Studies of appetite variation in juvenile Atlantic cod (*Gadus morhua L.*) using demand feeding systems

Helen Ablitt

This thesis is submitted for the degree of Doctor of Philosophy
Division of Ecology and Environmental Biology
Faculty of Biomedical and Life Sciences
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Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It is entirely my own composition and has not, in whole, or in part, been submitted for any other degree

Helen Ablitt

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Summary

This thesis covers two studies based on the feeding of juvenile Atlantic cod (*Gadus morhua*). The first study was carried out using an AQ1 interactive feedback feeding system and investigated their feeding patterns, both seasonal (chapter 2) and daily (chapter 3). The second study involved designing an on-demand feeding system for juvenile cod, then examining the process of the fish learning to use the feeder, focusing on the roles of individual fish.

The first trial was carried out in two sections, the first from March until August 2004 and the second form November 2004 until February 2005. Five 124 litre tanks were stocked with cod of 5 grams. The tanks were equipped with an AQ1 feeding system with infra red sensors at the outlet of each tank. The system added test feeds every 30 minutes and the feed input each minute was recorded to an attached computer. The sensors were affected by suspended solids in the water, causing false readings of waste pellets, causing under feeding. The data set was therefore cleaned up by removing affected days.

Chapter 2 focuses on the seasonal changes in feed consumption of the cod. The mean daily feed input (as a percentage of bodyweight per day, BWD) varied between 0 and 10%, with the mean being around 5%. The feed input (%BWD dropped to its lowest levels around early summer, the opposite result to that expected. The effects of temperature and photoperiod on feed input were also opposite to the expected outcome, when the day length was longer, and when the temperature was high, the feed intake dropped. There was a significant pattern of autocorrelation in feed input, leading to the conclusion that juvenile cod appetite is affected by a medium term internal rhythm.

Chapter 3 focuses on the daily feeding patterns of the juvenile cod. The main pattern found during the first part of the trial was a significant dawn peak when up to 40% of the days feed was consumed. The remaining part of the day was spent browsing. In the second part of the trial, there were a lot less definite patterns; there was a trend towards browsing over a full 24 hour day with heavier feeding nocturnally and short phases of crepuscular feeding, again with continuous light browsing at other times. The reasons for the differences in feeding pattern are discussed and the peak feeding hour and percentage nocturnal consumption are investigated.

Chapter 4 concentrates on the process of juvenile cod learning to use a demand feeder. Every group of fish learnt to use the demand feeder, though only one fish from each

group was responsible for activating the trigger. That fish was also found to be the boldest when tested in a novel object test and its growth was significantly higher than the other fish in the group, it was named the trigger fish. Another group emerged at the other end of the scale; they did not interact with the sensor at all, nor approached the novel object. They were significantly smaller than all the other individuals and gained the least weight, in some cases, the fish lost weight.

1.1 The Atlantic cod and its aquaculture

1.1.1 Distribution and habitat

The cod (*Gadus morhua*) is a gadiforme fish that is distributed across the Atlantic from Northwest to Northeast. Its distribution runs along the North American coast to the east and west coast of Greenland, around Iceland and the coasts of Europe from the Bay of Biscay to the Barents Sea, including the region around Bear Island (Froese and Pauley, 2008). The distribution and spawning areas of cod can be seen in figure 1.1. There are both migratory and coastal cod and the coastal juvenile cod, the subject of this thesis, inhabit the shallow littoral and sub littoral zone of 0-20 metres (Nordeide and Fosså, 1992). This area can be rocky and covered with algae which provides cover and harbours invertebrates and fish that form their diet (Nordeide and Fosså, 1992).

1.1.2 Challenges to wild cod stocks

Cod is an important commercial fish that is becoming increasingly rare in the wild due to over-fishing, exacerbated by global warming (O'Brian *et al.*, 2000, Sartoris *et al.*, 2003). Historically it was a bountiful species that had a huge impact on the economies of several societies, notably, the Basques, who, during the Middle Ages, were able to travel to uncharted waters by living off dried, salted cod and traded the preserved cod far beyond their natural boundaries (Kurlansky, 1999). Because the cod is a highly fecund and historically plentiful fish it was considered such a robust species that it would be impossible to over fish. According to Dumas, (1883) '*...it has been calculated that if no accident prevented the hatching of the eggs and each egg reached maturity, it would take only three years to fill the sea so that you could walk across the Atlantic dryshod on the backs of cod.*' (Alexandre Dumas, Le Grande Dictionary de Cuisine, 1873).

However, modern advances in industrial fishing technology and the growing human population have led to over-fishing of cod, leading to the crash of many populations. For example, in the North West Atlantic, the population has declined by 90% over the past 30 to 40 years (Hutchings, 2005). The exploitation of cod stocks has led to restructuring of the populations. Age and length at maturity is getting lower. For example, as Northern cod abundance has declined by 99% this has led to a fall of one year age at maturity of 50% of the populations' females since the 1970s (ibid).

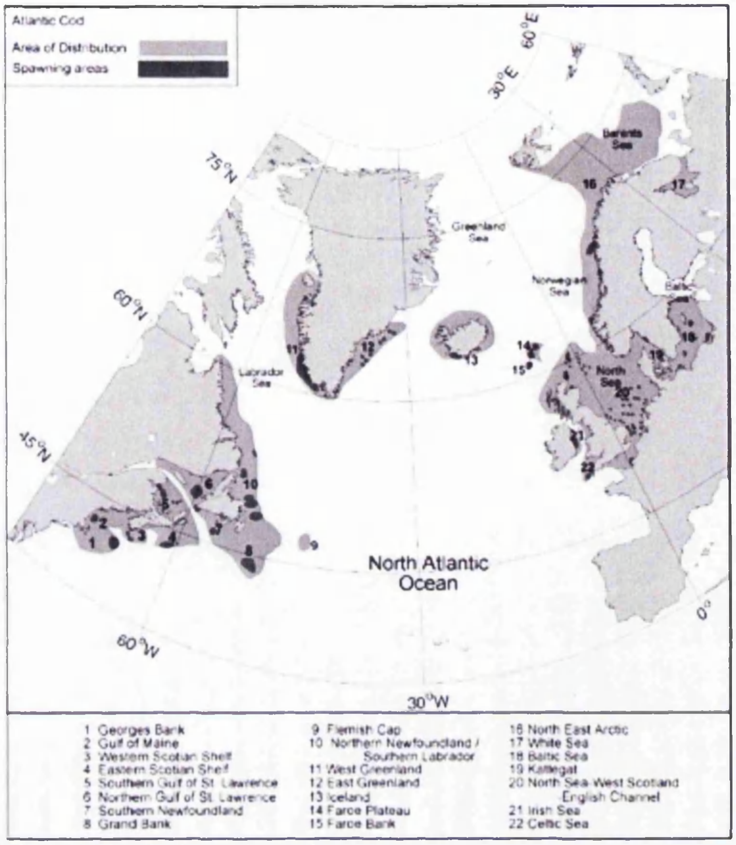


Figure 1.1. Distribution and spawning areas of the Atlantic cod. (From Drinkwater, 2005)

1.1.3 Possible impacts of climate change

Cod are found at a wide range of temperatures, being found in water between –1 and 20°C (Björnsson *et al.*, 2001). Differences in temperature can have a highly significant effect on growth. For example, Brander (1995) looked at the weight-at-age of 17 populations of cod from areas with different temperatures and found huge variation. For example, at four years old, mean weights varied from 0.61kg in fish from Labrador / Grand Bank where the mean temperature is 2°C, to 7.32kg in the Celtic sea, where the mean temperature is 11°C.

The surface temperature of the North Sea is predicted to rise by around 1°C by 2040 (Kell *et al.*, 2005). This will have a likely impact on cod stocks. Drinkwater (2005) and O'Brien *et al.* (2000) predict that cod in the Celtic and Irish Sea will disappear if the expected water temperature rise takes place and other southern populations will decline. The northern limits of the species are likely to progress further north and spawning sites may be established further north. Additionally migrations may stop if the arctic sea ice melts. This is because the winter migration to warmer waters is needed to stimulate gonadal growth. If the water temperature is consistently above 4°C (the critical temperature for gonadal development) no migration will be needed. Although there will be a loss of stocks at the southern limits, the higher temperatures will cause an overall increase in the total production of cod in the northern Atlantic (Drinkwater, 2005).

