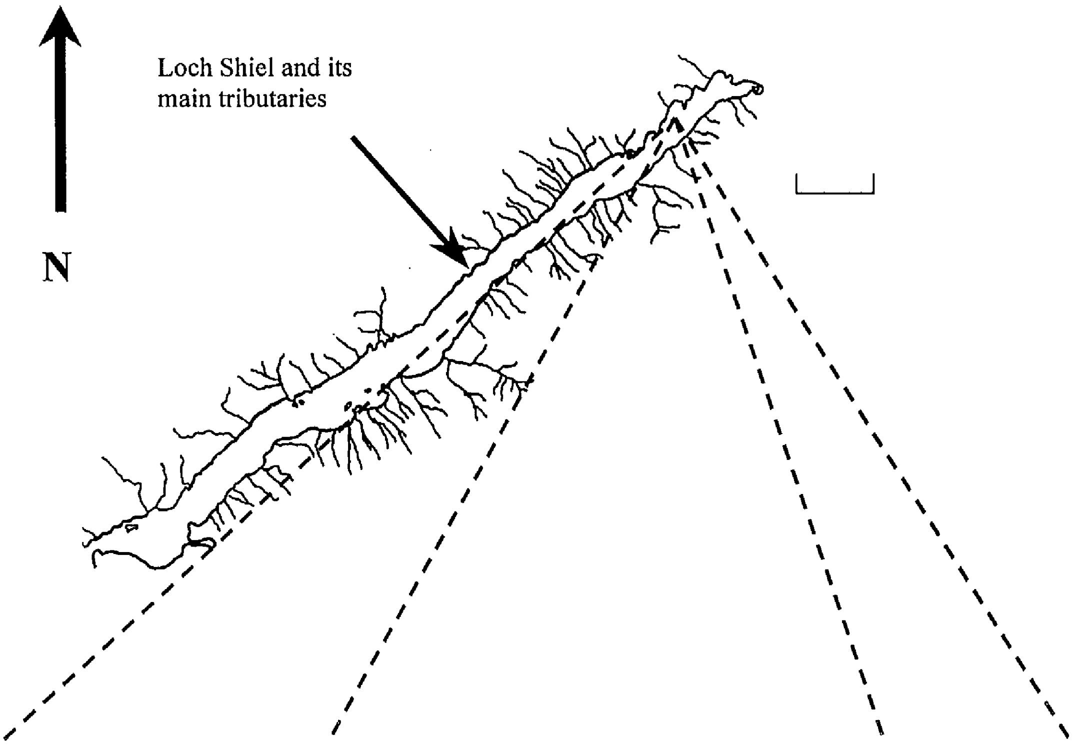
5a.2.3 Manipulation of the on-demand feeding data

On-demand feeding data were manipulated and transformed using the methodology outlined in Chapter 3a. When food was available during the artificially illuminated nocturnal period from the 16th October, both day and night were broken down into ten fractions for statistical analysis. For graphical representation the Relative Feed Delivery Index (see Fig. 5a.2 and Fig. 5a.3) was combined for both the diurnal and illuminated nocturnal period, breaking the portion of the feeding day and night into twentieths. The proportion of feed delivered by the on-demand feeders during the ambient day and illuminated night were also recorded when the fish were held under 24hr light.

5a.2.4 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0. Minitab Inc., Philadelphia) or SPSS (v.10-0. SPSS Inc., Chicago) for MS Windows. A non-parametric ANOVA (Kruskal-Wallis test) was used to test for possible differences in appetite across a day in the on-demand treatment. A post hoc multiple comparison test (Siegel and Castellan, 1988) was used to locate any differences observed. Data are presented as median values ± first and third interquartile ranges.

Differences in appetite between days were tested with a Univariate General Linear Model (GLM) model in SPSS version 10.0. Independent variables were: day, natural daylength (h), change in daylength from previous day (h), artificial daylength when under 24hr lights (h), change in artificial daylength from the previous day (h), water temperature at 2m depth (°C), water clarity (m), average daily wind speed (knots), and all variables with day as an interaction. The dependent variable was daily ration, which was transformed into amount fed as a percentage body weight day^{-1}. This was further transformed using the arcsin square root procedure to satisfy normality and homogeneity of variance requirements. A significance level of $P < 0.05$ was used for all statistical tests. Synchrony between cages for daily food delivery, from the beginning until the end of the experiment, was tested for using ANCOVA analysis in SPSS.



Imposed cage 1 Mean wet weight on 26.8.99 = 42.82g Stocking density = 4.79 kg m^{-3} No. of fish = 64,377	**On-demand cage 1** Mean wet weight on 26.8.99 = 43.81g Stocking density = 5.04 kg m^{-3} No. of fish = 66,230
On-demand cage 2 Mean wet weight on 25.8.99 = 43.81g Stocking density = 5.13 kg m^{-3} No. of fish = 67,491	**Imposed cage 2** Mean wet weight on 25.8.99 = 41.32g Stocking density = 4.70 kg m^{-3} No. of fish =65,517
Imposed cage 3 Mean wet weight on 24.8.99 = 43.06g Stocking density = 4.11 kg m^{-3} No. of fish = 55,027	**On-demand cage 3** Mean wet weight on 23.8.99 = 43.44g Stocking density = 3.95 kg m^{-3} No. of fish = 52,439

Fig. 5a.1 The location of the experimental production cages on Loch Shiel, showing initial fish numbers, stocking densities and average weights at the beginning of the experiment. Scale bar = 2 miles.

5a.3 Results

5a.3.1 Diurnal patterns of appetite

Atlantic salmon parr show a distinct peak in appetite across the day, although the timing of this peak differs between months and, in some cases, between cages (see Fig. 5a.2 and Fig. 5a.3). For this reason the feeding patterns of each cage will be reviewed independently, before examining each data set for synchrony between cages within the on-demand treatment.

5a.3.1.1 On-demand fed cage 1

During September this cage of salmon parr exhibited a highly significant morning peak in feed delivery immediately after first light (Kruskal-Wallis $H_{adj} = 27.67$, d.f. = 9, $P < 0.001$) but this peak was not maintained from the 1st – 15th October (Kruskal-Wallis $H_{adj} = 33.19$, d.f. = 9, $P < 0.001$). From the 16th October until the 8th November, food was made available throughout the day and artificially illuminated nocturnal period. There was no significant peak in appetite during the illuminated nocturnal period (Kruskal-Wallis $H_{adj} = 13.46$, d.f. = 9, $P = 0.143$), whereas a significant late morning peak in diurnal feed delivery was observed (Kruskal-Wallis $H_{adj} = 18.07$, d.f. = 9, $P = 0.034$).

5a.3.1.2 On-demand fed cage 2

During September this cage also exhibited a highly significant peak in appetite, although this occurred around midday (Kruskal-Wallis $H_{adj} = 22.12$, d.f. = 9, $P = 0.008$). The fish exhibited a significant morning peak in appetite during the first two weeks of October (Kruskal-Wallis $H_{adj} = 30.20$, d.f. = 9, $P < 0.001$). During the late October – November period there was no significant peak in appetite during daylight hours (Kruskal-Wallis $H_{adj} = 1.28$, d.f. = 9, $P = 0.998$) or the artificially illuminated nocturnal period (Kruskal-Wallis $H_{adj} = 12.04$, d.f. = 9, $P = 0.213$).

5a.3.1.3 On-demand fed cage 3

During September this group exhibited a highly significant morning peak in appetite and this peak was maintained until late midday before decreasing as dusk approached (Kruskal-Wallis $H_{adj} = 38.91$, d.f. = 9, $P < 0.001$). There was no significant peak in appetite during the first two weeks of October (Kruskal-Wallis $H_{adj} = 15.97$, d.f. = 9, $P = 0.067$). During the October – November period when fish were subject to artificial illumination, there was no significant peak in appetite during daylight hours (Kruskal-Wallis $H_{adj} = 10.83$, d.f. = 9, $P = 0.287$), or during the artificially illuminated nocturnal period (Kruskal-Wallis $H_{adj} = 10.08$, d.f. = 9, $P = 0.344$).

5a.3.1.4 Synchrony between cages within the on-demand feeding treatment

There was a high degree of synchrony between two of the three on-demand feeding cages in the time fish chose to feed under ambient lighting conditions and the period where fish were subject to artificial illumination during the nocturnal period (cages 1 and 3 respectively). Each on-demand fed group showed a similar trend in feeding time (although there were slight differences in the exact timing of feeding peaks in September, and Cage 3 did not exhibit a significant peak in group appetite during October); this general synchrony was evident throughout the experiment.

The second on-demand fed cage showed synchrony with the other two during September, although there was no synchrony in feeding patterns in the following months. This may have been an effect of the feeding unit, which also caused problems in the earlier freshwater trial (Chapter 4a), or it may be a group effect (see discussion for further details).

The amount of food eaten per day and night was compared when fish were held under artificial lights, to see if fish consumed food during the extended daylength period (see Fig. 5a.4). During the three week period from the 16th October until the 8th of November fish consumed on average

60 – 70% of the daily ration during the hours of natural daylight (natural daylight accounted for around 11hr of the total 24hr period); the rest was consumed during the artificially illuminated nocturnal period. The average temperature during this period when food was available over 24hr was 10.93 ± 0.49°C .

5a.3.2 Differences in appetite between days and the effect of environmental variables

Daily feed delivery was analysed in relation to a number of environmental variables using a Univariate General Linear Model in the SPSS statistical package (version 10.0). As in Chapters 3a and 4a, it should be noted that the daily feed delivery variable in each model is not independent as data is taken from each of three on-demand fed cages over an extended time period. Daily variation in feed delivery for each of the on-demand fed cages is plotted against environmental variables (Fig. 5a.5). Univariate GLM analysis showed natural daylength had a significant effect upon appetite for two of the three cages although the extent of its effect did differ between cages (Table 5a.2a,b,c respectively). Interestingly, for on-demand fed cage 2, average wind speed was the only significant predictor of amount eaten per day. The other variables measured did not significantly affect food demand. The model accounted for 66.1%, 32.7% and 46% of daily food demand for on-demand cages 1,2 and 3 respectively.

5a.3.3 Effect of cage or population upon daily feed delivery between cages

Although there were differences between cages on the amount of food delivered on any given day (see Fig. 5a.6), this variability may be absorbed over longer time periods. To compare the influence of either a feeding unit effect or biological effect upon daily feed delivery throughout the experiment, analysis of covariance was used (ANCOVA) with cage as a factor and date (Julian date) as a covariate (see Fig. 5a.6). The analysis was carried out on all experimental on-demand feeding data, from the beginning of September until the middle of November. As expected, there was a significant effect of date upon the amount of food delivered (ANCOVA: effect of date, $F_{1,119} = 67.00$ $P < 0.001$). There was a significant cage effect on overall feed delivery (ANCOVA: comparison of regression slopes, $F_{2,117} = 2.33$ $P = 0.102$, NS; comparison of regression elevations, $F_{2,119} = 8.99$ $P < 0.001$), which did not support the hypothesis that fish select similar rations over longer time periods.

To locate the observed difference in cage effect the ANCOVA analysis was repeated using data from only two on-demand fed cages. For on-demand fed cages 1 and 2 there was a significant effect of date upon the amount of food delivered (ANCOVA: effect of date, $F_{1,68} = 34.07$ $P < 0.001$). There was no significant cage effect on overall feed delivery (ANCOVA: comparison of regression slopes, $F_{1,67} = 3.73$ $P = 0.058$, NS; comparison of regression elevations, $F_{1,68} = 2.12$ $P = 0.15$), which supported the hypothesis that these caged groups select similar rations over longer time periods.

For on-demand fed cages 1 and 3 there was a significant effect of date upon the amount of food delivered (ANCOVA: effect of date, $F_{1,85} = 63.95$ $P < 0.001$). There was a significant cage effect on overall feed delivery (ANCOVA: comparison of regression slopes, $F_{1,84} = 0.40$ $P = 0.843$, NS; comparison of regression elevations, $F_{1,85} = 6.59$ $P = 0.012$), which did not support the hypothesis that these caged groups select similar rations over longer time periods.

For on-demand fed cages 2 and 3 there was a significant effect of date upon the amount of food delivered (ANCOVA: effect of date, $F_{1,84} = 38.39$ $P < 0.001$). There was a significant cage effect on overall feed delivery (ANCOVA: comparison of regression slopes, $F_{1,83} = 3.83$ $P = 0.054$, NS; comparison of regression elevations, $F_{1,84} = 14.77$ $P < 0.001$), which again did not support the hypothesis that these caged groups select similar rations over longer time periods.

This analysis showed there was asynchrony in the ration size selected over prolonged time periods between on-demand fed cage 3 and the other two on-demand fed cages, with on-demand fed cage 3 consuming a significantly greater ration per gram body weight relative to the other two cages.

Table 5a.2.a The results of a univariate General Linear Model comparing the daily feed intake of S1/2 Atlantic salmon parr in on-demand production cage 1, against environmental factors. $R_{2\ adj.}$ = 0.661, so the model accounts for 66.1% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Ambient daylength	3.684	13.590	0.001
Excluded from model			
Temperature	0.764	3.100	0.094
Change in daylength	0.960	3.733	0.068
Turbidity	0.004	0.136	0.717
Windspeed	0.0003	0.009	0.924

Table 5a.2.b The results of a univariate General Linear Model comparing the daily feed intake of S1/2 Atlantic salmon parr in on-demand production cage 2, against environmental factors. $R_{2\ adj.}$ = 0.327, so the model accounts for 32.7% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Windspeed	2.995	12.159	0.002
Excluded from model			
Ambient daylength	0.0003	0.011	0.919
Turbidity	0.179	0.721	0.406
Change in daylength	0.007	0.264	0.614
Temperature	0.005	0.175	0.681

Table 5a.2.c The results of a univariate General Linear Model comparing the daily feed intake of S1/2 Atlantic salmon parr in on-demand production cage 3, against environmental factors. $R_{2\,adj.}$ = 0.460, so the model accounts for 46.0% of daily variation in feed intake.