1.1.4 Importance of, and bottlenecks in, cod aquaculture

From discussion with cod farmers, farming of cod is being investigated in the United States of America, Canada, Norway and the United Kingdom. Cod mariculture is attractive to farmers in Northern Europe for a number of reasons; it is possible to grow cod in salmon cages already *in-situ*, the species will grow well at ambient temperatures, salmon market prices are falling and, because of its popularity and scarcity, cod prices are rising. However, because it is a novel species, a significant amount of research and development needs to take place before it becomes a viable species for farming.

According to the Food and Agriculture Organization of the United Nations (FAO), in 2006 the total UK aquaculture production of Atlantic cod was 543 tonnes and the Norwegian production was 11,087 tonnes. Figure 1.2a shows the total world production of cod, haddock (*Melanogrammus aeglefinus*) and hake (*Merluccius merluccius*) (fisheries and aquaculture). The level is relatively stable, although there were unexplained dips in 2000 and 2002. Figure 1.2b shows the percentage of cod production by aquaculture. There is an increasing trend, but aquaculture still only accounts for less than 0.1% of total production of cod, haddock and hake.

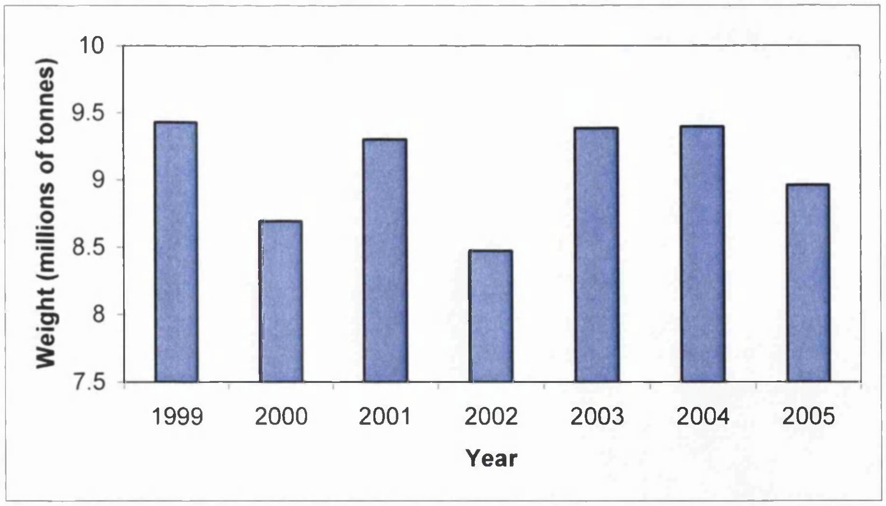


Figure 1.2a World capture fisheries of cods, hakes and haddocks from 1999 to 2005 (FAO, 2008)

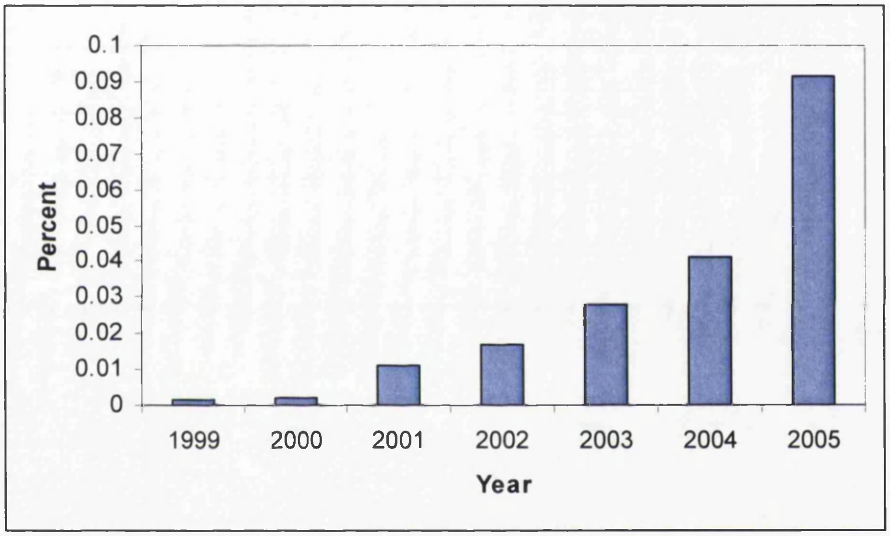


Figure 1.2b Percentage of the total annual cod, haddock and hake production that is produced by aquaculture from 1999 to 2005 (FAO, 2008)

Cod farming has made use of techniques from other cultured finfish species. On-growing technology uses techniques and infrastructure from salmon farming. Cod farming however has its own challenges, such as the tendency of the fish to bite the net cages, leading to escapes and a higher cost for net maintenance. To avoid such damage, some farms put balls and pieces of rope in the cages in order to distract the cod from the cage (Bourhill, personal communication). Another difference between cod and other species is their nutritional requirements. Cod muscle is very low in fat, and when they are fed on a salmon or trout diet, the fat is laid down in the liver

(Love, 1970; Dos Santos *et al.*, 1993). The fatty liver can however be used as an extra product by producing cod liver oil, which has slightly higher saturated fatty acids and linoleic acid than that produced by their wild counterparts, but nevertheless, shows promise as a product (Morais *et al.*, 2001).

Eggs from farm reared cod have lower hatching rates than those from wild cod (Salze *et al.*, 2005) with hatch rates from farmed fish varying from under 10% to over 90%. The same author writes that it has been deduced that nutrition, such as lipid and caratinoid content, plays a role in the hatch success.

Another challenge to cod farming is the fact that these fish would normally mature and spawn before they reach market size. This poses two main problems, the first being that maturation and spawning diverts energy and nutrients from the flesh to the developing gonads and flesh quality takes several months to recover. The second problem is that cod spawning in net cages will release millions of fertilised eggs into the surrounding waters. As farmed strains develop, they will become genetically distinct from the wild population and farmed populations become less fit, in farmed salmon, farm reared fish have poorer reproductive success (Fleming *et al.,* 1996) and offspring of wild x wild matings have significantly higher lifetime survival compared to those from farmed fish matings (Fleming *et al.*, 2000), so the large release of eggs will cause genetic pollution as is seen with salmon and trout farm escapees (Hindar *et al.*, 2006). This has been confirmed by a study by Jørstad *et al.*, (2008), who found that when a genetic marker was introduced to pen-held farmed cod, 20 to 25% of the larvae found in the surrounding area carried that genetic marker.

Other bottlenecks associated with the farming of cod are listed below:

1. *Filling the swim bladder as larvae.* When farming of marine fish, both northern species such as cod and Mediterranean species like Sea bass (*Dicentrarchus labrax*, and Sea bream (*Sparus aurata*) began, the larvae would often fail to inflate their swim bladder, which would lead to their dying of exhaustion, due to swimming to maintain their position in the water column. Failure to inflate the swim bladder was due to a layer of oil forming over the water surface keeping the larvae separate from the air and was overcome by adding a simple surface skimmer (Olsen *et al.*, 2005).

2. *Achieving the correct fatty acid profiles for larval rearing.* There are three essential fatty acids for larval cod: docosahexanoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA). Not only the levels of these nutrients, but also their ratio is important. Feeding an incomplete diet leads to retarded growth in cod and abnormal development such as spinal deformities (Kjørsvik *et al.*, 2004)
3. *Avoiding and controlling disease such as vibriosis.* One of the current bottlenecks of cod farming is infection with *Vibrio anguillarum.* It has long been associated with salmonid farming. There are commercial vaccines available though they are not 100% effective yet (Rodger and Colquhoun, 2008).
4. *Anorexia at transfer from hatchery to sea cage.* A proportion of cod develop anorexia at transfer to sea cages. They take feed into their mouth, but reject it and eventually die. In some farms they are simply graded out and culled but work is being planned in the area of investigating the characteristics of these individuals (Treasurer, personal communication).
5. *Flesh quality and melanosis.* This is the deposition of melanin in blood vessels leading to black lines that are visible against the white flesh. This is particularly a problem for companies marketing their cod as a high quality product, as such imperfections mean that the fillet is rejected and has to be used for lower priced products. It is thought that the melanosis is caused by high copper levels in feeds, stimulating tyrosinase, which in turn is responsible for laying down melanin (Cooper and Midling, 2007).

Thus there are still many problems to be solved while developing cod aquaculture. One important aspect of aquaculture for any species is effective feed management. This thesis is aimed at collecting and interpreting information on the feeding biology of cod that may help in developing effective feed management systems for the species.

1.2 The diet of wild cod

A study of the stomach contents of wild cod caught by bottom trawl in the Balsfjord, northern Norway across a full calendar year showed that they had ingested 72 different species. The main prey items identified were the prawn (*Pandalus borealis),* capelin (*Mallotus villosus*) and krill (*Thysanossa spp.*). Other stomach contents were made up of polychaetes, mysids, amphipods and the herring (*Clupea harengus*) (Klemetsen, 1982). It was noted that the krill was the only midwater prey item present, the other species being taken from the sea bed, leading to the conclusion that cod in this area are mostly benthic feeders. Brawn (1969) carried out a comprehensive study on the feeding behaviour of cod between 5 and 90cm in length. In general, the cod used sight to locate food items from the water column and movement of the feed item stimulated feeding. However, if a food was familiar, movement was not important; instead of sight, food items on the bottom and buried in the substrate of the tanks were detected by smell using the taste buds on the barbel and possibly by taste buds on the spines of the pelvic fins.

1.3 Diel rhythms of activity and feeding in fish

Fish usually have a daily activity pattern that allows them to feed and express other behaviours such as courtship and defending their territory whilst minimising the risk of predation. Table 1.1 details different species general feeding patterns (either diurnal or nocturnal) and gives the reference for each finding. In some cases a species is shown to be both diurnal and nocturnal, this is because some species switch activity patterns due to seasonal changes or life history decisions.

There have been a number of studies of feeding rhythms of gadoid fish. Although some of these studies have used only small numbers of fish, the results are very consistent, providing strong evidence that cod and other gadoids have a daily vertical migration and at times are nocturnal.

Historically, it was thought that cod was purely a visual predator and that it was impossible for them to feed in the dark (Bateson, 1889-90) but in his study of cod feeding, Brawn (1969) writes that some live feed was taken at night. In addition, Clark and Green (1990) and Klemetsen (1982) also state that cod are capable of feeding over all 24 hours. In Klemetsen's paper, it was found that the 24 hour darkness of the arctic winter did not influence the stomach contents of wild cod. This is thought to be because the low temperature in the area studied probably meant that the cod were only feeding lightly throughout the trial and so only ever had partially full stomachs.

Species	Diurnal or Nocturnal	Reference
Rainbow trout (*Oncorhynchus mykiss*)	Diurnal	Boujard and Leatherland, 1992b Sanchez Vazquez and Tabata, 1998 Chen *et al.*, 2002
Rainbow trout (*Oncorhynchus mykiss*)	Diurnal and nocturnal	Alanärä and Brännäs, 1997
Tilapia (*Oreochromis niloticus*)	Diurnal	Toguyeni *et al.*, 1997
Catfish (*Hoplosternum littorale*)	Nocturnal	Boujard and Leatherland., 1992
European catfish (*Silurus glanis*)	Nocturnal	Boujard, 1995
European catfish (*Silurus glanis*)	Diurnal and nocturnal	Bolliet *et al.*, 2001
Senegal sole (*Solea senegalensis*)	Nocturnal	Bayarri *et al.*, 2004
European seabass (*Dicentrarchus labrax*)	Diurnal and nocturnal	Sanchez Vazquez *et al.*, 1995
Goldfish (*Carassius auratus*)	Diurnal and nocturnal	Sanchez Vazquez *et al.*, 1996
Yellowtail (*Seriora quinqueradiata*)	Diurnal and nocturnal	Kohbara *et al.*, 2000

Table 1.1 Summary of broad daily feeding pattern of different species of fish

Løkkeborg and Fernö (1999) studied food search behaviour in wild Atlantic cod in a fjord in northern Norway. They tagged the fish by offering them baited tags which were ingested; the fishes movements could then be tracked while they carried out 'normal' behaviour. This trial found that cod are most active throughout the day, but can also consume food through the night. A slower swim speed was recorded at night, but this could be because a slower speed is needed to maximise the probability of finding food by olfactory means.

An investigation into the vertical distribution patterns of juvenile European hake (*Merluccius merluccius*) found that these fish display a nocturnal migration away from the sea bed by 10-20 metres and then return to the bottom during the day (Bozzano *et al.*, 2005) Similar results were found for juvenile cod and haddock on the eastern Georges Bank. According to Lough *et al.*, (1989) 'During the day, demersal juvenile cod and haddock remain within a few centimetres of the bottom… where they maintain their position by swimming continually in the current;' at night they rise off the bottom to feed on invertebrates (Lough *et al.*, 1989). The same paper observes that the coloration of the juvenile fish mimics the appearance of the gravel bottom so they keep close to the gravel substrate to ensure good camouflage. It was found that their stomach contents were almost entirely composed of krill which were abundant close to the bottom after sunset.

In Kiel Bay in the Baltic, food intake of juvenile cod from 11 to 35cm (believed to be between year 0 and 2) was investigated by looking at stomach contents of fish caught by trawl. The results showed that there were two main feeding peaks during the day, one before sunrise and a second before and around sunset (Arntz 1973). In November a larger proportion of food was taken during the morning, whereas in March the largest fish took more in the evening while the other size classes had roughly equal feeding peaks at dawn and dusk. Clark and Green (1990) investigated the activity of 3 year old cod in Conception Bay, Newfoundland using sonic telemetry. They found that during the summer the cod were nocturnally active, displaying similar behaviour to that described in the papers cited above. Thus they migrated each day from the deep, cold water where they were inactive to the warm shallow water where they fed. When the summer thermocline disappeared, fish stayed in the shallow water, feeding continuously over the 24 hour period. The authors of this paper hypothesised that the switch from nocturnal to continuous feeding may be a predator avoidance strategy.

Løkkeborg and Fernö (1999) monitored 6 cod between approximately 40 and 55cm in a small fjord in Norway by allowing them to ingest transmitters wrapped in bait. The fishes' swimming speed was monitored to investigate their searching patterns when offered lines of mackerel flesh as bait across the seabed. It was found that the cod exhibited a diel activity pattern with more movement and a larger distance covered during daylight hours. However, searching for food continued over 24 hours, with fish detecting baits by the chemical plumes that were given off by the bait that could be detected by olfaction throughout the night as well as during the day. In a similar study investigating the response of haddock and cod to baited hooks, a comparable diel pattern was found, with an increase in activity in the morning and a decrease at dusk. The pattern followed the changing dawn and dusk in September and December (Bromley *et al.*, 1997). Thus the existing literature on feeding patterns in cod suggests that the fish have the capacity to feed at night as well as by day. They often show dawn and dusk peaks but this is variable. It is appropriate to learn whether similar patterns are shown by farmed fish when feed is made readily available throughout the day, so that feed can be provided at the times of peak hunger for farmed fish.

1.4 Flexibility of daily feeding patterns in fish

Table 1 shows that a number of species can feed either diurnally or nocturnal at different stages in their life according to environmental conditions. There are many fish species that show such flexibility in feeding patterns. This is a complex topic. This section first describes some examples of the flexibility, both in wild fish and in captivity, underlying mechanisms and finally comments on possible functions. The environmental conditions that control when circadian activities occur are known as synchronisers or zeitgebers.