Factor	**Mean square**	***F***	***P***
Included in model			
Ambient daylength	4.515	8.972	0.005
Excluded from model			
Windspeed	1.232	2.556	0.119
Temperature	0.320	0.657	0.423
Change in daylength	0.438	0.898	0.350
Turbidity	0.001	0.013	0.910

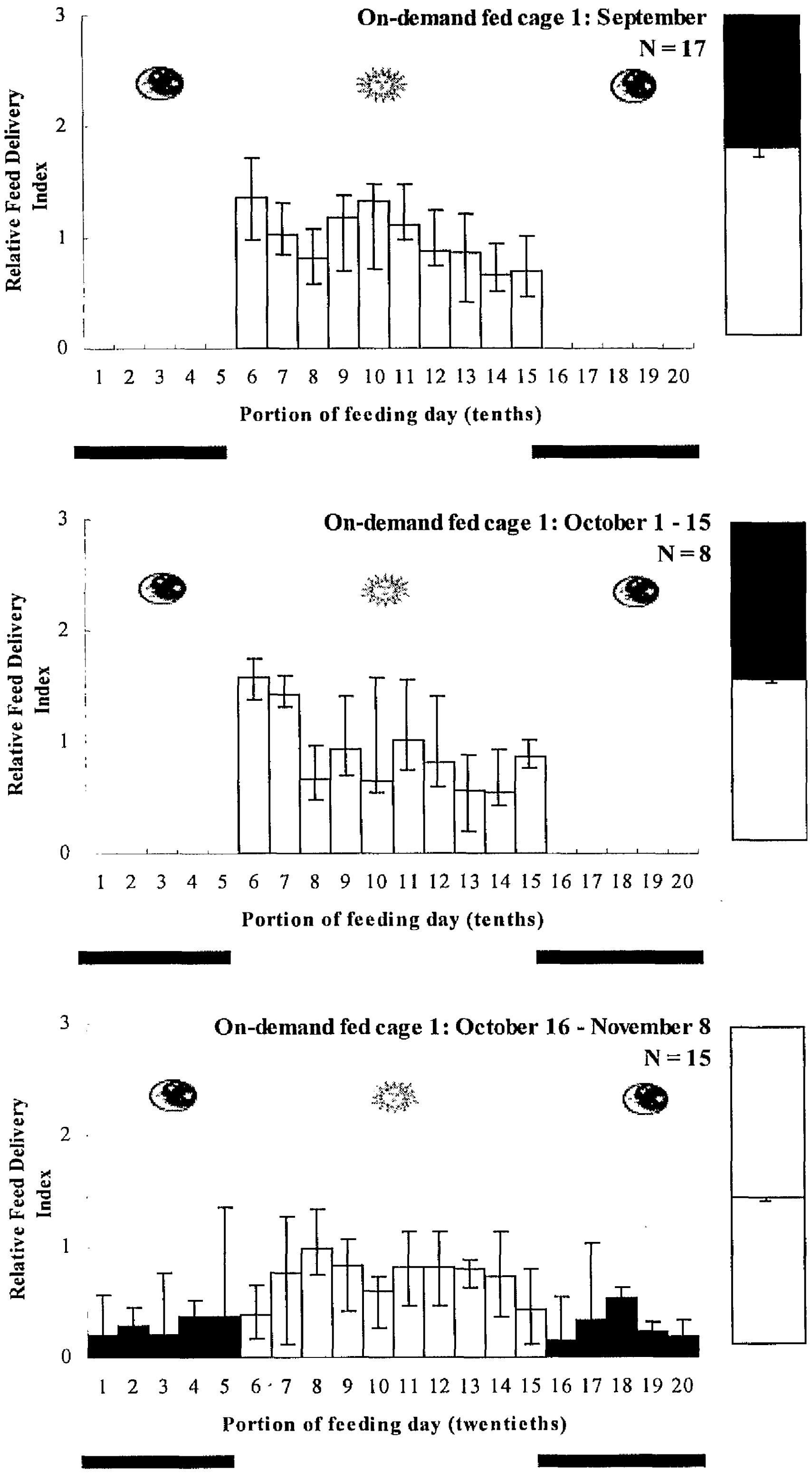


Fig. 5a.2 The relative daily feed delivery of S1/2 parr fed on-demand in production cages. Feed delivery began and ceased at dawn and dusk, until the cages were subject to artificial nocturnal illumination from October 16th, when food was also available through the night. Error bars indicate interquartile range. N = sampling days month^{-1}. Block on right represents average daylength month^{-1}, and its error bar indicates standard error of the mean. Refer to text for RFDI definitions.

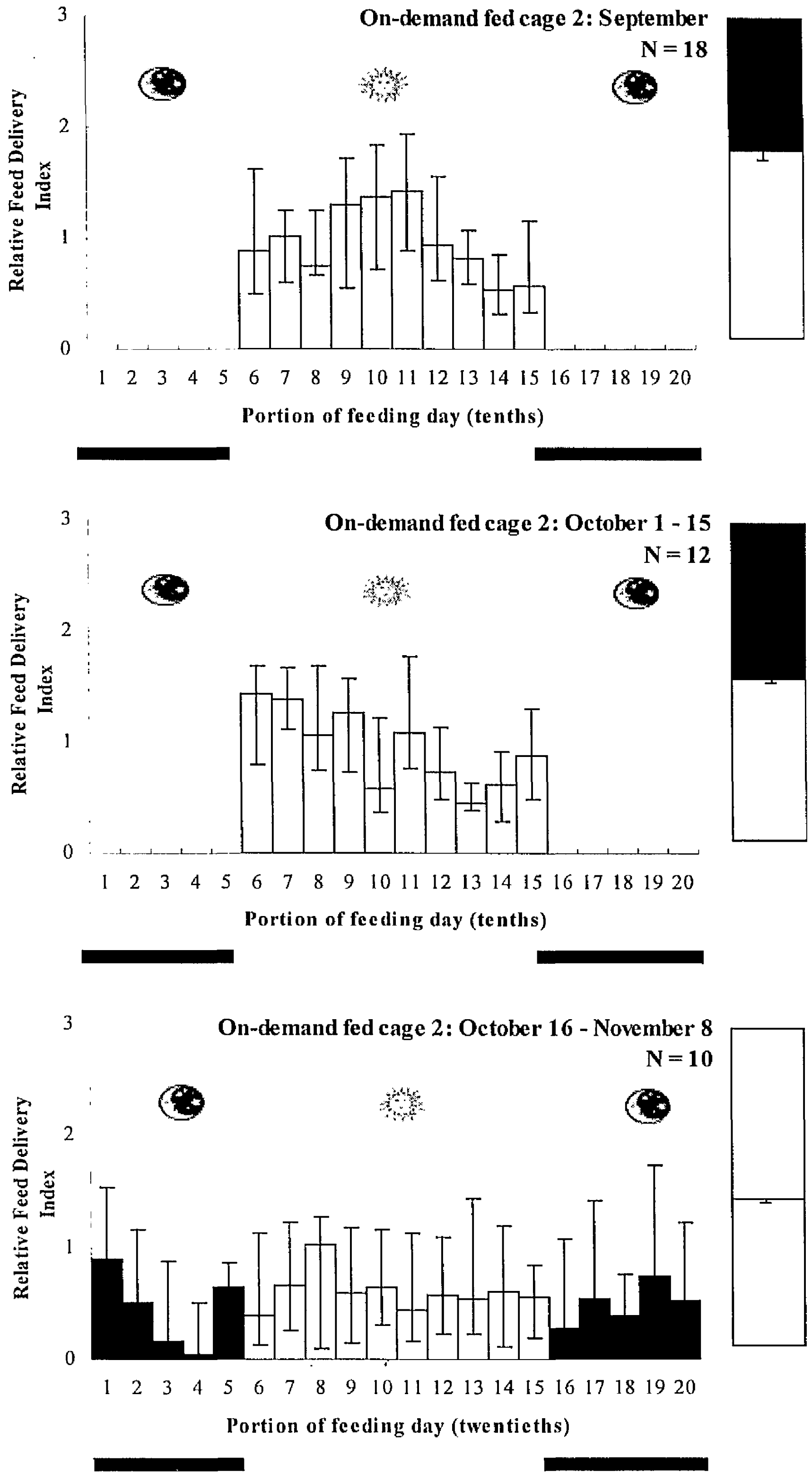


Fig. 5a.2 cont. The relative daily feed delivery of S1/2 parr fed on-demand in production cages. Feed delivery began and ceased at dawn and dusk, until the cages were subject to artificial nocturnal illumination from October 16th, when food was also available through the night. Error bars indicate interquartile range. N = sampling days month^{-1}. Block on right represents average daylength month^{-1}, and its error bar indicates standard error of the mean. Refer to text for RFDI definitions.

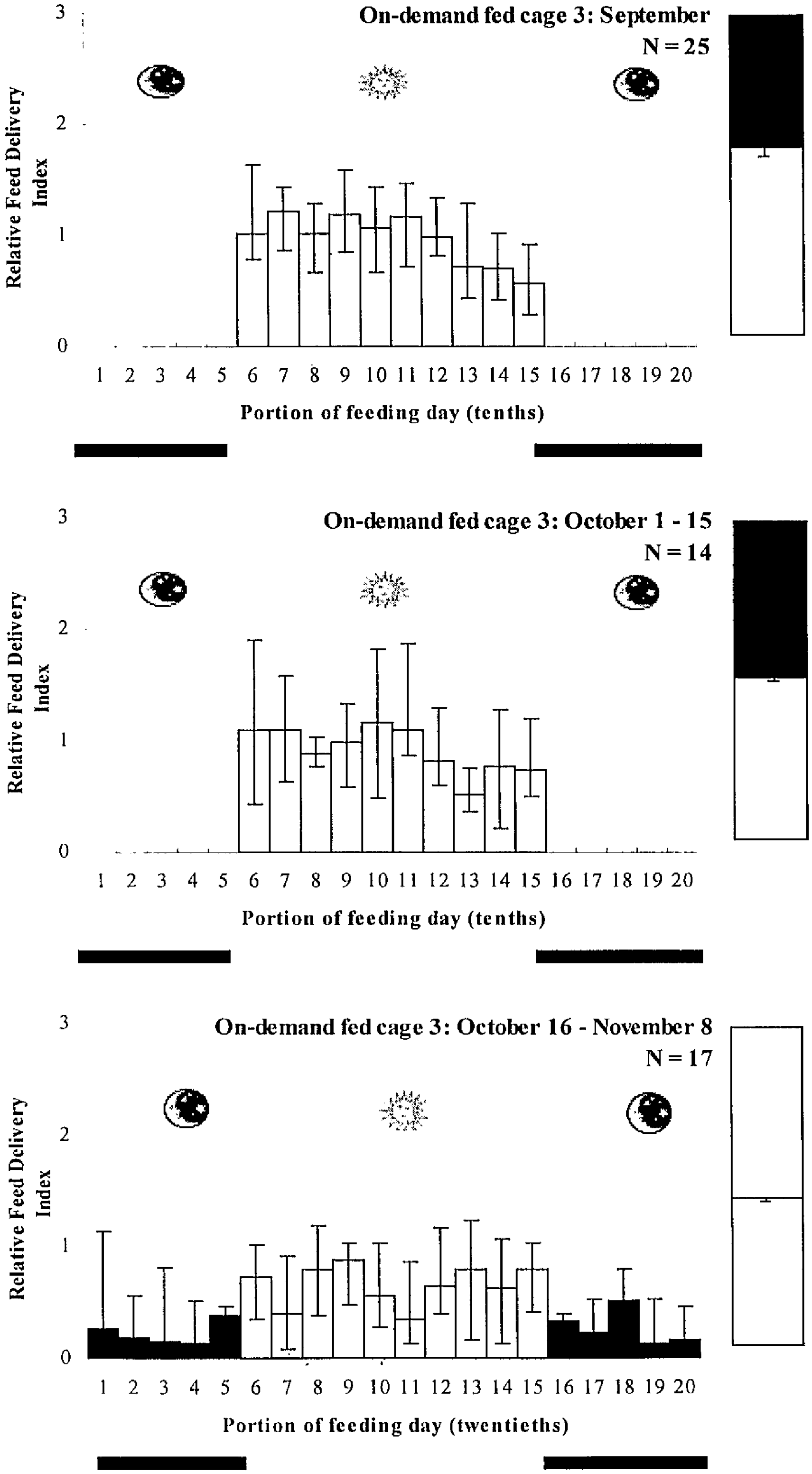
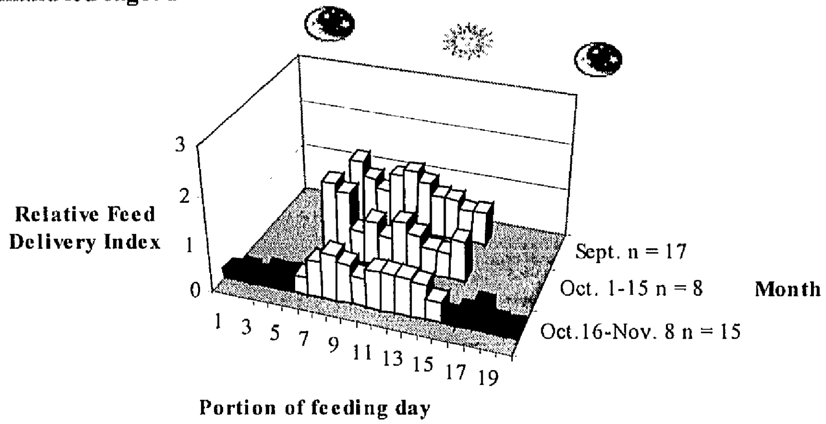
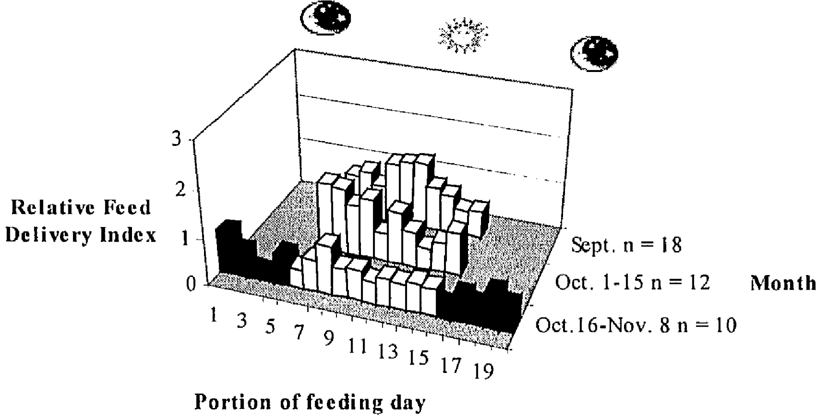


Fig. 5a.2 cont. The relative daily feed delivery of S1/2 parr fed on-demand in production cages. Feed delivery began and ceased at dawn and dusk, until the cages were subject to artificial nocturnal illumination from October 16th, when food was also available through the night. Error bars indicate interquartile range. N = sampling days month^{-1}. Block on right represents average daylength month^{-1}, and its error bar indicates standard error of the mean. Refer to text for RFDI definitions.

On-demand fed cage: 1



On-demand fed cage: 2



On-demand fed cage: 3

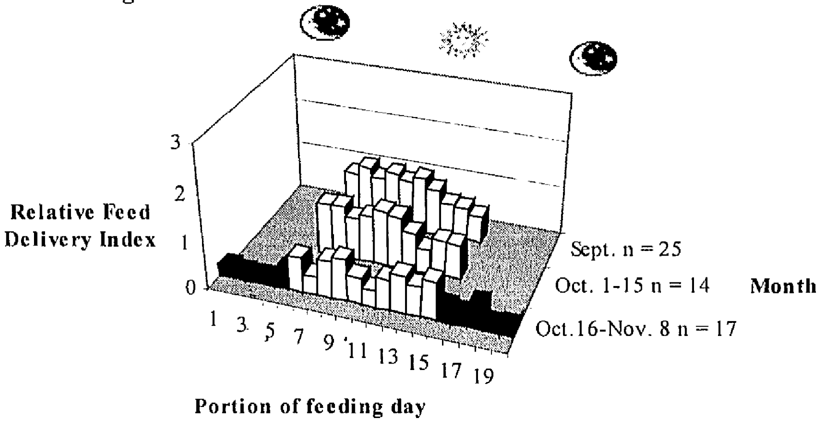


Fig. 5a.3 The relative daily feed delivery of S1/2 parr fed on-demand in production cages. Feed delivery began and ceased at dawn and dusk (until the cages were subject to artificial nocturnal illumination from October 16th, when food was also available through the night. n = sampling days month^{-1}). Images of the sun and moon represent diurnal and nocturnal periods.

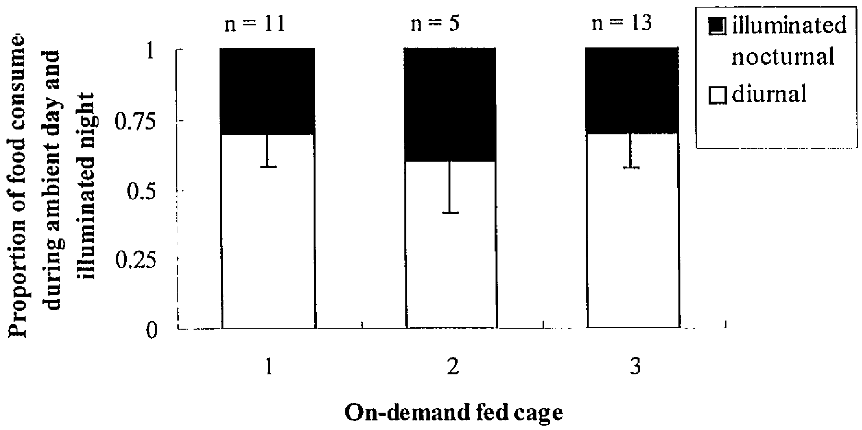


Fig. 5.4 The proportion of food delivered by on-demand feeders during the ambient day and illuminated night for S1/2 parr held under an artificial photoperiod in production cages. Error bars indicate standard deviation and n = number of continuous sampling days during the light manipulation period. Average temperature for the manipulation period was 10.93 ± 0.49 degrees centigrade.

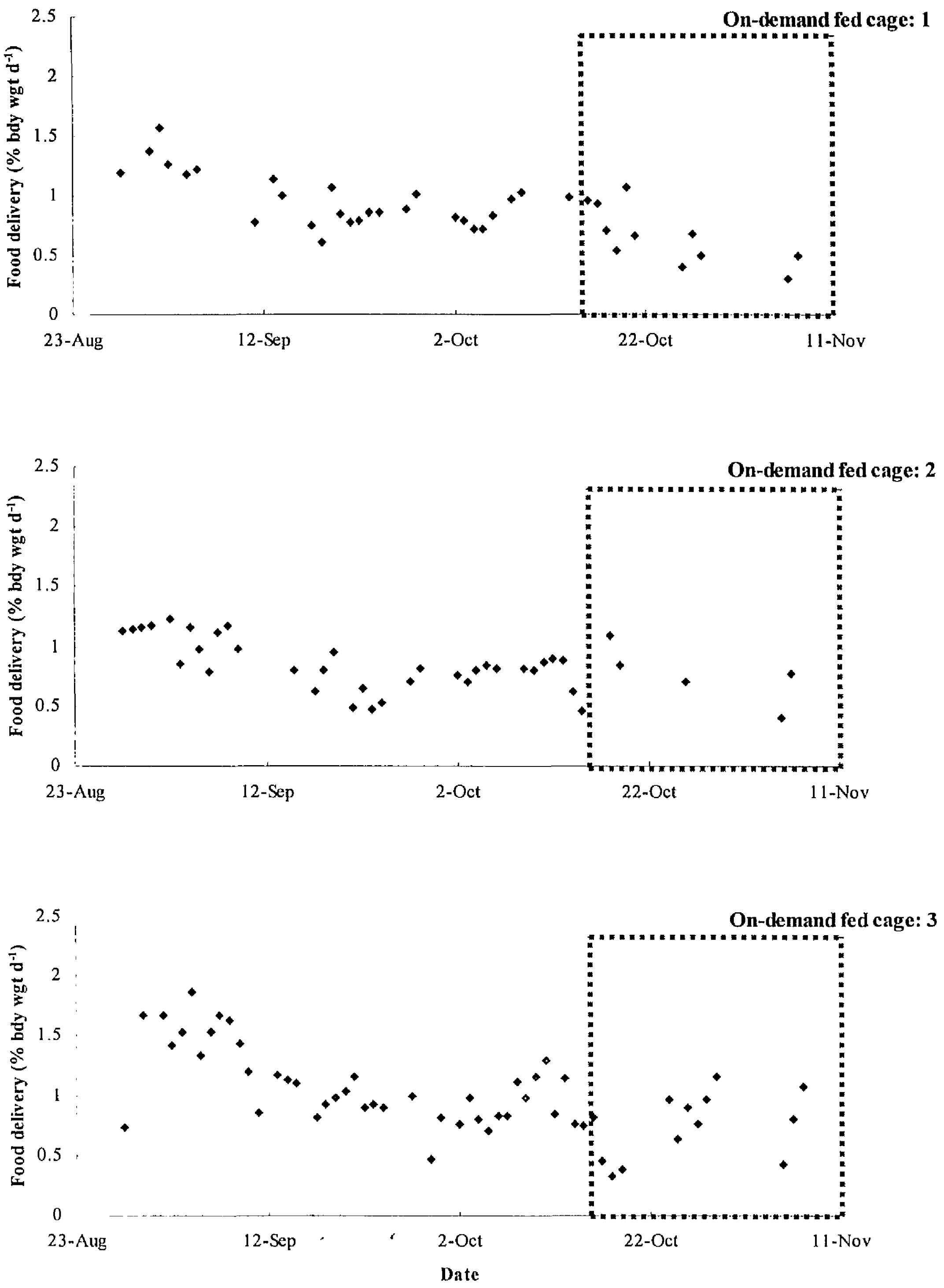


Fig. 5a.5 Daily and seasonal variation in the daily appetite of on-demand fed S1/2 parr (as percentage body weight day^{-1}). Also shown is the period where fish were subject to a 24hr light regime (dashed rectangle). Fish were transferred to sea cages during the second and third weeks of November.

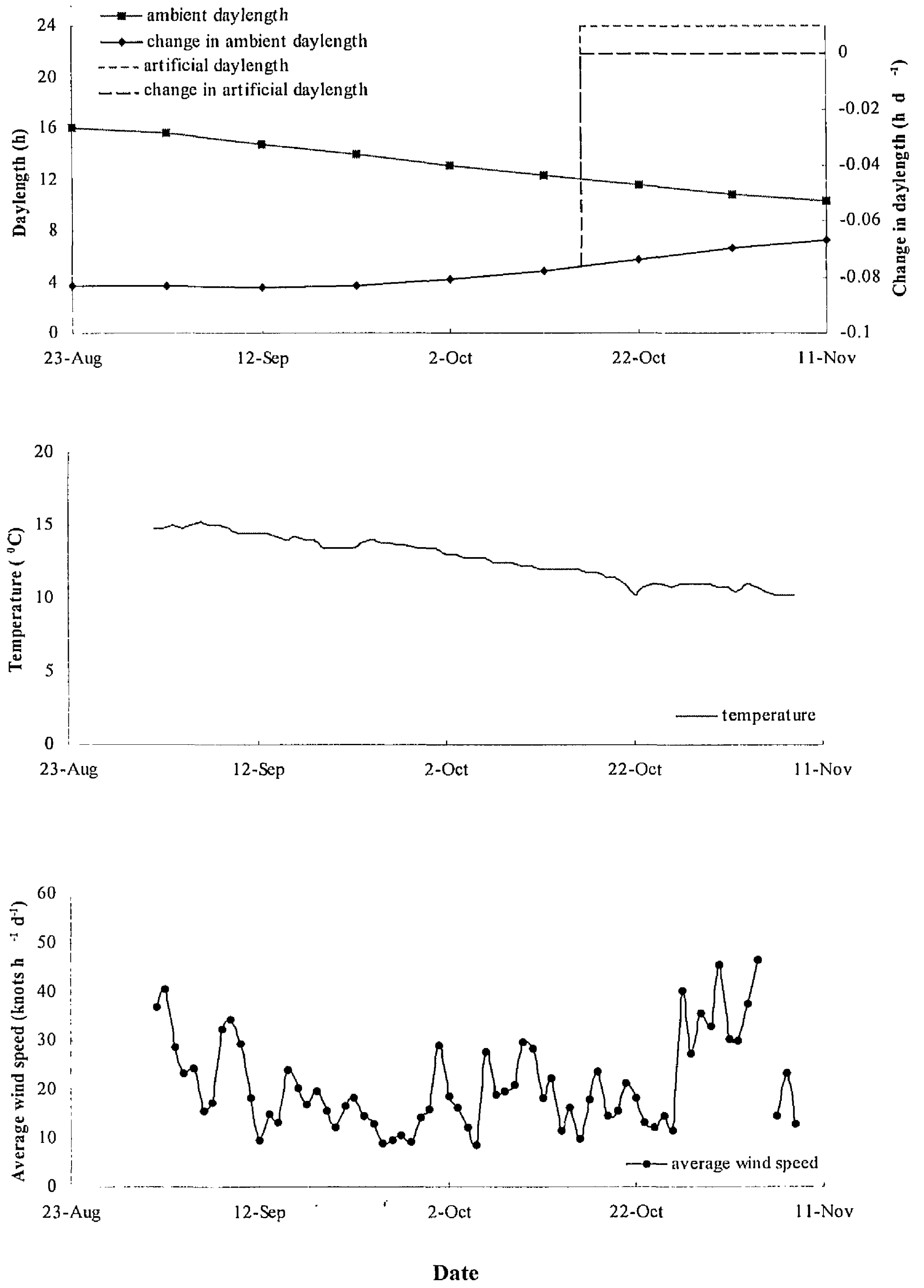


Fig 5a.5 cont. Daily variation in environmental conditions: daylength, change in daylength (including the length of the artificial photoperiod), water temperature and average wind speed day^{-1}.

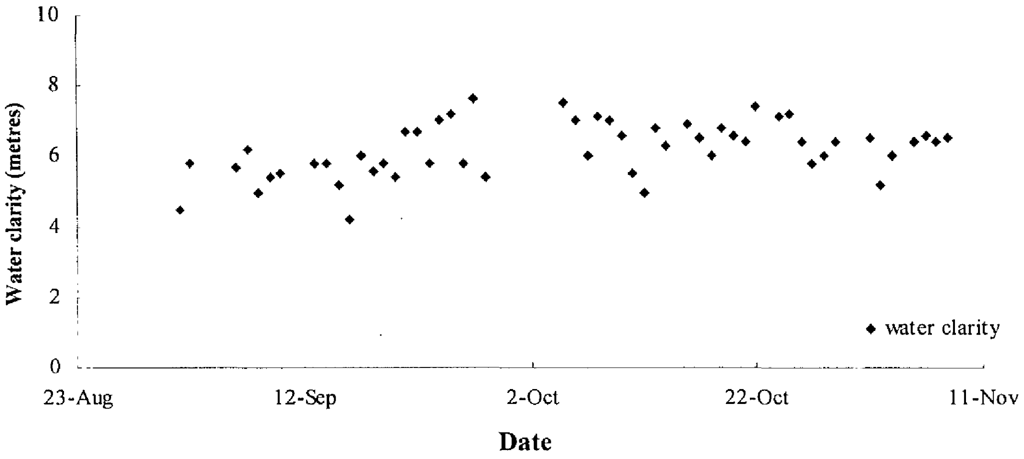


Fig 5a.5 cont. Daily variation in environmental conditions (water clarity measured with a secchi disc)

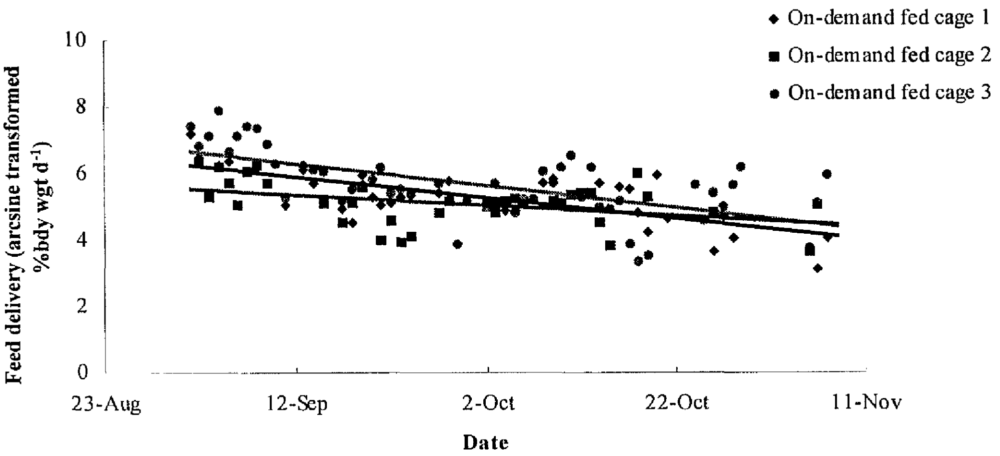


Fig. 5a.6 The differences between the amount of food selected by three groups of on-demand fed S1/2 parr in production cages for the duration of the study. Data points represent daily feed delivery (arcsine square root transformed % bdy wgt day^{-1}). Also shown are the linear regression lines for each cage. Fish were held under an ambient photoperiod until the 16th October when they were subject to a 24hr light regime.

5a.4 Discussion

A primary objective of fish farming is to develop feeding management strategies that can optimise growth performance and feed conversion ratios. An aquaculturist may be able to achieve this by matching feed delivery to times of peak appetite.

5a.4.1 Variability in daily feed intake and environmental factors

Parr fed on-demand show clear differences in daily feed intake with time. Some of this variability may be related to endogenous factors such as differences in stomach fullness (see Grove et al., 1978; Ruohonen et al., 1997) and environmental perturbations (see Smith et al., 1993) or a combination of both. There is a decrease in daily feed intake during autumn until the end of the trial for all cages within the on-demand fed treatment. This decreased feeding has been observed in other studies and has been associated with a reduction in feeding motivation (Metcalfe et al., 1986; Simpson et al., 1996; Amundsen et al., 1999). It was also observed in the S1 feed trial (chapter 4a). Univariate GLM analysis showed natural daylength was the only significant predictor of daily feed consumption for two of the three cages and accounted for 66% and 46% of daily variation respectively; wind speed was the only predictor for the third cage and accounted for only 32% of daily variation in feed intake. This third cage (on-demand cage 2) was fitted with the feeding unit that caused problems earlier in this trial and in chapter 4a. Earlier experiments in this thesis found that natural daylength was a significant predictor of daily feed intake in S1 parr and post-smolts held in cages. A study by Smith et al., (1993) also found daylength and the change in daylength were the only significant predictors of daily appetite in 2-5kg Atlantic salmon in sea cages. Furthermore, daylength, windspeed and rainfall were the only significant predictors of daily food consumption in sea bass, *Dicentrarchus labrax*, held in ponds (Bégout Anras, 1995).

It was initially surprising that artificial daylength was not a significant predictor of daily food delivery. It should also be noted that daily food delivery and appetite did not escalate with an increase in daylength as has been noted by other studies (Komourdjian et al., 1976; Higgins and Talbot, 1985; Villarreal et al., 1988). Daily food consumption was closely related to ambient daylength and even though the fish consumed 30-40% of food at night, it appears they only extended the time required to consume their daily ration, rather than taking the opportunity to consume extra food during the extended photoperiod.

If there were differences in the light intensity between the artificially illuminated nocturnal period and the ambient day, the nocturnal light intensity may not have been sufficient to override existing feed requirements to elicit an increase in appetite. It is highly unlikely that this is the case as the underwater lights are of sufficient intensity to accelerate smoltification. The duration of the artificial lighting regime may have been too short to instigate a change in appetite (see Villarreal et al., 1988; Boujard and Leatherland 1992*a*, who noted that photoperiod does not affect growth over short time periods). In addition, directional changes in photoperiod, where there is a decrease or increase in daylength in relation to that previously experienced (Bromage et al., 1993) may act as a signal in entraining feeding responses. The effects of decreasing daylength during autumn, followed by continuous light under the accelerated smoltification regime may not be sufficient to alter the feeding activity of the fish. A further suggestion is that daily food requirements may be under endogenous circannual control. It has been reported that the diel behaviour of brown trout, *Salmo trutta*, changes with season even when held under a constant 12L: 12D photoperiod (Eriksson, 1978), indicating there may be a seasonal circannual component regulating food requirements. In this study the constant photoperiod again may not override this endogenous component.