The seasonal and diel feeding pattern of small yellow croaker (*Pseudosciaena polyactis*) was investigated by Xue *et al.*, (2005). Over the year there were differences in the level of stomach fullness, with cod caught in summer and autumn having higher mean fullness than those caught in spring and winter. The percentage of empty stomachs dropped during the summer and autumn. The diel feeding patterns also varied through the seasons, the main pattern being a morning and evening peak

Some fish species show dual activity rhythms (Aranda *et al.,* 1999a, b), meaning that their daily activity rhythm inverts with the seasons. These fish include Atlantic salmon (*Salmo salar*) (Amundsen *et al.*, 2000, Metcalfe *et al.*, 1998) and the sea bass (*Dicentrarchus labrax*). For example the sea bass is generally nocturnal during the winter and diurnal during the rest of the year. (Sanchez-Vazquez *et al.*, 1995a,b) Trials in the laboratory have shown that although the light dark cycle controls daily feeding rhythms a change in photoperiod alone does not cause this switch (Aranda *et al.*, 1999a). Nor did varying the temperature or a combination of temperature and photoperiod induce nocturnal feeding (Aranda *et al.*, 1999b). It is probably influenced by other changes such as food availability or an endogenous circadian control. Sea bass from the Mediterranean eat different prey in different seasons, namely sardines during the summer and autumn, mullet in the spring and annelid worms and small fish in the winter (Madrid *et al.,* 2001). The availability of these prey items could account for the seasonal change in feeding rhythm.

There have been a number of studies of the mechanisms controlling feeding rhythms.

Studies have shown that fish activity generally follows a circadian rhythm based on daily patterns of light and dark but their activity patterns can be altered by other environmental stimuli (Boujard and Leatherland, 1992a). For example, temperature fluctuations, tidal rhythms and other more subtle changes will control when appetite peaks. It was thought that the pineal gland alone controls circadian feeding patterns by excreting melatonin. However, after pinealectomy there was no change to the feeding rhythm of rainbow trout and melatonin was still found in the bloodstream (Sánchez Vázquez *et al.*, 2000). This would indicate that there is another site of melatonin production, that is responsible for fishes diel rhythms.

Alanärä, (1992) writes that in rainbow trout fed by demand feeder, as temperature increases, the number of demands increases. However, as the temperature increases the FCR (feed conversion ratio) increases suggesting that although the demands for feed increase, the feed is not all consumed, or that digestion and utilisation were inefficient. As this trial took place in net-pens, any excess feed was lost through the net and the fish did not get another chance to consume it as they would in tanks.

The feeding pattern of yellowtail (*Seriola quinqueradiata*) was measured using a demand feeding system in net cages by Kohbara *et al.* (2003). It was found that temperature plays a significant role in determining the number of daily demands; when temperature was higher than 18°C there were significantly more feed demands than when temperature was below 18°C.

One of the zeitgebers of the many fishes diel rhythms is in fact the availability of food. When sea bass in their nocturnal phase had their food availability limited to daylight hours some fish were unable to change their behaviour to diurnal. This indicates that the light-dark zeitgeber is the more powerful. In a farm situation this would mean that, if no night feed were given, a proportion of the stock would be unable to feed satisfactorily leading to a lowered productivity.

African catfish (*Clarius gariepinus*) have poor eyesight. They rely on their barbels, covered in taste buds, to detect food by smell; they are also reported to have the ability to detect electrical fields. This means that they do not need light and so can be nocturnal. When food is provided only during the day their feed intake drops and when they have access to food throughout the 24 hour cycle, the majority is taken through the night (Hossain *et al.*, 1999).

Amundsen *et al.* (2000) found that Atlantic salmon parr in sub-arctic rivers switch to nocturnal feeding during autumn due to increased availability of benthic prey. Benthic invertebrates such as caddis fly larvae are more active at night, coming out of hiding places and so can be found more easily. Salmon are usually visual predators, and can only feed during darkness if the prey is on the river bed when they switch to detecting prey by olfactory means (Jørgensen and Jobling, 1992)

1.5 Individual variance in diel feeding patterns

Circadian rhythms in fish are highly variable; in some tests a proportion of individual fish exhibited different rhythms from the majority of the other fish in the experiment (Reebs, 2003). One important determinant of such variability is competitive interactions between fish which often take the form of dominant-subordinate relationships. This refers to a relationship in which, following a series of aggressive encounters, one animal after a series of defeats defers to the other which may then gain preferential access to resources (Huntingford and Turner, 1987). Even though ample food is delivered to farmed fish, there is still aggression and the establishment of dominance hierarchies. For example, in a group of Atlantic salmon with access to a demand feeder, a group of the smaller fish fed at night while the larger fish fed during the day (Kadri et al, 1997b). In farming situations, the fish may form different levels of dominance, sometimes according to size and aggressive disposition. A measure of the level of aggression amongst farmed fish is fin damage (Turnbull *et al.*, 1998). Noble *et al.*, (2007a) writes that salmon allowed to self feed had lower levels of fin damage compared to those given a fixed ration ever ten minutes. It was also found that smaller fish had more fin damage.

Night feeding in some species, such as rainbow trout can be used by subordinate fish in order to consume feed unhindered by the dominant fish that keep them from eating during daylight hours of the day (Chen *et al.*, 2002). If this is the case it may be advantageous to provide feed nocturnally as well as diurnally for subordinate fish in order to increase the overall growth of the stock and reduce variability of size, which may reduce aggression.

It is thought that by becoming nocturnal some species are avoiding predators (Metcalfe *et al.*, 1998). In Atlantic salmon it appears that nocturnal feeding by juvenile fish depends on whether they are going to smolt in year one or in a later year. Year one smolts need a higher growth rate and so eat during the day when there is a higher predation risk, whilst those fish that are going to smolt in the following years, not needing to grow as fast, become nocturnal to lower the risk of predation.

Aggression caused by restricted feeding has also been seen to cause fin damage in cod (Hatlen *et al.*, 2006), with slower growing fish having more fin damage than their faster growing counterparts. In other fish species restricting feeding also led to more fin damage and also exacerbated the situation by causing more variation in growth between fish. However, it was noted that aggression was present in a group of non-fed fish at the same level as those that were fed, so it was concluded that aggression happens regardless of the presence of food. One of the main methods of decreasing aggression around feed is to distribute the feed over a large area. This will avoid competition and spread the fish across a wider area and so reduce contact (Alanärä *et al.*, 2001). Work done on Atlantic salmon and sea bass suggest another method for reducing aggression is to use a demand feeding system that can provide food for individual fish within the group so no fish is hungry (Andrew *et al.*, 2002; Noble *et al.*, 2007)

By mapping a species' feeding pattern and behaviour, then using this information to build a feeding regime, considerable economic and environmental benefits can be gained. By feeding when the fish's appetite is at its height the maximum feed quantity is consumed and there is less wastage. For example, Azzaydi *et al.*, (2000) demonstrated that European sea bass, fed nocturnally during winter in accordance with their natural feeding pattern had significantly better feed conversion ratios (FCRs) and higher specific growth rate (SGRs) than diurnally fed sea bass. The increased growth happens despite sea bass's reduced pellet catching ability when fed nocturnally (Rubio *et al.*, 2003).

When fish are fed regular large meals, anticipatory behaviour occurs where the swimming speed increases and the fish swim higher in the water column (Sánchez Vázquez and Madrid, 2001). Feeding using an Aquasmart™ system or other device that feeds to the fishes appetite reduces anticipatory behaviour and the fish stay at a lower level. As mobile phase fish lice rise to the surface during the day, fish that spend more time high in the water column have a greater chance of sea lice attachment, so removing anticipatory behaviour keeps the fish lower and reduces the level of sea lice attachment (Lyndon and Toovey, 2000).

Fish do not always switch to a feeding pattern that is optimal for their production, for example the Florida pompano, *Trachinotus carolinus*, preferentially feeds early in the day. However the best growth occurs when fish are fed in the evening when compared with morning feeds. This indicates that the fishes preferred eating time might not always give the best growth (Heilman and Spieler, 1999).