5a.4.2 Daily feeding rhythms under ambient and artificial photoperiods

This current study indicates that fish fed on-demand in freshwater production cages do not feed throughout a day i.e. fish do exhibit daily rhythms of feed consumption. Under ambient photoperiods in September, all replicates within the on-demand fed treatment exhibited a significant peak in feed delivery after first light. During the first two weeks of October, under an ambient photoperiod, two of the three groups of fish showed no significant pattern in feed delivery, whereas the third group (on-demand cage 2) again exhibited a morning peak in feeding (see Figures 5a.2 and 5a.3). During the period of photoperiod manipulation from mid-October, two cages did not exhibit any patterns in diurnal feed delivery, with the third replicate exhibiting a peak in activity late in the morning (on-demand cage 1). Although fish consumed 30-40% of daily ration during the artificially illuminated nocturnal period, fish did not display any significant nocturnal peaks in feed activity in any of the replicates. Jørgensen and Jobling (1992) reported that Atlantic salmon parr show no general time preferences for feeding under continuous light conditions in summer, a similar pattern to this trial. They suggested feeding might become arrhythmic when food is continuously available under constant lighting conditions. A further suggestion is that feeding is not under endogenous control and can be affected by sudden changes in external conditions. The endogenous nature of a rhythm is unmasked under constant environmental conditions, such as continuous light. If it persists but deviates slightly from its normal synchronisation then it is a free-running rhythm under endogenous control (Madrid et al., 2001). Rainbow trout (Adron et al., 1973; Sánchez-Vázquez and Tabata, 1998), sea bass (Sánchez-Vázquez et al., 1995*a*) and goldfish, *Carassius auratus* (Sánchez-Vázquez et al., 1996) exhibit free running feeding rhythms under constant light. Atlantic salmon parr retain a feeding rhythm for only a few days (Ali, 1964) or not at all (Richardson and M^{c}Cleave, 1974) under constant lighting conditions. In the present study there was no fixed daily rhythm expressed in two of the three replicates under constant lighting conditions in October and early November. Under ambient conditions in October and November (see Chapter 4a) salmon parr shift their feeding rhythms from the morning to afternoon. The fish in this present study may already be exhibiting a shift in feeding rhythm when subject to 24hr artificial lighting. The results of this current experiment should therefore not be used to suggest that feeding is under endogenous or exogenous control, due to the timing of the photoperiod manipulation.

The fish in this trial exhibited similar feeding patterns to the S1 fish in the previous experiment (chapter 4a) when held under ambient environmental conditions, with an early morning peak in September. Interestingly, these fish were from a different stock and larger than those in the experiment described in Chapter 4a, and were held under higher stocking densities. This may indicate stocking density and broodstock strain do not influence daily patterns of feeding in parr held in freshwater cages. Brännäs and Alanärä (1996) found stocking density did not affect the self-feeding activity of Arctic charr, *Salvelinus alpinus*, whereas in rainbow trout, *Oncorhynchus mykiss*, it was highest at a stocking density of 30 kg m^{-3}, much higher than encountered in this experiment.

As in chapter 4a, there was asynchrony in the diurnal patterns of feeding between replicates. This is not uncommon and has been reported in rainbow trout (Boujard and Leatherland 1992*a*; Alanärä and Brännäs, 1994), Arctic charr (Brännäs and Alanärä, 1993) and sea bass, *Dicentrarchus labrax* (Sánchez-Vázquez et al., 1995*a*,*b*) and may be a group effect or an effect of the feeder. The on-demand feeder that exhibited asynchronous feeding times in the previous trial (chapter 4a) also operated the cage that was least harmonious with the other replicates in the current study (on-demand cage 2). Cage location can be ruled out as a possible cause as cage positions were different to the previous trial; this may mean the sensor associated with this on-demand feeder was less sensitive than those used in the other replicates.

Feeding did occur during the period of artificial nocturnal illumination and the amount of food consumed was 30-40% of the total circadian feed delivery. The temperature during this period was around 11°C; near, but not below, the temperature threshold when parr switch from being diurnal to nocturnal (Fraser et al., 1993). Some fish, but not all, may be switching their foraging behaviour around this time, accounting for the incidence of nocturnal feeding. Intraspecific competition can lead to heterogeneity in feeding patterns within a group and may mean that some fish are choosing to feed at night when given the opportunity. Brännäs and Alanärä (1997) reported that individuals within a group of rainbow trout feed at different times under self-feeding conditions, with the majority of fish exhibiting diurnal feeding patterns and others feeding outwith these times. This may be occurring in on-demand fed freshwater cages when fish are given the opportunity to feed throughout a 24hr period. Another possibility is that fish may wish to feed at night but have problems in successfully obtaining food items, as pellets are not silhouetted against the surface because of the sub-surface lighting. The results of the present trial do not support this, as the fish do feed under artificial lighting conditions. Furthermore, rainbow trout decrease feeding activity at night under conditions of constant dim light (Boujard and Leatherland 1992*a*) and Higgins and Talbot (1985) showed less then 25% of daily food consumption occurred during ambient nocturnal conditions.

5a.4.3 Synchrony in feed intake over longer periods

As stated in chapter 4a, replicate cages may exhibit large variation in daily feed intake under identical environmental conditions; this may be a group effect associated with fluctuating stomach fullness and the time required for appetite to return (Grove et al., 1978; Ruohonen et al., 1997). This could account for some of the daily variability in feed delivery between replicates. If fish are allowed to feed to satiation for prolonged periods of time this variation may disappear as it can be hypothesised that similar groups will select similar rations. In this study only two on-demand fed replicates selected similar rations over the duration of the experiment, with the third replicate selecting a significantly greater ration over time (see Figure 5a.6).

5a.4.4 Conclusions

Cage held groups of Atlantic salmon do not exhibit a propensity to feed at all times of the day when fed on-demand. During September fish in all replicates showed a significant early morning peak in appetite. Under constant lighting conditions (as part of accelerated smolt strategy) the fish generally exhibited no peaks in feeding during the ambient diurnal or artificially illuminated nocturnal period although there was some asynchrony between replicates. Fish also consume around 30-40% of their daily ration during the illuminated nocturnal period. A farmer should be wary about modifying existing feeding techniques to attempt to feed fish at night as they exhibit arrhythmic patterns; if a farmer fails to synchronise feed delivery with appetite at night this could lead to pellet wastage and increased FCR's (Thorpe and Cho, 1995). In any case, Fraser and Metcalfe (1997) found nocturnal feeding, even when preferred, was relatively inefficient. In this experiment natural daylength was the only significant predictor of daily food requirement in two of the replicates. Artificially increasing daylength in autumn did not elicit an increase in daily appetite.

Chapter 5b: The effect of feeding regime on the growth and production of Atlantic salmon parr held in freshwater production cages under an artificial photoperiod regime

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5b.1 Introduction

5b.1.1 Aims and content

The aim of this final experimental chapter was to assess the impact of feeding regime upon growth performance and feeding efficiency when juvenile Atlantic salmon parr were held in production cages under artificial lighting (as part of an accelerated smoltification strategy). It evaluated existing feeding regimes by comparing them with on-demand feeding systems and provided recommendations for improved feeding practice by utilising the data from the on-demand feeders.

5b.1.2 Feeding regimes and feed management

Feed regimes and feeding management govern the size and spatio-temporal delivery of food to fish; together with the frequency, duration and rate of feed supply (Talbot, 1993; Talbot et al., 1999). An aquaculturist must consider each of these options when designing and implementing a feed management strategy, as they can have a profound effect upon the profitability and sustainability of an aquaculture venture.

Ration size and feed intake can vary between meals (Juell et al., 1994; Ang and Petrell, 1997) and days (see Grove et al., 1978; Juell et al., 1993; Blyth et al., 1993, 1999). A farmer may find this variation difficult to control and integrate into feed management using existing feeding techniques based upon feed tables. The optimal ration is primarily governed by exogenous factors such as water temperature and photoperiod (Brett, 1979), but can also be influenced by:

wave action (Bégout Anras, 1995; Juell, 1995); increased wind speed and rainfall (Bégout and Lagardère, 1993; Juell, 1995); dissolved oxygen concentrations (Thetmeyer et al., 1999); increased ammonium concentrations from waste products (Beamish and Tandler, 1990) and turbidity (Berg and Northcote, 1985; Ang and Petrell, 1997). Fish size and recent feeding history may also affect feed intake (Brett, 1979; Talbot et al., 1984; Juell et al., 1994). Erroneous assessments of ration size can lead to over or underfeeding and neither is desirable for effective sustainable aquaculture. Overfeeding increases food wastage (Thorpe and Cho, 1995), which is deleterious upon water quality and fish health (Wedemeyer, 1997) and decreases feeding efficiency (Talbot et al., 1999). Underfeeding reduces the growth potential of cultured fish and increases competition (see M^{c}Carthy et al., 1992) and may also decrease feeding efficiency (Cho, 1992).

The spatio-temporal delivery of food can influence competition (Thorpe and Huntingford, 1992; Grant, 1993; Ryer and Olla, 1995, 1996*b*). Spatially limiting a feed resource can increase competition and lead to increased levels of aggression and food monopolisation by better competitors (Metcalfe et al., 1992; Grant, 1993; Grant and Guha, 1993). This disproportionate food acquisition has been implicated in growth depensation (Symons, 1968; Davis and Olla, 1987; Ryer and Olla, 1995). Temporal clumping of food can reduce aggression by making the feed resource less defensible (Davis and Olla, 1987). Appetite can also vary temporally across a day (Kadri et al., 1991) and synchronising feed delivery with appetite can improve the growth performance and nutrient utilisation of a number of commercially reared fish (for example, Noeske et al., 1981; Noeske-Hallin et al., 1985; Boujard et al., 1995). Feed tables and many existing feeding systems do not account for this daily variation in appetite and may be potentially deleterious to growth performance.

Feed delivery rate can affect the nutrient output of a farm and intraspecific competition. A high feed delivery rate may increase pellet wastage, as fish may not be able to consume the entire ration before it passes through the rearing system (Ang and Petrell, 1998). A low or predictable feed delivery rate can increase competition and the number of aggressive interactions between conspecifics, as the feed resource is easily defensible (Thorpe and Huntingford, 1992; Grant, 1993; Ryer and Olla, 1995). Many salmonids can achieve optimal growth when fed between one and four times a day, depending on fish size (Grayton and Beamish, 1977; Juell et al., 1994). Meal duration may be a less important parameter of feed management as it has been shown to have no effect on growth or FCR in adult Atlantic salmon (Sveier and Lied, 1998). This further supports the hypothesis that feed regime has little effect on growth performance if the fish are fed to daily satiation (Juell et al., 1994; Juell, 1995).

5b.1.3 The impacts of feeding regimes upon farm performance

All the above examples demonstrate that the choice of feeding regime and feed management strategy can affect growth performance, uniformity of growth, growth rate, and the cost of growth. These factors can govern the profitability and both the economic and environmental sustainability of a farming venture. If a farmer can improve upon any of these factors, it is possible to increase feeding efficiency and profits. Efficiently improving growth performance can help a farmer achieve a better return on their current standing investment and culture facilities. Prudent feed management strategies can decrease competition for food within a rearing system and reduce growth depensation, allowing a farmer to produce fish of a more uniform size, in accordance with market demands. Farmers can also realise the full growth potential of fish by delivering enough food to efficiently achieve optimal growth whilst being aware of situations when food can be wasted.

The recent commercial deployment of interactive on-demand feeding systems where fish regulate the timing, frequency and size of feed delivery can give aquaculturists the opportunity to evaluate existing feed management strategies and improve feeding methods.

5b.1.4 Objectives of the experiment

In the following experiment I will examine the effect of feeding regime upon the growth performance of parr held in freshwater cages under artificial lighting in winter (as part of an accelerated smolt strategy). I will also evaluate the effectiveness of current feeding techniques by comparing an existing automatic feeding system (that delivers a fixed ration at regular intervals throughout a day) with an on-demand feeding system, which matches feed delivery to appetite. A farmer can then assess the effects of differing ration size and temporal presentation upon production performance. The questions I will address in this experiment are: -

i) How does the daily ration of an imposed regime compare with that of fish fed on-demand?
ii) Does a feeding regime affect growth performance and growth rate?
iii) Do feeding regimes affect the uniformity of growth within a cage?
iv) Do feeding strategies affect feeding efficiency?

The experiment was designed as a full-scale production trial, under the constraints of in-house husbandry practices to produce data that is directly applicable to freshwater cage aquaculture. By evaluating existing feeding regimes with data produced by novel on-demand feeding systems a farmer can address how feeding strategies may be improved, to implement more economically and environmentally acceptable feeding practices.

5b.2 Materials and methods

5b.2.1 Fish husbandry

Fish husbandry used in this experiment is described in Chapter 5a and Fig. 5a.1 outlines the experimental set-up. At period T_1 farm management made the decision to attempt to restrict the growth of fish fed to the imposed regime, as the farm was approaching its production tonnage limit (D. Cahill, pers. comm.). This meant these fish were subject to a feed table/restricted regime (feeding to tables until the experimental midpoint where fish were then fed a restrictive ration). This regime affected a number of production parameters (see discussion).

5b.2.2 Sampling methods for morphological studies

Each group of fish was sampled on a monthly basis from late summer (23rd August 1999) until one week prior to smolt transfer on the 8th November 1999. The timing of these samples will be referred to as T_0, T_1, and T_2 respectively (after Jørgensen et al., 1996). During sampling, the entire group of fish were crowded into a small area of the cage and netted out at random. The procedure was carried out using the same equipment and person at each sampling point to reduce sampling error. The number of fish removed from the cage was initially 300 fish per cage and rose by 100 fish for each subsequent sample to attempt to improve sampling accuracy (see Chapter 4b). Fish were netted out of the cages and anaesthetised using benzocaine. Excess water was drained off the fish before they were weighed (wet weight to nearest 1.0g) and lengthened (fork length to nearest 0.5cm).

5b.2.3 Statistical analysis

Statistics were performed using either the computer packages Minitab (v. 11-0. Minitab Inc., Philadelphia) or SPSS (v. 10-0. SPSS Inc., Chicago) for MS Windows. Data were natural log transformed to satisfy normality and homogeneity of variance requirements. If the data could not be transformed non-parametric statistics were used. The accuracy of the sampling method was evaluated using 95% confidence intervals for each sample mean. Differences in growth between individual cages between treatments were tested for using a non-parametric Kruskal Wallis test. Within group variability in size was calculated using Coefficient of Variation: CV = (S.D. / Mean wet weight (g)) x 100. Where S.D. is standard deviation. Differences between the pooled Coefficient of Variation data for each treatment were tested for using a Mann Whitney test. Condition factor for individual fish was calculated as: $(W / L.^3)$ x 100, where W is wet weight (g) and L is fork length (cm). Differences in the mean condition factor of individual imposed and on-demand cages were calculated using a non-parametric Kruskal Wallis test (after Bolger and Connolly, 1989). Differences in median daily ration per growth period were calculated for each individual imposed and on-demand cage using a Mann Whitney test. Specific Growth Rate (SGR, a function of % body weight per day) was calculated using [(ln Final weight (g) – ln Initial weight (g))/ Time in days] x 100. Where ln is natural log. Feed Conversion Ratio (FCR) was calculated using the formula: Amount Fed (kg)/ Wet weight gain (kg), for each growth period. Differences in SGR and FCR per growth period for all replicates within each treatment were tested for using a Mann Whitney test. Data are presented as means ± S.E. unless stated in the legend. Growth and conditional factor data, although based upon non-parametric statistics, is presented as means ± S.E. in line with current practice at Marine Harvest Ltd, the industrial CASE partner (allowing farmers to directly compare this data set with existing findings). A significance level of $P < 0.05$ was used for all statistical tests.