1.6 Demand feeders and their use in agriculture and research

One way to feed fish at the most effective times is to use demand feeder systems that are based on the principle that supply of feed is regulated by the demand of the fish (Alanärä *et al.*, 2001). Demand feeders are used widely in agriculture, both for production and research. They are a useful tool for mapping an animal's or group of animal's feeding pattern if they are connected to a logger device.

In aquaculture, fish can be hand fed, fed by automatic feeder or by a demand feeder. The advantages and disadvantages of different methods of feeding are listed in table 1.2

Simple on-demand feeders are used in agriculture, for example the NOSLOC^{TM} poultry feeder is a simple device. It releases feed when the nozzle is tapped by the bird's beak. The feeder can be set up at specific heights to allow only the target species to feed, saving feed waste and discouraging vermin as well as providing environmental enrichment for the birds. These feeders are popular with 'back yard' poultry owners and game bird producers in New Zealand and around 10,000 have been sold (personal communication, Colson, 2008).

Feeding method	Advantages	Disadvantages
Hand feeding	Inexpensive Can set feed level by fish's reaction	Labour intensive Cannot see fate of feed unless underwater cameras are employed
Automatic feeding	Relatively inexpensive Low labour requirement	No taking into account the environmental conditions or fish's appetite Can generate a lot of waste feed
Demand feeding by self feeder	Feeds to the fish's appetite Can be inexpensive Low labour requirement	Some systems can suffer from accidental actuations
Demand feeder by interactive feedback system	Feeds to the fish's appetite Take account of environmental conditions, for example, tides and atmospheric pressure. Low labour requirement Reduces feed waste	Expensive Moderate level of technical difficulty

Table 1.2 Advantages and disadvantages of some different feeding systems for aquaculture

1.6.1 Use of demand feeders in research

Self feeders are used to measure feed intake of a test species. For example, Thanos *et al.*, (2008), used obese and lean Zucker rats (*Rattus norvegicus*) trained to operate a push lever to obtain food. Injection with different levels of two dopamine receptor antagonists caused a reduced level of feeding and lever presses, suggesting that dopamine receptors may be involved in food search behaviour. Much of the information on daily feeding rhythms described in section 1.4 is based on data generated using demand feeders.

1.7 Aims and thesis plan

The main aim of the work described in this thesis was to investigate the seasonal and daily feeding patterns of juvenile cod in an aquaculture environment, using an interactive feedback feeding system. Another aim was to design an on-demand feeding system for juvenile cod and to investigate the process by which fish learn to use the sensor.

Chapter 2 focuses on the growth and production of juvenile cod throughout the year, taking into account the feed conversion ratio (FCR) and feed intake. Effects of temperature and photoperiod were investigated and the presence of longer term feeding patterns was assessed using time series analysis.

Chapter 3 concentrates on the daily feeding patterns of juvenile cod throughout the year. The focus was on factors such as the proportion of daily feed taken during the night and the peak hour for feeding. Again, the effects of temperature and photoperiod were taken into account.

Chapter 4 focuses on the production of an on-demand feeding system for juvenile cod and charts their learning progress. The fish were classified as to the level of involvement in triggering the feeder and then correlated for size, risk taking as determined by a novel object test and stress levels by whole body corticosteroid assay.

Chapter 2 Annual feeding patterns in farmed juvenile cod

2.1 Introduction

As discussed in Chapter 1, the rate at which food is taken by juvenile cod on any given day is influenced by a number of physical factors, the main ones being temperature and photoperiod. Other influential factors are wind speed, light intensity, air pressure, dissolved oxygen, other water quality parameters, stocking density and level in social hierarchy. By taking account of these conditions, farmers could in principle, predict the amount of feed to offer the fish each day (Kestemont and Barras, 2007).

2.1.1 Measuring feeding patterns

Farmers and researchers can measure fishes' appetite with a variety of methods and devices (Alanärä, 1996). Most simply the fish can be fed to apparent satiation by hand and the amount of feed taken, recorded systematically. For example, Grove *et al.* (1978) hand fed rainbow trout to satiation to measure their gastric emptying rate. More recently, Tripple and Neil (2003) used hand feeding to assess the effects of photoperiod and light intensity on growth and activity of juvenile haddock. Gastric evacuation and feed intake can be measured by a number of methods including feeding a known quantity at set times, then euthanasing the fish and dissecting the digestive tract to measure the progress of the ingested food at set time periods after feeding (Riche *et al.*, 2004), X radiography (Grove *et al.*, 1978) or gastric lavage (dos Santos and Jobling, 1991). Kadri *et al.* (1991) used direct observations of salmon in net pens consuming pellets to estimate feed intake.

Demand feeders involve individual fish activating a feeder by pressing a lever or biting a switch. This can be purely mechanical, where a lever is pressed by the fish, which directly releases feed without any recording, which is relatively low cost so available to the majority of farmers (Alanärä, 1996). A more sophisticated kind of self feeder is electronic, where the sensor is attached to a computer or data logger that can record the number and times of feeder activations as well as activating a feeder. This is particularly useful for researchers as it gives a record of fishes' feeding history. Electronic feeders are much more expensive than mechanical feeders so are only available to larger farms and institutions. Rubio *et al.* (2004) used this type of self feeder under both laboratory and farming conditions for raising sea bass. They used a string sensor that had to be bitten by the fish to release feed. It was concluded that this type of trigger would be suitable for commercial production of sea bass.

Another type of demand feeder is an interactive feedback system, where uneaten feed is monitored to assess the fishes appetite level and a feeder activated accordingly. Such systems usually have an infra red sensor at the bottom of a cage or at the outlet of a tank. A commercial version of this is the AQ1 system (AQ1 Systems Pty., Hobart). A diagram of this system is shown in figure 2.1.

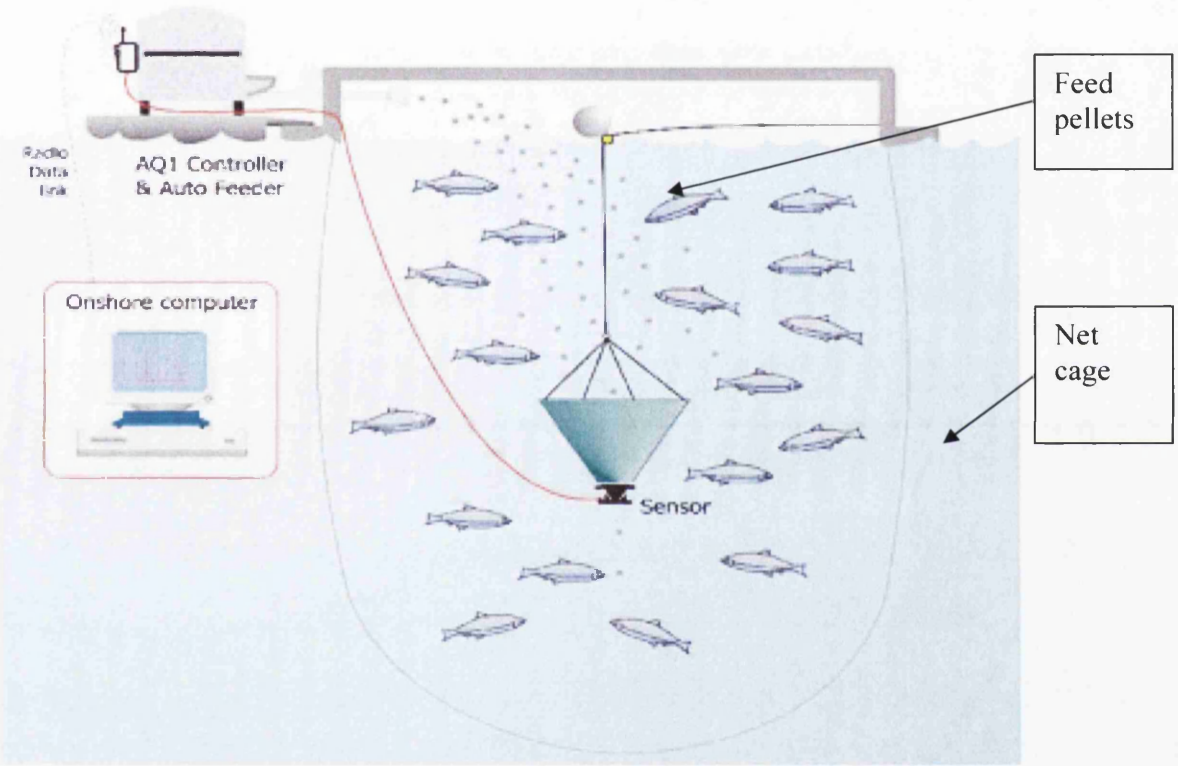


Figure 2.3 A schematic diagram of the AQ1 system (from www.AQ1systems.com)

After food has been released into the tank, the number of uneaten pellets is counted by the sensor towards the bottom of the cage. If no, or a low number of pellets have been detected, more will be released as it would appear that the fish have eaten the majority of pellets and therefore are hungry. When more than a set threshold number of pellets are detected it is assumed that the fish are no longer hungry, so feeding is stopped. This type of system is often used in larger farms as the main feeding system and the most modern systems also take account of factors such as tides, temperature, wind speed and air pressure. The AQ1 system was used by Andrew *et al.* (2002) to compare the swimming speed of Atlantic salmon, European sea bass and gilthead sea bream when using either the AQ1 system or normal automatic feeding. It was found that the swimming speed of the control salmon that were fed by hand was significantly higher at feeding times than the demand fed fish. It was suggested that there was less competition in the AQ1 fed system as food was provided at irregular times so the more dominant fish did not dominate the feeder and all the fish had a chance to feed (Kadri, Personal communication). The results for the sea bass and bream were similar to those of the salmon. There are advantages and disadvantages to each system as research tools. Mechanical demand feeders are cheap and simple to use, while the feedback feeders are expensive and need a trained workforce. If a feedback system fails, it would have to be fixed by a trained electronic engineer. A mechanical feeder may accidentally trigger with wave movement or by fish swimming too close. Feedback systems such as the AQ1 have to be set up to take account of environmental conditions if they are to run efficiently. For example if a strong tide occurs that is not taken account of, pellets will be carried out of the cage leaving fewer to go through the sensor. The system will then add even more feed leading higher feed wastage and thus increasing pollution of the surrounding environment.

2.1.2 Variation in food intake in fish

Using the various methods for measuring appetite, there have been many studies of annual variation in feed intake in various groups of fish.

Feeding frequency in Salmonids Salmonid fish have been extensively farmed and accordingly, there has been much research done on their feeding patterns and appetite. Sæther *et al.* (2007) investigated the seasonal feed consumption of Arctic charr (*Salvelinus alpinus*). It was found that the highest feed intake was at mid summer and the lowest in autumn. This seasonal feeding pattern continued regardless of a continuous 12:12 LD photoperiod and constant temperature (4°C). Smith *et al.* (1993) found that Atlantic salmon held in sea cages also had seasonal changes in appetite showing a reduction of appetite from autumn to winter, then a rapid increase from late winter. These changes were closely related to changes in photoperiod.

In rainbow trout (*O. mykiss*) appetite was directly related to gastric emptying time, which fluctuates according to temperature and fish size (Grove *et al.*, 1978).

Feed intake depends on other processes occurring within the fish. For example, in Atlantic salmon, gonadal development, smolting and its associated changes in osmoregularity capacity, silvering, migratory behaviour and endocrine shifts, will all affect the amount of feed consumed (Erriksson and Lundqvist, 1982). Such changes are controlled to some extent by photoperiod changes but if fish are kept in 12:12 LD they will run to their own rhythms. Arnesen *et al.* 2003 studied the feed intake of Atlantic salmon that were transferred to sea water at different stages of smoltification. Smolts transferred to sea water had suppressed appetites at day 3 post transfer, but by day 30 returned to feeding normally.

In a study of the feed intake of Atlantic salmon, the fish were given either simulated natural light or 24 hour light. The fish exposed to natural light had a lower feed intake from April to August. However, from August until November, the fish given 24 hour light consumed more feed (Nordgarden *et al.* 2003). Elliot (1975) found that the maximum weight of feed consumed each day by brown trout (*Salmo trutta*) was positively correlated with both the weight of the fish and temperature. The larger the fish, the less the relative feed intake (percentage body weight per day). If the temperature was raised from 10°C to 15°C the relative feed intake approximately doubled (Figure 2.2).

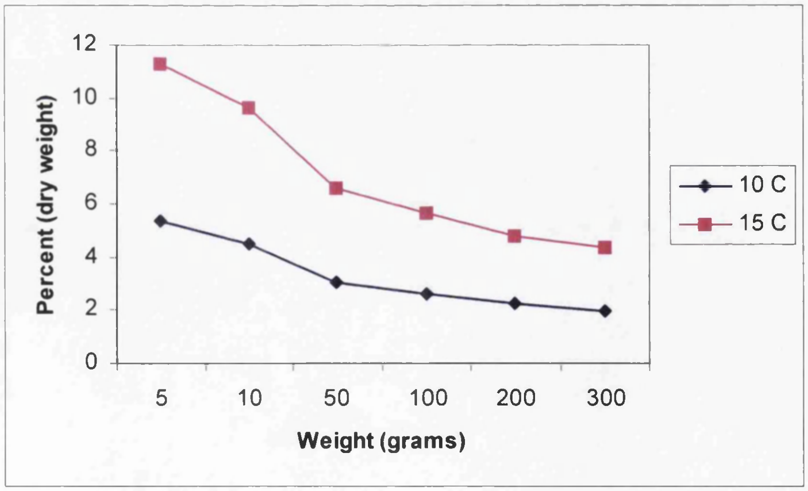


Figure 2.2 The dry weight of feed eaten in a day by trout in relation to body weight expressed as a percentage of the dry weight of the trout at 10 and 15°C (after Elliot, 1975).

Feeding frequency in sea bass Sea bass are generally nocturnal during the winter and diurnal during the rest of the year (Anthouard *et al.*, 1993)). Trials have shown that, a change in photoperiod alone does not cause this switch (Aranda *et al.* 1999a). Nor does varying the temperature or a combination of temperature and photoperiod induce nocturnal feeding (Aranda *et al.* 1999b). Instead it is probably due to other factors such as food availability or an endogenous circadian control. Sea bass from the Mediterranean eat different prey in different seasons, taking sardines (*Sardina pilchardus*) during the summer and autumn, mullet (*Mugil cephalus*) in the spring and annelid worms and small fish in the winter (Madrid *et al.* 2001). This could account for the seasonal change in feeding rhythm as the prey species in the summer are active by day, whereas annelid worms tend to be nocturnal.

Feeding frequency in gadoids: The majority of cod aquaculture is currently based in net cages where fish are exposed to ambient light and temperature conditions, although supplementary lighting may be used in order to interrupt maturation and improve growth and feed conversion ratio (FCR) (Davie *et al*, 2007, Taranger *et al.*, 2006, Hemre *et al.*, 2002). As the temperature increases, feed intake increases up to the point of optimum temperature (Jobling, 1988).

Smith *et al.*(2007) charted seasonal changes in stomach contents of wild cod from Cape Cod (Massachusetts, USA). There were two periods of increased feeding when schools of small pelagic fish arrived in the area. These two arrivals coincided with spawning and over wintering and the author speculate that these life history events have evolved to coincide.

2.1.3 Effects of temperature and photoperiod on food intake in fish

In the wild cod are exposed a wide range of temperatures being found in water between –1 and 20°C with spawning occurring in the narrow band of 5-8°C (Björnsson *et al.*, 2001). Björnsson and Steinarsson (2002) produced a model of cod growth at different temperatures. They found that optimal temperature for growth decreases with the weight of cod for fish between 50 and 5000g. Aranda *et al.* (1999a) found that increasing the water temperature for sea bass lead to increased feeding demands. In general, as temperature increases the energy demands of fish go up. For example figure 2.3 shows the effect of fish size and temperature on food intake (kilojoules per day) of cod.