5b.3 Results

5b.3.1 Sampling accuracy

In response to problems encountered in chapter 4b with the sampling regime, sample size was increased with every monthly sampling period (where possible n = 300 at T_0, n = 400 at T_1, n = 500 at T_2). To evaluate whether this sample mean was close to the true population mean, 95% confidence intervals were calculated (Zar, 1999) for corresponding imposed and on-demand fed cages for each sampling period (see Fig. 5b.1). Even though the 95% confidence interval was outwith the ± 2 grams of the sample mean for period T_2, there was little overlap between treatment and the previous sampling period; any differences between treatments can therefore be interpreted with a degree of confidence.

5b.3.2 Ration differences per growth period between feeding regimes

The results of Mann Whitney analyses to compare the rations between feeding regimes per growth period are shown in Table 5b.1. There were significant differences in ration between feeding treatments (see Fig. 5b.2). In all cages within each treatment, the fish under the imposed regime were overfed in the growth period up to T_1. During the following period, up to T_2, two of the three cages of fish under the imposed regime were significantly underfed in comparison with their corresponding on-demand cages. There was no significant difference in ration between feeding treatments for the third pair of cages at period T_2. The results of this chapter suggest the existing technique, using cessation in surface feeding activity to determine group satiation, is inaccurate in freshwater production cage culture. The period T_0 - T_1, where fish under the imposed regime were overfed, suggests that this was a period of inefficient feeding as there was generally no difference in growth between feeding treatments. During the final period of the experiment, where on-demand fed fish exhibited significantly better growth, differences in ration for two of the three replicates can account for the disparity, as fish under the imposed regime were underfed.

5b.3.3 Effect of feeding regime on growth

Generally, the choice of feeding regime did not have a significant effect upon growth (mean wet weight) until period T_2, where there were differences between corresponding imposed and on-demand cages (see Table 5b.1 and Fig. 5b.3). Statistical analysis was carried out on individual corresponding imposed and on-demand cages, although the data between replicates was pooled for graphical representation to aid clarity (Fig. 5b.3). The first pair of treatment cages did not have any significant differences in growth until period T_2, where fish fed on-demand grew better than those under the imposed regime (see Table 5b.1 for results of Kruskal-Wallis analyses). For the second pair of treatment cages, the fish fed on-demand had significantly better growth at period T_0, but there were no significant differences in growth at period T_1. At period T_2, on-demand fed fish again grew significantly better than those fed to an imposed regime. There was no significant difference in growth between the fish held under each feeding regime at the start of the trial, for the third pair of treatment cages. By the latter two sampling periods T_1 and T_2, the fish fed on-demand exhibited significantly better growth.

5b.3.4 Effect of feeding regime on growth heterogeneity

There were no significant differences in growth heterogeneity between feeding treatments for the duration of the experiment (see Table 5b.2 for results of statistical analyses). There was a trend for lower size heterogeneity for periods T_1 and T_2 in the on-demand groups, although this difference was not significant. Growth heterogeneity increased as the experiment progressed in both feeding treatments (Fig. 5b.4).

5b.3.5 Effect of feeding regime on mean condition factor between treatments

Feeding regime did have a significant effect upon condition factor between treatments but this differed with both the growth period and treatment replicate. Statistical analysis was carried out on individual corresponding imposed and on-demand cages (see Table 5b.1), but the data between replicates was pooled for graphical representation, to aid clarity (Fig. 5b.5). Condition factor remained relatively homogenous for the duration of the trial for both treatments.

For the first pair of treatment cages there was no significant difference in condition factor until period T_2, where fish fed on-demand had a significantly better condition factor. Fish under the imposed regime exhibited significantly better condition factor for the periods T_0 and T_1, in the second pair of treatment cages. In the final period of the experiment, on-demand fed fish had a better condition factor. There were no significant differences in condition factor between treatments, for periods T_0 and T_1, in the third pair of treatment cages, although by the final growth period, T_2, on-demand fed fish had a significantly better condition factor.

5b.3.6 Effect of feeding regime upon FCR and SGR

Although there were no significant differences between treatments for FCR there was a general trend that fish under the on-demand treatment fed more efficiently (see Table 5b.2 and Fig. 5b.6). A low number of replicates because of production constraints decreased the power of the experimental design (Jobling, 1995; Gardeur, 2001; Hoenig and Heisey, 2001) and may not have provided the means to demonstrate significant treatment effects (Searcy-Bernal, 1994).

There were no significant differences in SGR between treatments for the duration of the experiment (see Table 5b.2) although fish fed on-demand demonstrated better SGR for period T_1 -

T_2 (see Fig. 5b.7). This may have been due to the imposed regime underfeeding the fish at this point.

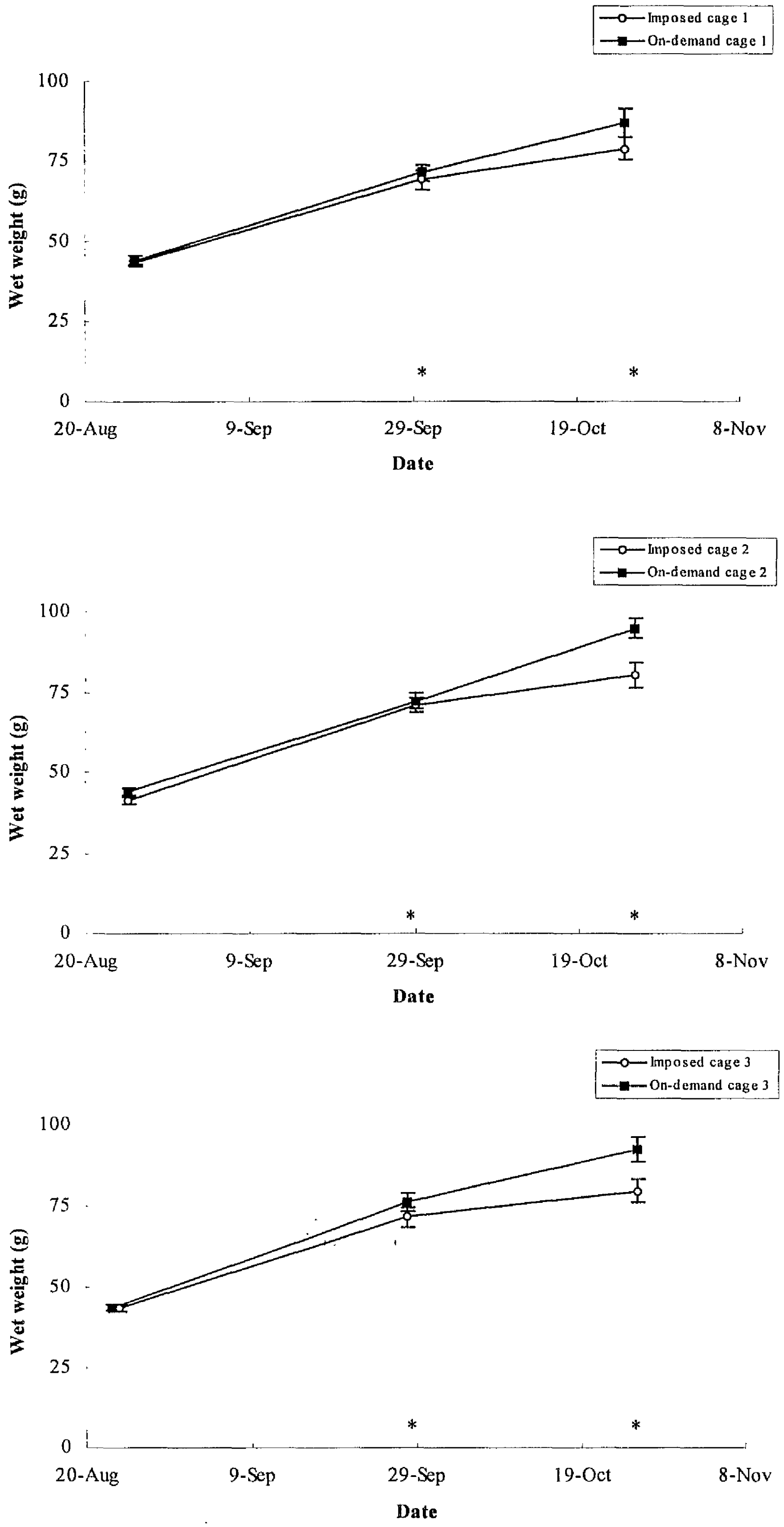
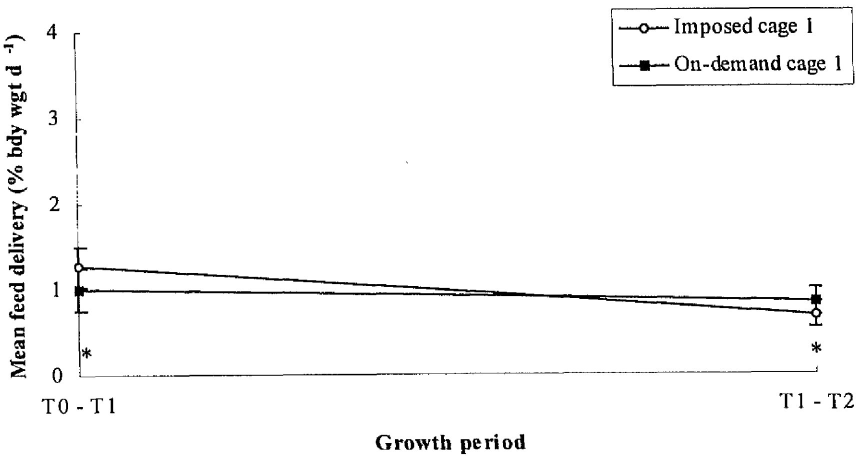
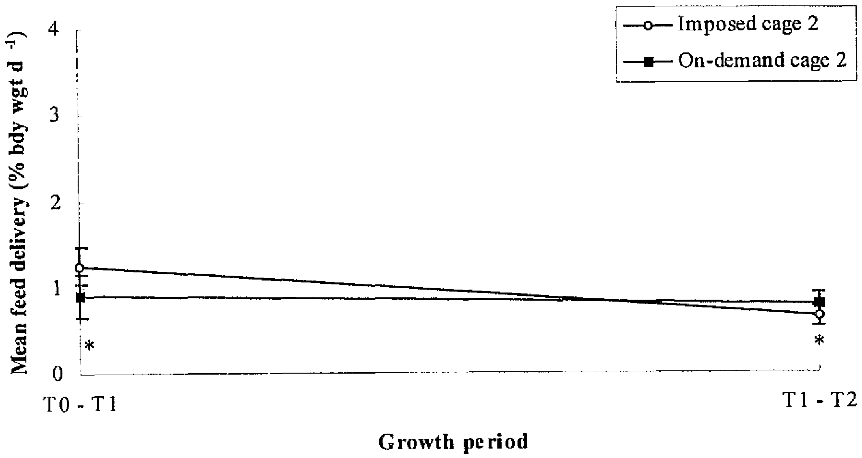


Fig. 5b.1 The effect of feeding regime upon the growth of S1/2 parr in production cages. Growth is expressed as mean wet weight (grams) and error bars indicate 95% confidence intervals. * indicates samples with confidence intervals outwith ± 2 grams of sample mean.





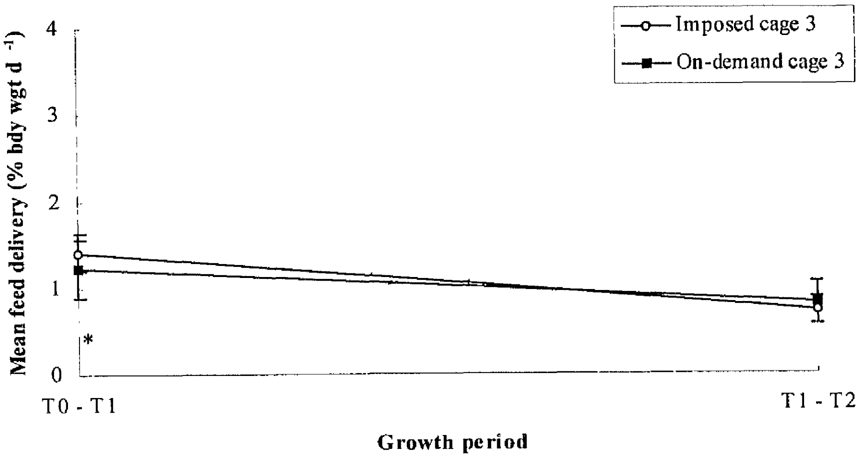


Fig. 5b.2 The differences in feed delivery between the on-demand and imposed feeding regimes. Data points represent mean daily fed delivery per growth period and error bars indicate standard deviation. * indicates significant differences at $P < 0.05$.

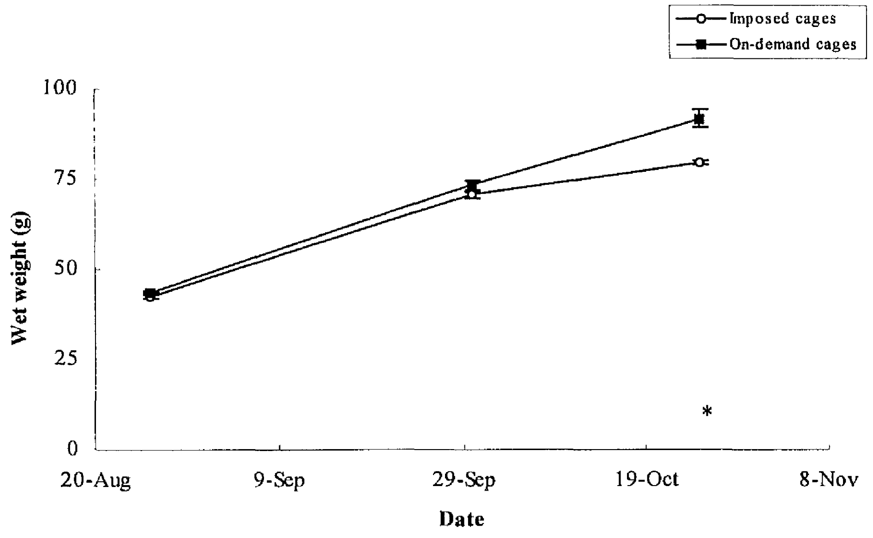


Fig. 5b.3 The effect of feeding regime upon the growth of S1/2 parr in production cages. Growth is expressed as mean wet weight (grams) and error bars indicate standard error of the mean. Data from replicates within treatments are combined to aid clarity. * indicates significant differences between treatments (for each replicate) at $P < 0.05$.

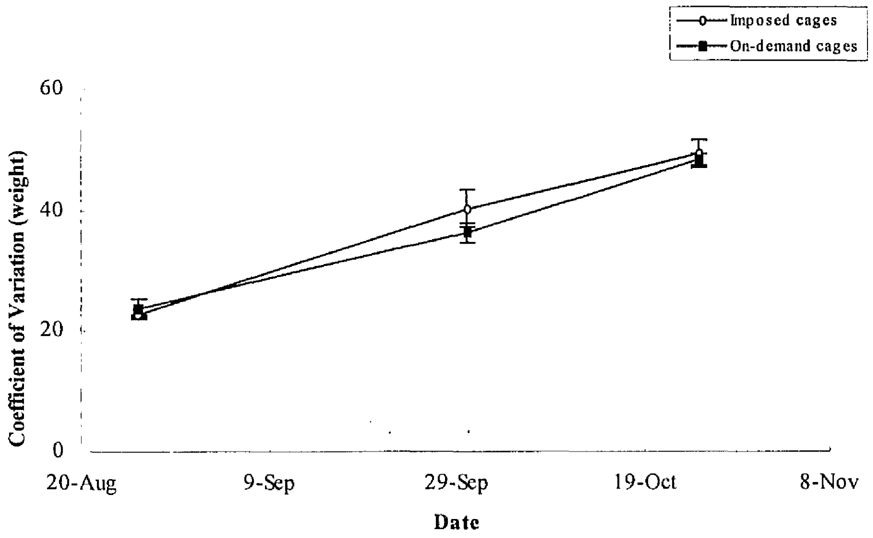


Fig. 5b.4 The effect of feeding regime upon the size variation of S1/2 parr in production cages. Size variation is expressed as coefficient of variation (wet weight) and error bars indicate standard error of the mean. Data from replicates within treatments are combined to aid clarity.