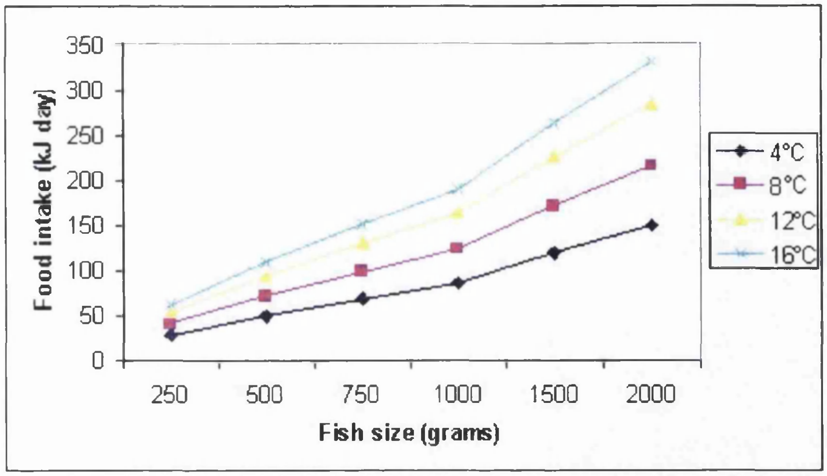


Figure 2.3 The effect of fish size and temperature on food intake of cod. Daily feed intake in relation to fish weight at four temperatures (after Jobling 1988)

Photoperiod is one of the most studied external cues that entrain the internal time keeping system of organisms (zeitgeber) for feed intake in most fish species. In cod, it is known to be the most important cue for maturation and spawning (Skjæraasen *et al.* 2004). The onset of maturation causes a decrease in appetite and generally increasing photoperiod stimulates increased feed intake whilst decreasing day length reduces feed intake (Kestermont and Baras, 2007). Smith *et al.*, (1993) found that the appetite of caged Atlantic salmon was more strongly correlated with photoperiod and change in photoperiod than other factors such as temperature. Continuous light gives the highest growth and therefore food intake in juvenile haddock (*Melanogrammus aeglefinus*) (Trippel and Neil, 2003). However, continuous light is known to decrease FCR and increase growth of Atlantic salmon (Nordgarden *et al.*, 2003).

2.1.4 Medium term appetite rhythms

Time series analysis has been used in modelling growth rates and food intake rhythms of brook trout (*Salvelinus fontinalis*) by Wafa *et al.* (2004). This group found periodicity of 4 -6 days in the rate of increase of the length of the fish, 5-10 days in the rate of increase of the mass. Feed intake also showed periodicity of 6 and 20 days that was not linked with growth rate.

As well as seasonal rhythms of feed intake fish show regular changes of appetite over periods of days to weeks probably related to stomach fullness and energy storage. Bailey and Alanärä, (2006) performed time series analysis on demand feeding data to explore medium term appetite patterns in rainbow trout (*O. mykiss*). The results showed that the feeding level of the groups of fish peak every second day probably because this it the time allowed for full gastric evacuation. Figure 4 shows the speed of gastric evacuation of rainbow trout of different weights at a range of temperatures.

A number of papers (including Lambert and Dutil (2001) and Lambert *et al.*, (1994)) state that cod can be fed every second day with little impact on the growth rate as 'it may take several days for the food consumed in a single meal to be emptied from the stomach' (Jobling, 1988). Currently on cod farms it is usual to feed every day as it is thought that in a cage holding thousands of fish there are always going to be hungry fish (Bourhill 2007, Personal communication).

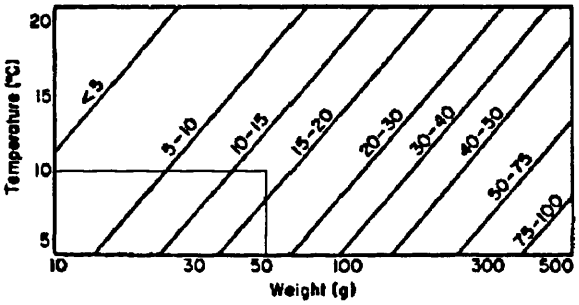


Figure 2.4 Gastric emptying times for rainbow trout of different sizes at different temperatures. For example, the thin black line marked on the graph shows the example of a 50 gram fish at 10°C that would take 15 hours to empty. This chart can also be used to predict feeding frequency for rainbow trout. Grove *et al.* (1978).

Indeed, Solberg *et al.* (2006) found no significant difference between the weight of cod (with a start weight of 500 grams) fed two or three times a week compared to fish fed five times per week. The main difference found in this trial was a larger amount of feed was given to the fish fed five times a week, which led to a higher FCR, though it is not known if this was due to higher levels of waste feed or if it was biologically higher, i.e., was more feed consumed for the same growth rate as those fed less?

2.2 Aims

As this review makes clear, much less is known about the appetite rhythms in cod and the factors that influence them than in other species. With this background, the overall objective of the present study were:

- To use the AQ1 interactive feeding system to monitor daily feed intake in juvenile cod across a full year.
- To chart the annual feeding pattern of juvenile cod held in tanks.
- To investigate the influence of temperature and photoperiod on feed intake.
- To perform time series analysis to examine periodicity in the feed intake

2.3. Methods

This study was carried out at the Marine Environmental Research Laboratory, Machrihanish, Scotland (55° 26' 14.00" N, 005° 41' 11.00" W). The husbandry was carried out in accordance with Stirling University's standard practices which included a program of size grading to maintain stocking densities at commercial levels and reduce aggression between larger and smaller fish.

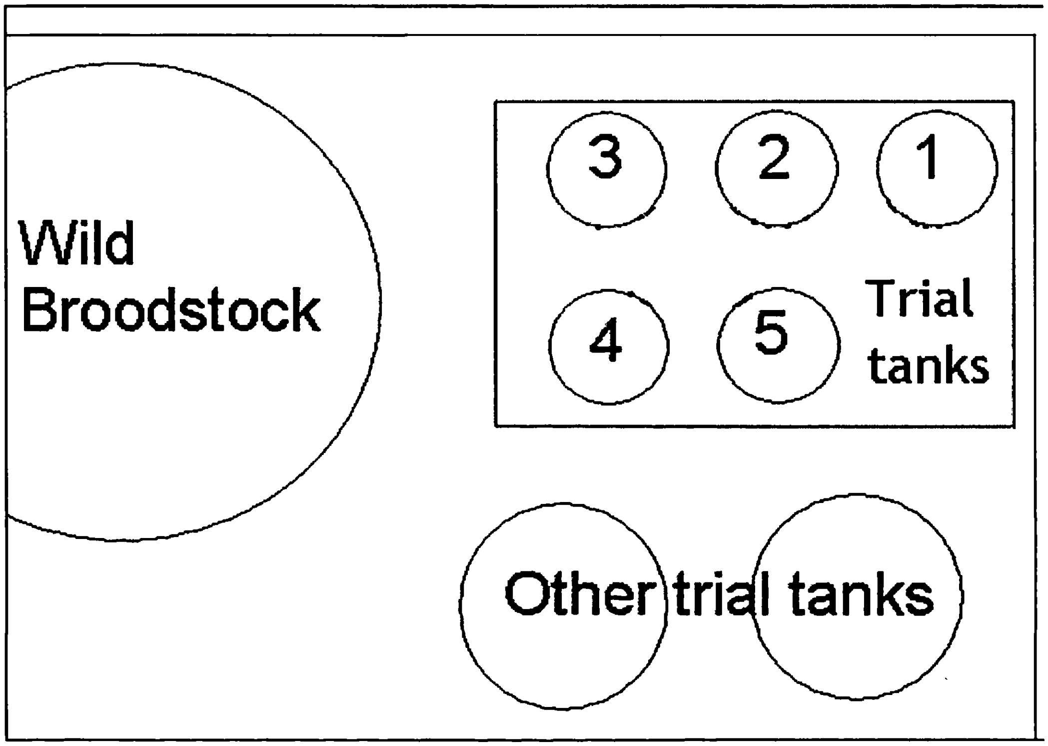


Figure 2.5 A diagram of the layout of experimental tanks used for this trial in relation to other tanks in the area (not to scale but the trial tanks were of 1 metre diameter). The diagram shows proximity of the trial tanks to other tanks and a large broodstock tank. Tanks 1, 2 and 3 were less disturbed than 4 and 5 because there was a walkway between tanks 4 and 5 and the other tanks.