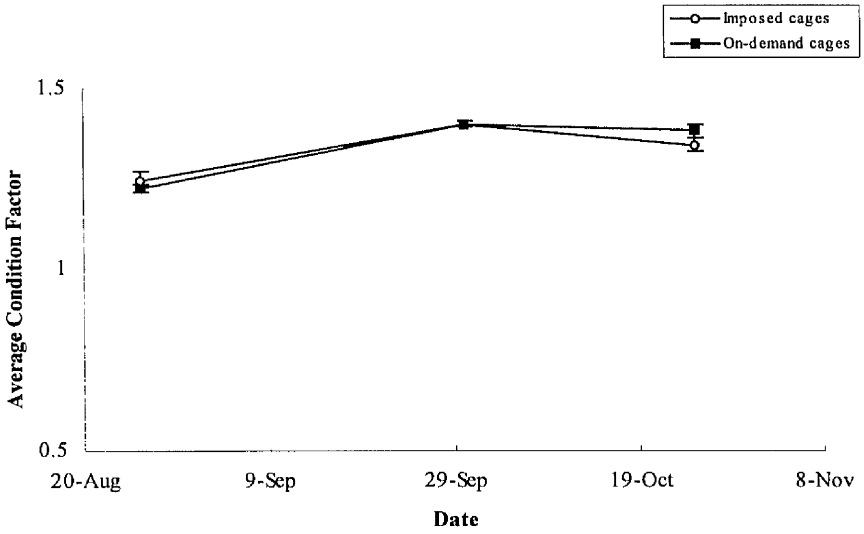


Fig. 5b.5 The effect of feeding regime upon the condition factor of parr in production cages. Data from replicates within treatments are combined to aid clarity and error bars indicate standard error.

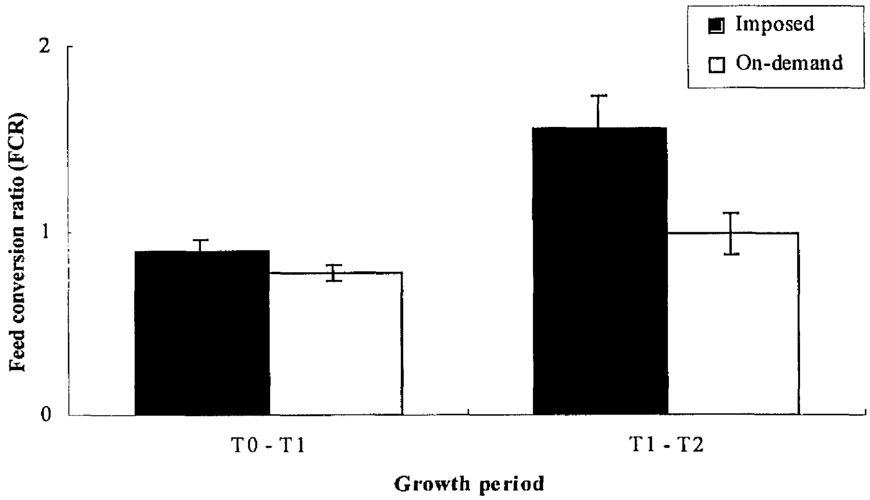


Fig. 5b.6 The effect of feeding regime upon the feed conversion ratio (FCR) of S1/2 parr held in production cages. Data points represent mean FCR (all replicates combined within each treatment) for each growth period. Error bars indicate standard error of the mean.

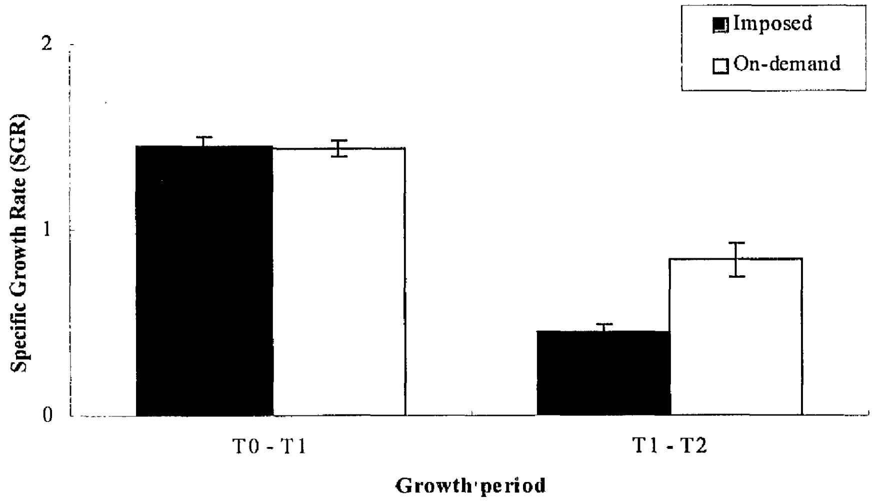


Fig. 5b.7 The effect of feeding regime upon the Specific Growth Rate (SGR) of S1/2 parr held in production cages. Data points represent mean SGR (all replicates combined within each treatment) for each growth period. Error bars indicate standard error of the mean.

Table 5b.1 Results of Kruskal-Wallis analyses for comparing growth and condition factor between the corresponding individual treatment cages. Also shown are the results of Mann Whitney tests to compare mean daily feed delivery between treatments per growth period. All significant results atT_1 denote periods where fish in the on-demand treatment selected a significantly smaller daily ration than those fed to an imposed regime, whereas significant results at period T_2 denote when the imposed regime underfed fish in comparison to those fed on-demand.

Cage treatment	Growth period	Significant differences between replicates in:		
		Mean weight	**Condition factor**	**Ration**
On-demand and Imposed cages: 1	T_0	No ($H_{adj} = 0.80$, d.f. = 1, $P = 0.371$)	No ($H_{adj} = 0.01$, d.f. = 1, $P = 0.926$)	
	T_1	No ($H_{adj} = 3.05$, d.f. = 1, $P = 0.081$)	No ($H_{adj} = 0.78$, d.f. = 1, $P = 0.376$)	Yes ($W = 1099$ $P = 0.001$)
	T_2	Yes ($H_{adj} = 11.18$, d.f. = 1, $P = 0.001$ - on demand bigger)	Yes ($H_{adj} = 7.58$, d.f. = 1, $P = 0.006$ - on-demand better)	Yes ($W = 430$ $P = 0.004$)
On-demand and Imposed cages: 2	T_0	Yes ($H_{adj} = 10.19$, d.f. = 1, $P = 0.001$ - on-demand bigger)	Yes ($H_{adj} = 17.27$, d.f. = 1, $P < 0.001$ - imposed better)	
	T_1	No ($H_{adj} = 5.35$, d.f. = 1, $P = 0.388$)	Yes ($H_{adj} = 5.70$, d.f. = 1, $P = 0.017$ - imposed better)	Yes ($W = 1223$ $P < 0.001$
	T_2	Yes ($H_{adj} = 34.33$, d.f. = 1, $P < 0.001$ - on-demand bigger)	Yes ($H_{adj} = 25.91$, d.f. = 1, $P < 0.001$ - on-demand better)	Yes ($W = 485$ $P < 0.001$)
On-demand and Imposed cages: 3	T_0	No ($H_{adj} = 0.09$, d.f. = 1, $P = 0.761$)	No ($H_{adj} = 3.24$, d.f. = 1, $P = 0.072$)	
	T_1	Yes ($H_{adj} = 10.64$, d.f. = 1, $P = 0.001$ - on-demand bigger)	No ($H_{adj} = 2.65$, d.f. = 1, $P = 0.103$)	Yes ($W = 1173$ $P < 0.019$)
	T_2	Yes ($H_{adj} = 36.04$, d.f. = 1, $P < 0.001$ - on-demand bigger)	Yes ($H_{adj} = 19.09$, d.f. = 1, $P < 0.001$ - on-demand better)	No ($W = 663$ $P = 0.053$)

Table 5b.2 Results of Mann Whitney analyses for comparing growth uniformity, FCR and SGR between treatments per growth period. N = 3 in all cases. Each data point represents data from the previous growth period.

Cage treatment	**Growth period**	**Significant differences between replicates in:**		
		Growth uniformity	**FCR**	**SGR**
All On-demand and Imposed cages	T_0	No ($W = 10.0$ $P = 1.000$)		
	T_1	No ($W = 13.0$ $P = 0.383$)	No ($W = 14.0$ $P = 0.184$)	No ($W = 11.0$ $P = 1.000$)
	T_2	No ($W = 11.0$ $P = 1.000$)	No ($W = 15.0$ $P = 0.081$)	No ($W = 6.0$ $P = 0.081$)

5b.4 Discussion

5b.4.1 Differences in ration between feeding regimes

An aim that has been stated in earlier chapters (and which has been a primary objective of this thesis) is to evaluate the accuracy of existing feeding techniques for determining satiation and daily feed requirements. This can be attained using on-demand interactive feeding systems. A farmer can adapt existing feed practices to account for the fish's requirements, rather than impose a regime that is best suited for farm management. The results of this experiment suggest the existing feeding regime, based upon a combination of in-house feed tables and hand feeding, does not accurately match daily feed requirements of S1/2 parr during late autumn and early winter, when compared with an on-demand feeding regime (see Fig. 5b.2 and Table 5b.1). Other studies have demonstrated that monitoring surface feeding activity as an indicator of satiation can be inaccurate in Atlantic salmon cage culture (Ang and Petrell, 1997; Talbot et al., 1999), so the observed disagreement between feeding treatments is not surprising. The imposed regime fed significantly more food at the start of the trial during late summer, early autumn (period $T_0 - T_1$). These results are similar to those of Chapter 4b, where S1 parr were also overfed at this time. A farm management decision was made at this point to attempt to restrict the growth of fish fed to the imposed regime, as the farm was approaching its production tonnage limit (D. Cahill, pers. comm.). This meant two of the three imposed cages received significantly less food in comparison to on-demand fed fish in the month prior to smolt transfer (period $T_1 - T_2$). This underfeeding significantly affected a number of production parameters, which will be discussed later.

5b.4.2 How does feeding regime affect growth?

The amount of food delivered, and the way in which this ration is offered to fish can affect growth performance and changes in the gross nutritional balance of fish. Many studies have demonstrated that if the timing of feed delivery is preferentially matched to appetite, it is possible to improve the growth performance of many cultured species (see, Noeske et al., 1981; Sundaraj et al., 1982; Noeske and Spieler, 1984; Kerdchuen and Legendre, 1991; Boujard et al., 1995; Gelineau et al., 1996; Chen and Purser, 2001). This hypothesis is attractive for an aquaculturist, and feeding practices should attempt to integrate diel variations in appetite into feed management (Spieler, 1977, 1990).

This present experiment indicates that the choice of feeding treatment does have a significant effect upon weight gain, with fish fed on-demand exhibiting better growth performance than those fed a combination of hand-feeding and predetermined ration. The timing of this effect differed between treatments, but by period T_2, all on-demand replicates exhibited significantly better growth than their corresponding controls (see Fig. 5b.3and Table 5b.1). This is not surprising, as the fish fed to the imposed regime were underfed during this period. The first pair of treatment cages did not exhibit significant differences in growth until periodT_2. At the start of the trial, the fish fed on-demand were significantly larger than their controls in the second pair of treatment cages. This was not the case at period T_1, where there were no significant differences in weight between treatments, but by period T_2 on-demand fed fish were significantly heavier. For the third pair of treatment cages, significant differences in growth were apparent at periods T_1 and T_2. The sampling method was adapted in this trial, to attempt to circumvent some of the problems encountered in Chapter 4b (where sample size was too small to account for the size variation within the sample). Although there were large 95% confidence intervals around each sample mean, there was little overlap between treatments orthe previous sampling point (see Fig. 5b.1) and the results can therefore be interpreted with some confidence.

For the period $T_0 - T_1$ the results of this trial suggest on-demand feeding strategies can match the growth performance of the existing fixed ration feeding regime. After this point it appears that the farm management decision to underfeed the fish was detrimental to growth. This negative effect of food restriction upon growth has been reported in other studies (Quinton and Blake, 1990; Thorpe and Huntingford, 1992; Jobling 1994, 1995; Sæther and Jobling, 1999; Gaylord et al., 2001;). This underfeeding should not be interpreted as indicative of the usual feeding practice of the farm, as it was enforced by biomass tonnage constraints. However, it has produced some interesting results.

5b.4.3 Effect of feeding regime upon growth depensation and condition factor

Growth depensation can be influenced by monopolisation of food by stronger competitors (Thorpe et al., 1990; Jobling, 1995). This food monopolisation and growth heterogeneity can be influenced by two primary variables in an aquacultural environment, access to food and group size (Li and Brocksen, 1977; Jobling and Baardvik, 1994). Growth heterogeneity increased in both feeding treatments as the trial progressed (Fig 5b.4), and there were no significant differences between treatments for the duration of the trial (see Table 5b.2). This suggests that there was no difference between access to food between treatments and that underfeeding between periods T_1 and T_2 did not affect growth depensation. These results have also been reported in other studies (Boujard et al., 1996; Jobling et al., 1999) and are in accordance with Chapter 4b on S1 parr, and may be associated with group size (Li and Brocksen, 1977). This suggests behavioural interactions do not govern feed intake in fish in a freshwater cage culture environment; high intraspecific variation in growth rate and metabolic rate (Cutts et al., 1998) may play greater role in regulating growth heterogeneity in freshwater cages. Many other studies have demonstrated that restrictive rations increase growth heterogeneity (M^{c}Carthy et al., 1992; Brännäs and Alanärä, 1994; Jobling and Koskela, 1996; Fontaine et al., 1997; Johansen and Jobling, 1998; Shelverton and Carter, 1998; Sæther and Jobling, 1999), whereas feed regime did not have an effect on the size heterogeneity of small groups (n = 40) of greenback flounder, *Rhombosolea tapirina* (Chen and Purser, 2001).

Condition factor is a non-intrusive indicator of gross nutritional balance (De Silva, 1985) inferring heavier fish are in better condition (Bolger and Connolly, 1989). By the end of the trial, after the underfeeding period in the imposed regime, on-demand fed fish displayed a significantly better condition factor (see Table 5b.1 and Fig. 5b.5). This shows that a feed regime can significantly affect nutritional balance if fish are underfed and supports the findings of Ng et al, (2000), who reported that underfeeding tropical bagrid catfish, *Mystus nemurus*, can significantly decrease condition factor.

5b.4.4 Effect of feeding regime upon FCR and SGR

Atlantic salmon no longer command an attractive market price and in an increasingly competitive market, farmers can improve production efficiency by implementing better feed management strategies. Overfeeding fish is detrimental to the economic sustainability of an aquaculture venture, as it increases pellet wastage (Thorpe and Cho, 1995) and can be potentially detrimental to the environment. Fish in the current trial were overfed in late summer, but underfed in early winter, when fed to the imposed regime. As a consequence, feed conversion ratios (FCR's) were generally lower in fish fed on-demand (see Fig. 5b.6) although this difference was not significant (see Table 5b.2). This is because both overfeeding and underfeeding can increase FCR (Cho, 1992; Thorpe and Cho, 1995). Underfeeding can increase FCR in rainbow trout, *Oncorhynchus mykiss* (Kindschi, 1988) and in the Channel catfish, *Ictalurus punctatus* (Gaylord et al., 2001). The improved feeding efficiency of the on-demand strategy may be due to the system reducing pellet wastage by synchronising feed delivery to appetite, whilst terminating it when the fish are no longer hungry (also see Azzaydi et al., 1999).

Growth rate decreased in both feeding treatments as winter approached (Fig. 5b.7). This is in accordance with other studies on salmon parr (Higgins and Talbot, 1985; Jørgensen and Jobling, 1992) and has been associated with a decreased feeding motivation as winter approaches (see

Simpson et al., 1996). The fish fed on-demand did appear to have better SGR than those fed to the imposed regime at period T_2, although this difference was not significant. This can be attributed to underfeeding (Weatherley and Gill, 1987; Jobling, 1994, 1995; Gelineau et al., 1998; Nakagawa et al., 1999) and a farmer is failing to realise the full growth potential of the fish by imposing this tonnage constraint. Furthermore, automated fixed ration feeding regimes in tanks can also reduce the growth rate of sea bass, *Dicentrarchus labrax*, in comparison to fish fed on-demand (Paspatis et al., 1999).

5b.4.5 Conclusions

Fish under the imposed feeding regime were overfed at the start of the experiment, but underfed at the end. On-demand fed fish exhibited significantly better growth performance than fish under existing feeding regimes and farm management constraints at the end of the trial. This difference was caused by intentionally underfeeding the imposed fed fish in response to a management directive to restrict production tonnage. The results should therefore not be used to evaluate normal farm feeding practices, although they did reveal some interesting insights into the effects of underfeeding in a freshwater cage environment. Underfeeding did not have any significant effect upon the growth heterogeneity of fish between treatments. It did increase FCR and decrease SGR, although this difference was not significant. It also decreased the gross nutritional balance of fish in comparison with those fed on-demand.

Chapter 6: General Discussion

6.1 Synopsis of aims and questions

The overall aim of this project was to identify appetite rhythms of Atlantic salmon in production cages using recently developed on-demand feeding systems. These systems present farmers and researchers with an opportunity to compare the effect of an on-demand feeding regime upon the growth performance and social interactions of fish within rearing facilities, against existing feeding techniques. With these objectives, experiments were designed employing a three-fold approach:

i) To identify appetite rhythms in groups of fish held in both freshwater and seawater cages.

ii) To evaluate the effect of feeding regimes that match feed delivery to appetite upon growth performance, growth heterogeneity and feeding efficiency.

iii) To obtain information on how farmed salmon respond to and compete for food when expressing natural appetite rhythms.

Aquaculture feed management needs information on these objectives in order to develop and improve existing husbandry systems. This will help a farmer boost efficiency and productivity, whilst minimising intraspecific competition and adverse environmental effects. It will also help culturists produce fish of more uniform size and quality. In this chapter I will review the main results and conclusions of the previous chapters, outlining their implications for aquaculture.

6.2 Investigating feeding rhythms in cage culture

With recent advances in feeding system development, farmers can improve the efficiency of feeding management strategies. It is difficult to accurately match feed ration to appetite, as fish do not show a propensity to feed at all times during a day. In fact, many commercially important fish species show marked variations in appetite over the course of a day, and this can vary with season (Landless, 1976; Boujard and Leatherland 1992*b*; Juell et al., 1993; Blyth et al, 1993, 1999). This can lead to underfeeding, which stimulates competition (Symons, 1968; Thorpe and Huntingford, 1992; M^{c}Carthy et al., 1992), or overfeeding, which increases food wastage, costs and environmental impacts (Thorpe and Cho, 1995). Interactive feedback systems, where fish can passively govern the timing and duration of feed delivery via their appetite requirements, can counteract some of these problems by integrating daily feeding variability into husbandry strategies. Furthermore, delivering feed preferentially across a day in relation to changes in appetite may improve the efficiency of feed management strategies (Spieler, 1977, 1990; Azzaydi et al., 1999). A greater understanding of appetite rhythms can allow a farmer to adapt existing feeding systems to deliver food at times of peak appetite. A number of endogenous, behavioural and environmental variables can affect feeding rhythms and daily feeding requirements; these will be reviewed in the context of cage culture, with an intention to provide information on which factors can best predict daily feed intake. In all three trials the fish did exhibit daily rhythms of feeding and appetite when fed on-demand, and did not show a propensity to feed at all times during a day. Clear and distinct meal periods have also been observed in other salmonids (Boujard and Leatherland 1992*a*) ,and have been attributed to the presence of a well-defined stomach where food is stored and digested. In addition to investigating whether fish exhibit feeding rhythms in cages I examined two subsidiary objectives:

i) Do fish fed on-demand show any variations in daily ration and how do environmental factors influence this variability?

ii) Are feeding patterns affected by competition? Kadri et al. (1997*a*) reported that in autumn, small groups of Atlantic salmon parr refrain from feeding at first light because of aggression.

6.2.1 Feeding rhythms in freshwater cages under ambient environmental conditions

Atlantic salmon parr exhibit flexible feeding patterns in freshwater cages, which change as the season's progress. Although there was asynchrony between some replicates (see chapters 4a and 5a for a full review) general trends did emerge. In late summer and early autumn S1 fish exhibited a significant morning peak in appetite. This peak was not retained during November and shifted to around midday in December. In January and February this peak occurred later in the afternoon, and in March the fish extended their feeding period, whilst maintaining a mid-afternoon peak in appetite.

Daily feeding intensity and ration requirement varied with season. There was a decrease in daily feed intake during autumn and an increase in feed intake with the onset of spring. This is similar to other findings in Atlantic salmon parr (Komourdjian et al., 1976; Higgins and Talbot, 1985; Villarreal et al., 1988; Stephansson et al., 1990; Jørgensen and Jobling, 1992). The only environmental variable that significantly predicted daily feed intake in all three replicates was daylength and this accounted for approximately 70% of the daily variability. This is in support of other findings in salmon held in cages (Smith et al., 1993) and is not surprising, as daylength is suggested to be a primary entraining agent in regulating feeding rhythms and feed requirements (Eriksson and Alanärä, 1992; Boujard and Leatherland, 1992*a*,*b*). There was no significant cage effect upon daily feed requirements over prolonged periods; if there is disparity in the amount of food delivered between replicates on any given day, this is absorbed over longer time intervals. Further, fish fed on-demand commenced feeding immediately after first light during autumn.

6.2.2 Feeding rhythms in freshwater cages under artificial photoperiod regimes

Photoperiod manipulation during winter allows a farmer to produce out-of-season smolts by artificially increasing daylength after a short winter period (Clarke, 1989; Duston and Saunders, 1990; Berge et al., 1995; Duncan et al., 1998). As a subsidiary, increasing daylength can act as a trigger for increasing appetite and feed intake (Higgins and Talbot, 1985) and the growth potential of fish may be improved if culturists utilise this extended daylength and make food available to fish throughout the illuminated nocturnal period.

Fish consume about 30-40% of their total daily ration during the illuminated nocturnal period in late October and early November. The temperature around this time was 11°C, which was near, but not below, the threshold where fish switch to nocturnal feeding preferences (Fraser et al., 1993, 1995). The extended 24hr light regime does not appear to trigger an increase in daily feeding requirements; fish only extend their existing daily ration throughout the lengthened photoperiod. During the ambient light regime prior to photoperiod manipulation in September, the fish exhibited a significant morning peak in feeding activity. This was a similar pattern to that exhibited by parr under ambient environmental conditions (chapter 4a), even though these fish were held under higher stocking densities and were bred from a different strain. This may indicate that stocking density and fish strain does not affect feeding patterns of fish in freshwater cages. Although there was asynchrony between replicates, the general trend was that fish did not exhibit any pattern of feed intake during the time of photoperiod manipulation. Jørgensen and Jobling (1992), reported that parr do not exhibit a preferential feeding time under constant lighting conditions and suggested feeding may become arrhythmic. Other authors have found a rhythm is only retained for a few days (Ali, 1964) or not at all (Richardson and M^{c}Cleave, 1974). The endogenous nature of a rhythm is revealed under constant environmental conditions, such as continuous light. If it persists but deviates slightly from its normal synchronisation then it is a free-running rhythm under endogenous control (Madrid et al., 2001). As the time of photoperiod manipulation coincided with a period when the fish shift their feeding rhythms under ambient conditions (chapter 4a), I do not wish to suggest that this arrhythmic feeding pattern could be indicative of an exogenous factor controlling feeding.

Daily feed requirements decreased in autumn and were not affected by the artificial change in daylength. This autumnal decrease in feed requirements has been noted in other studies and is associated with a decreased feeding motivation (Metcalfe et al., 1986; Simpson et al., 1996; Amundsen et al., 1999). Univariate GLM analysis showed ambient daylength was the only significant predictor of daily feed intake in two replicates. These analyses accounted for 66% and 46% of daily feeding variability respectively. Wind speed was the only significant predictor of daily ration in the third replicate, although the model only accounted for 32% of daily variability. This cage was controlled by an on-demand feeder that had been problematic in this trial and an earlier one (chapter 4a), which may have contributed to this surprising and somewhat erroneous result.

To my knowledge, there have been no other studies on the feeding rhythms of parr in production cage culture, under both ambient and manipulated lighting regimes. These studies have produced data that are directly transferable to culture conditions and can act as a basis for developing and improving existing feeding regimes, by giving an accurate representation of daily feed requirements and peaks in appetite.

6.2.3 The feeding rhythms of post-smolts in sea cages

This small scale production study (chapter 3a) demonstrated that cage held fish exhibited an early morning peak in feeding activity for two months after smolt transfer (in relation to later periods of the day), and this peak was significant. This significant peak in feeding after first light was not retained during June. These results are in accordance with many other studies, which have reported a significant morning peak in feeding when adult salmon are held in cages (Kadri et al., 1991; Blyth et al., 1993, 1997, 1999; Juell et al., 1994). Univariate GLM analysis showed salinity and daylength were the best predictors of daily feed intake, although the model only accounted for around 20% of the daily variability. This indicates that factors outwith those measured in the trial have a greater effect upon daily feed requirements. Interestingly, the daily on-demand ration selected by the fish was considerably less than feed table recommendations for post-smolts.

6.3 Implications for aquaculture

6.3.1 Effect of feeding regime upon growth and production

Ration size, food dispersal and presentation ultimately regulate satiation levels, which firstly affects growth performance and secondly may affect competition amongst conspecifics and stress levels. A large portion of this thesis has been devoted to evaluating how an on-demand feeding regime can affect the growth performance of fish when compared with existing feeding systems. It is not considered appropriate to use the results of this thesis to support or reject the hypothesis that feeding fish to preferred appetite rhythms improves growth (Spieler, 1977, 1990; Azzaydi et al., 1999), as the ration size between feeding regimes was not matched. Future work should include a comparative study of the effect of meal timing on the growth of salmon in cage culture. In each of the trials the spatial component of food delivery was matched between treatments; this allowed me to compare the effect of a combination of the following factors: -

i) Variability in ration between an on-demand regime, where fish select their feed requirements and a pre-determined in house feeding regime.

ii) Effect of temporal variability in feed delivery in relation to appetite.

Using this approach a farmer can evaluate the accuracy of existing feed tables in determining daily ration whilst comparing fixed ration feeding systems against those which feed on-demand.

6.3.2 The effect of feeding regime upon the growth and production of parr

In late summer, there were marked differences in the amount of food delivered to the freshwater cages when fish selected their ration using on-demand feeders, compared with a regime based upon predetermined fixed rations (see chapter 4b and 5b). This shows that current in-house feeding tables do not accurately match ration to the daily feed requirements of fish in cages and overfeed fish at this time. Although there was marked differences in ration, there was little difference in growth between feed regimes (with the exception of when S1/2 fish were underfed in the imposed feeding regime; a farm directive due to production tonnage constraints). This suggests that fish fed on-demand achieved similar growth performance as fish fed to existing feeding strategies. There was no difference in growth heterogeneity between treatments in each trial. This was initially surprising; as other studies in this thesis have reported that feeding on-demand reduces competition (see chapter 3b and 4c), which has been associated with growth depensation (Jobling, 1995). This suggests that inter-individual differences in metabolic and growth rate regulate growth heterogeneity more than behavioural interactions in freshwater cages. Growth depensation may therefore be difficult to eradicate using behaviourally based feeding methods.

The imposed regime, which delivered predetermined daily rations, consistently overfed the fish in late summer. As this oversized ration was not delivered preferentially in relation to appetite, the 'small and often' feeding regime may have been underfeeding the fish at times of peak requirement whilst overfeeding at other times. There were periods during a day when the fish did not consume food (see 4a and 5a) and the imposed regime may increase pellet wastage by offering food around these times; this can increase FCR (Thorpe and Cho, 1995). Small rations delivered regularly can also increase competition and lead to an increase in swimming activity (Niimi and Beamish, 1974), which is energetically costly (see Chapter 4c).

6.3.3 The effect of feeding regime upon the growth and production of post-smolts

The results of the post-smolt study (chapter 3b) were somewhat different to those of the freshwater trials. The fish fed an imposed regime using current techniques for determining satiation were consistently underfed in comparison to those fed on-demand. This restrictive ration was deleterious upon growth performance, which is in accordance with other studies (Quinton and Blake, 1990; Thorpe and Huntingford, 1992; Jobling, 1995; Nakagawa et al., 1999). This underfeeding also increased growth depensation in comparison to fish fed on-demand (see Jobling, 1995; Kadri et al., 1996). There were also slight differences in feeding efficiency, with the on-demand regime achieving marginally better FCR's. The results of this study suggest a farmer can optimise feeding efficiency and growth performance by feeding post-smolts on-demand, in synchrony with their appetite rhythms and daily feeding requirements.

6.3.4 The effect of feeding regime upon fish farm economics

Feeding management can have a profound effect on the profitability of a fish farm venture, with feed costs accounting for up to 60% of the overall running costs of farm (Blyth et al., 1993). Food must not be wasted, as it is potentially deleterious to the environment (it can increase the nutrient output of a farm) and is expensive (costs in 2001 are between £700-£1000 tonne^{-1}, D. Mitchell, pers. comm.). Although there were no biologically significant differences in FCR between imposed and on-demand feeding regimes in the trials that permitted statistical analysis (Chapter 4b and 5b), any observed trends can have an impact on profits.

Fish fed on-demand in the Loch Eil post-smolt trial grew significantly better than fish fed to the imposed regime (with a 6% difference in mean wet weight after 3 months). The on-demand feeding regime also achieved an FCR that was around 10% better than the imposed feeding regime (see Fig. 6.1).

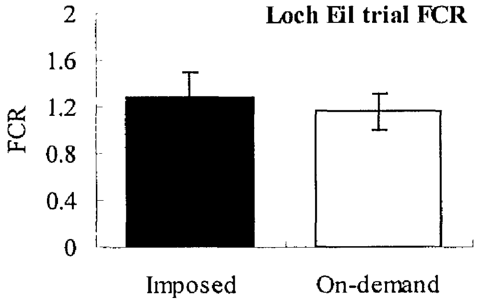


Fig. 6.1 The effect of feeding regime on FCR in Atlantic salmon post-smolts in sea cages. Data represents mean ± S.D. n = 1.

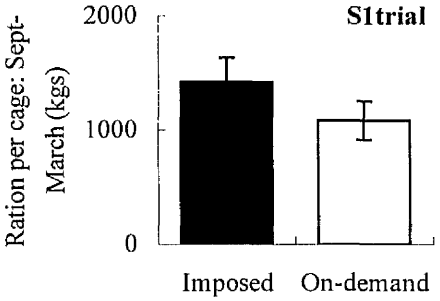


Fig. 6.2 Differences in the amount offered between each feeding regime replicate in Atlantic salmon S1 parr for the duration of the experiment. Data represents mean ± S.E. n = 3.

There was generally no significant difference in growth (weight gain) between S1 parr fed under imposed and on-demand regimes (see Chapter 4b). An observer could therefore determine the economic efficiency of each regime by comparing the amount of food offered for the duration of the trial by each cage (as there were no differences in growth performance; note that FCR was not used as a suitable comparison due to sampling error, see Chapter 4b). The on-demand feeding regime was around 25% more efficient than the imposed regime whilst achieving the same growth performance. From September until March the three on-demand fed cages collectively saved around 1 tonne of food when compared to the three cages fed to the imposed regime (a saving of up to £1000). This can have a marked impact upon the efficiency and profitability of a farming venture.

A farm management decision to restrict the growth of PP1 fish under the imposed feeding regime for the final month of the trial (due to production tonnage constraints, as stated earlier), led to significant differences in weight gain between treatments. At the end of the two month trial, on-demand fish were around 15% heavier than fish fed to the imposed regime. The on-demand regime, which matched feed delivery to group appetite, also fed fish more efficiently.

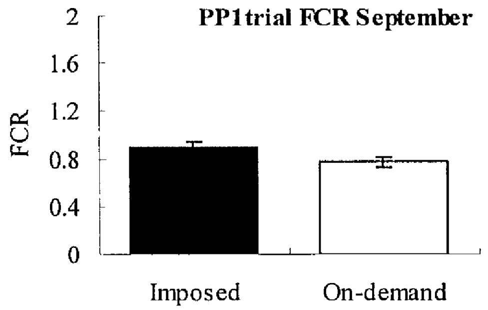


Fig. 6.3a The effect of feeding regime on FCR in Atlantic salmon PP1 parr. Data represents mean ± S.D. and denotes the growth period where the imposed regime attempted to feed to satiation using a combination of tables and hand feeding. n = 3.

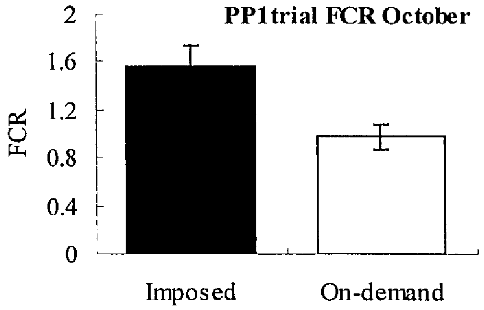


Fig. 6.3b The effect of feeding regime on FCR in Atlantic salmon PP1 parr. Data represents mean ± S.D. and denotes the growth period where the imposed regime underfed fish to restrict growth performance. n = 3.

During September, when the PP1 fish under the imposed feeding regime were fed to apparent satiation using a combination of feed tables and hand feeding, there was a 14% difference in FCR between treatments. When the imposed regime underfed fish during October, there was a 37% difference in FCR between treatments; suggesting that further to impeding growth performance, underfeeding can be deleterious to the efficiency of feed management strategies (see also Cho, 1992).

6.3.5 Effect of feeding regime upon social interactions

As stated throughout this thesis, the primary objective of aquaculture feed management is to supply an accurate ration, in order to maximise growth and minimise competition around feeding times. On-demand feeding systems can provide information on how culturists may achieve these objectives. My experiments have shown that on-demand feeding can reduce competition for a feed resource amongst Atlantic salmon parr and post-smolts held in cages. Fish fed to the imposed feeding regime exhibited increased swimming speeds and more complex swimming manoeuvres around meal times, in comparison with those fed on-demand. This increased activity can be energetically costly and deleterious to growth (see Beamish, 1978; Krohn and Boisclair, 1994; Tang and Boisclair, 1995; Farrell et al., 1997; Gregory and Wood, 1999) and suggests that on-demand feeding reduces the competitive value of the feed resource. On-demand feeding also reduces the incidence of overt aggression around meal times (as competition can regulate temporal access to a feed resource, Kadri et al., 1997*b*).

The prevalence of fin damage in fish can be used as an indicator of the levels of aggression and competition between feeding regimes. The on-demand feeding regime significantly reduced the prevalence of fin damage in comparison with the imposed feeding regime. It also influenced which parr were affected by fin injury within the commercial scale populations of S1 fish by significantly reducing the prevalence of fin damage in smaller individuals within the group. This can enhance the market appeal of whole fish for the consumer whilst improving welfare considerations for both parr and post-smolts, as competition is a potential stressor for fish (Wedemeyer, 1997). An aquaculturist should strive to reduce the effects of intraspecific competition, as it can cause a rapid elevation in plasma cortisol levels (Pottinger and Pickering, 1992), which are reportedly deleterious upon tissue repair (Gregory and Wood, 1999) and growth (Barton et al., 1987).

Table A1. Monthly sample weight data showing original mean, standard deviation and sample size for Glenfinnan S1 fish (Chapter 4). Also shown is the corrected sample data (see Chapter 4b results for calculations): the refined sample mean, refined sample size and the fish weights that were excluded from the refined data. Also excluded were precocious parr as these have differing growth patterns to non-maturing fish (shown as weights that fall within ± 3 S.D. but are excluded from the refined mean in the outliers column).

Cage	Sample	Uncorrected mean wet weight (g)	Range (mean ± 3 S.D.)	Original sample size	Weight of outliers	Refined mean wet weight (g)	Refined sample size
Imposed: 1	T_{A0}	23.57	1.73 – 45.41	200		23.57	200
	T_{A1}	35.78	2.03 – 69.53	250	70	35.64	249
	T_{A2}	46.94	7.31 – 86.57	250	90,91,94	46.39	247
	T_{A3}	47.58	-5.01 – 100.17	250	140,185	46.65	248
	T_{B0}	48.18	-4.23 – 100.59	250	36, 101, 127, 128	47.16	246
	T_{B1}	54.80	-0.85 – 110.45	250	111,112,123,140,	53.71	246
	T_{B2}	60.37	-32.51 – 153.25	250	166, 176, 220, 225, 284	57.23	245
On-Demand: 1	T_{A0}	25.56	2.04 – 49.08	200	2	25.67	199
	T_{A1}	36.04	5.71 – 66.37	250		36.04	250
	T_{A2}	48.02	-1.48 – 97.52	250	18, 107, 107, 122	47.24	246
	T_{A3}	50.83	-2.36 – 104.02	250	207	50.20	249
	T_{B0}	50.73	6.18 – 95.28	250	103, 123	50.23	248
	T_{B1}	58.02	-4.47 – 120.51	250	121, 130, 145, 166, 174	56.20	245
	T_{B2}	60.92	-3.97 – 125.81	250	128, 134, 141, 141	59.70	246
Imposed: 2	T_{A0}	23.47	5.8 – 41.14	200		23.47	200
	T_{A1}	37.35	3.75 – 70.95	250	3, 74, 75, 78	37.02	246
	T_{A2}	44.99	-1.66 – 91.64	250	101, 145	44.36	248
	T_{A3}	46.4	5.15 – 87.65	250	91, 113, 168	45.86	247
	T_{B0}	50.21	8.03 – 92.39	250	98, 105	49.8	248
	T_{B1}	58.03	-6.8 – 122.86	250	130, 147, 171, 207	56.31	246
	T_{B2}	57.84	4.89 – 110.79	250	113, 119, 124, 141	56.76	246
	T_{C0}	49.12	29.65 – 68.59	250	29, 70	49.12	248
	T_{C1}	54.21	32.79 – 75.63	250	77	54.12	249
On-Demand: 2	T_{A0}	21.28	-1.19 – 43.75	200		21.28	200
	T_{A1}	34.99	1.12 – 68.86	250	86	34.78	249
	T_{A2}	44.15	1,28 – 87.02	220	32, 36, 95, 97, 115,	43.44	215
	T_{A3}	46.92	1.2 – 92.64	250	102, 104	46.47	248
	T_{B0}	48.74	2.6 – 94.88	250	96, 104, 117	48.05	247
	T_{B1}	53.18	-3.64 – 110	250	113, 121, 125, 130	52.06	246
	T_{B2}	56.88	-11.88 – 125.64	250	126, 130, 131, 131, 242	54.94	245
	T_{C0}	48.69	28.8 – 68.58	375		48.69	375
	T_{C1}	54.22	31.78 – 76.66	250		54.22	250

Table A1 cont. Monthly sample weight data showing original mean, standard deviation and sample size for Glenfinnan S1 fish (Chapter 4). Also shown is the corrected sample data (see Chapter 4b results for calculations): the refined sample mean, refined sample size and the fish weights that were excluded from the refined data. Also excluded were precocious parr as these have differing growth patterns to non-maturing fish (shown as weights that fall within ± 3 S.D. but are excluded from the refined mean in the outliers column).

Cage	Sample	Uncorrected mean wet weight (g)	Range (mean $\pm$ 3 S.D.)	Original sample size	Weight of outliers	Refined mean wet weight (g)	Refined sample size
Imposed: 3	T_{A0}	21.46	5.86 –37.06	200	40	21.37	199
	T_{A1}	32.74	5.98 – 59.5	250	5	32.84	249
	T_{A2}	42.44	8.09 – 76.79	250	14, 78	42.42	248
	T_{A3}	44.56	3.13 – 85.99	250	3, 92, 118	44.24	247
	T_{B0}	48.18	4.53 – 91.83	250	93, 99, 101, 112, 134	46.96	245
	T_{B1}	55.85	4.52 – 107.18	250	109, 124	55.36	248
	T_{B2}	55.67	4.13 – 107.21	250	112, 113, 115, 119, 159	54.28	245
	T_{C0}	39.55	24.34 – 54.76	375	23, 57, 58	39.50	372
	T_{C1}	46.57	28.33 – 64.81	250	26, 71	46.55	248
On-Demand: 3	T_{A0}	22.34	3.98 – 40.7	200		22.34	200
	T_{A1}	33.94	7.96 – 59.92	250	7, 63	33.93	248
	T_{A2}	42.01	6.97 – 77.05	250	81	41.85	249
	T_{A3}	44.62	4 – 85.24	250	22, 41, 90, 92, 93, 96, 100	43.71	243
	T_{B0}	46.99	9.58 – 84.4	250	85, 113	46.57	248
	T_{B1}	51.04	0.52 – 101.56	250	138, 168	50.22	248
	T_{B2}	53.98	7.21 – 100.75	250	119, 134, 140	53.05	247
	T_{C0}	40.38	24.81 – 55.95	250	24, 56, 56, 57, 57	40.19	245
	T_{C1}	46.38	20.59 – 67.17	250	16	46.50	249

Table A2. Monthly sample weight data showing the refined mean, its standard deviation and 95% confidence interval for Glenfinnan S1 fish (Chapter 4). Also shown is the Coefficient of Variation (mean wet weight).

Cage	Sample	Refined mean wet weight (g)	Refined standard deviation	Mean weight confidence interval (95%)	Refined Coefficient of Variation
Imposed: 1	T_{A0}	23.57	7.28	1.01	30.89
	T_{A1}	35.64	11.06	1.37	31.03
	T_{A2}	46.39	12.32	1.54	26.56
	T_{A3}	46.65	14.07	1.75	30.16
	T_{B0}	47.16	12.84	1.60	27.23
	T_{B1}	53.71	16.55	2.06	30.81
	T_{B2}	57.23	21.15	2.65	36.96
On-Demand: 1	T_{A0}	25.67	7.68	1.07	29.92
	T_{A1}	36.04	10.11	1.25	28.05
	T_{A2}	47.24	14.89	1.86	31.52
	T_{A3}	50.20	14.19	1.76	28.27
	T_{B0}	50.23	13.78	1.72	27.43
	T_{B1}	56.20	16.38	2.05	29.15
	T_{B2}	59.70	19.53	2.44	32.71
Imposed: 2	T_{A0}	23.47	5.89	0.82	25.10
	T_{A1}	37.02	10.22	1.28	27.61
	T_{A2}	44.36	13.79	1.72	31.09
	T_{A3}	45.86	12.77	1.59	27.85
	T_{B0}	49.8	13.33	1.66	26.77
	T_{B1}	56.31	16.59	2.07	29.46
	T_{B2}	56.76	15.54	1.94	27.38
	T_{C0}	49.12	6.25	0.78	12.72
	T_{C1}	54.12	7.01	0.87	12.95
On-Demand: 2	T_{A0}	21.28	7.49	1.04	35.20
	T_{A1}	34.78	10.84	1.35	31.17
	T_{A2}	43.44	12.60	1.68	29.01
	T_{A3}	46.47	14.44	1.80	31.07
	T_{B0}	48.05	14.09	1.76	29.32
	T_{B1}	52.06	16.88	2.11	32.42
	T_{B2}	54.94	17.47	2.19	31.80
	T_{C0}	48.69	6.63	0.67	13.62
	T_{C1}	54.22	7.48	0.93	13.80

Table A2 cont. Monthly sample weight data showing the refined mean, its standard deviation and 95% confidence interval for Glenfinnan S1 fish (Chapter 4). Also shown is the Coefficient of Variation (mean wet weight).

Cage	Sample	Refined mean wet weight (g)	Refined standard deviation	Mean weight confidence interval (95%)	Refined Coefficient of Variation
Imposed: 3	T_{A0}	21.37	5.05	0.70	23.63
	T_{A1}	32.84	8.77	1.09	26.71
	T_{A2}	42.42	11.12	1.38	26.21
	T_{A3}	44.24	12.44	1.55	28.12
	T_{B0}	46.96	11.72	1.47	24.96
	T_{B1}	55.36	16.27	2.02	29.39
	T_{B2}	54.28	14.07	1.76	25.92
	T_{C0}	39.50	4.84	0.49	12.25
	T_{C1}	46.55	5.76	0.72	12.37
On-Demand: 3	T_{A0}	22.34	6.12	0.85	27.39
	T_{A1}	33.93	8.32	1.04	24.52
	T_{A2}	41.85	11.44	1.42	27.34
	T_{A3}	43.71	11.60	1.46	26.54
	T_{B0}	46.57	11.54	1.44	24.78
	T_{B1}	50.22	14.11	1.76	28.10
	T_{B2}	53.05	13.11	1.63	24.71
	T_{C0}	40.19	4.70	0.59	11.69
	T_{C1}	46.50	6.67	0.83	14.34

Table A3. Monthly sample weight data showing the mean, its standard deviation and 95% confidence interval for Glenfinnan PP1 fish (Chapter 5b). Also shown is mean condition factor, the Coefficient of Variation (mean wet weight) and sample size.

Cage	Sample	Mean wet weight (g)	Standard Deviation	Mean weight 95% Confidence Interval	Mean Condition Factor	Coefficient of Variation	Sample size
Imposed: 1	T_0	42.82	9.62	1.09	1.21	22.47	300
	T_1	68.85	30.08	2.95	1.39	43.69	400
	T_2	78.62	41.41	3.63	1.38	52.67	500
On-Demand: 1	T_0	43.81	11.71	1.33	1.21	26.73	300
	T_1	71.01	27.36	2.73	1.40	38.53	385
	T_2	86.80	43.88	4.30	1.42	50.55	400
Imposed: 2	T_0	41.32	9.21	1.04	1.29	22.29	300
	T_1	70.99	24.20	2.37	1.42	34.09	400
	T_2	80.40	36.20	3.17	1.32	45.02	500
On-Demand: 2	T_0	43.81	9.97	1.15	1.23	22.76	290
	T_1	72.25	23.87	2.35	1.39	33.04	397
	T_2	94.78	44.71	3.92	1.37	47.17	500
Imposed: 3	T_0	43.06	9.91	1.12	1.21	23.01	300
	T_1	71.33	30.90	3.03	1.39	43.32	400
	T_2	79.31	39.98	3.50	1.31	50.41	500
On-Demand: 3	T_0	43.44	9.37	0.92	1.23	21.57	400
	T_1	76.14	28.22	2.77	1.41	37.06	400
	T_2	92.56	43.71	3.83	1.35	47.22	500

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