The trial was broken down into 2 parts. Part 1 ran from 6th March 2004 until 24th August 2004. The fish had a mean start weight of 4.16 grams. Part 2 started with a second batch of fish with a mean start weight of 5 grams on 12th November 2004 and continued until 14th February 2005. In part 1 as the fish grew they were graded twice. A diagram of the changes due to grading is shown in figure 2.6.

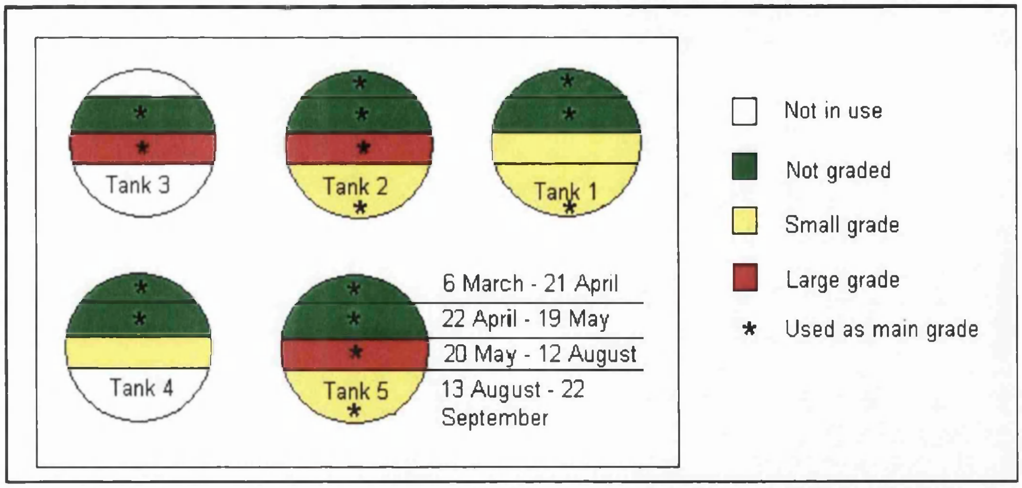


Figure 2.6 Diagram of dates and grade sizes in each tank in part 1 of the trial. Green indicates that the fish were not graded, red denotes the large grade and yellow the small grade. Grading occurred on 19th May and 12 August. The asterisk shows which tanks were focused on as the main grade in the different time sections.

In order to maintain biomass at standard levels in each tank and to keep fish in groups containing similarly sized fish, they were graded twice and redistributed three times in part 1 of the trial. The dates of the changes were 21st April (redistribution), 19th May (grade) and 12th August (grade). From the beginning of the trial to the redistribution 4 out of the five tanks were being used. At that point the fish were split across the five tanks. At the first grade (19th May the fish were graded into large and small grades. The large fish were distributed across tanks 2, 3 and 5 while the small grade was split between tanks 1 and 4. At this point just the large grade was analysed. On 12th August some of the largest grade of fish had out grown the tanks and so all the fish were graded and any over 50grams were removed from the trial. The remaining fish under 50 grams were distributed across tanks 1, 2 and 5 and those tanks were then used for analysis.

2.3.1 Housing conditions

Five one-metre diameter round tanks, each with a volume of 124 litres were stocked initially with 300 cod (supplied by Machrihanish Marine Farm Ltd.) in each (mean weight 4.2 grams) on 3rd March 2004. These fish were from an out-of-season batch produced through photoperiod manipulation. As the trial progressed the fish were graded using a manual grader and the number in each tank reduced in order to avoid overcrowding.

The water supplying the tanks was pumped ashore through a pipeline set 30m off the beach. The water was screened through a 5mm mesh, filtered to 100μm through a drum filter and was delivered to a header tank using a submersible pump from whence it flowed by gravity to the tanks. The water arrived at the tanks at ambient sea temperature and salinity.

A 1.9 metre tall wooden frame was constructed around the tanks and this was covered with black-out plastic to minimise disturbance and allow the photoperiod to be uninfluenced by room lights. The photoperiod was controlled by an Electrovent™ dawn dusk dimmer unit connected to two dimmable fluorescent strip lights which were suspended along the central line of the frame. The photoperiod was updated weekly to reflect the ambient light conditions.

2.3.2 The AQ1 feeding system

Each tank had an Aq1 unit controlling their respective feeders. Figure 2.7 shows a simplified schematic diagram of the system with arrows representing the data flow. They were fed on the Ewos Marine diet following the standard Ewos feed table.

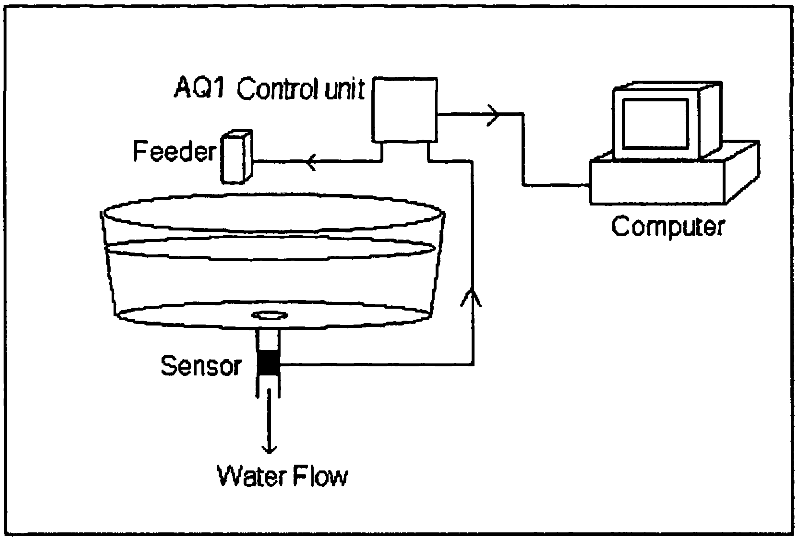


Figure 2.7 Schematic diagram of the AQ1 system, the arrows represent the data flow.

Programming the AQ1 system is reasonably complex but essential if the system is to work well, both for feed management and research. It is therefore described in some detail here.

Setting	Meaning
Start time	The clock time that a part of the program begins
End time	The clock time that a part of the program ends
Sense high	The maximum number of pellets counted before the system stops feeding or reduces feed level
Sense low	The maximum number of pellets counted before the system increases feed level
Feeder setting: time	The amount of time the feeder is switched on for each actuation
Minimum feed	The minimum amount of feed that can be delivered per feeder actuation
Maximum feed	The maximum amount of feed that can be delivered per feeder actuation
Minimum sleep	The minimum length of time that the system will remain inactive between test feeds
Maximum sleep	The maximum length of time that the system will remain inactive between test feeds
High repeat	Limits the maximum number of feeder actuations that can occur at once
Gain	The sensitivity of the sensor
Sense time	The time (in seconds) that the sensor is active
Depth	Depth of the sensor
Sink rate	Rate of sink of the pellet
Pellet size	Entered for user reference only

Table 2.1 Some settings and their meaning for the AQ1 system.

The AQ1 system is programmed using the settings listed in table 2.1. A 24 hour period could be broken down into sections with different program settings for each section. These sections were defined by the start time and end time. At the beginning of part 1 of this trial, the day was broken into three sections; the time from midnight to sunrise, from sunrise to sunset then from sunset until midnight. Feed was introduced every 15 minutes during the day and 30 minutes at night at the beginning, then, 3 weeks into part 1, the algorithm was changed so that the same program, introducing feed every 15 minutes ran throughout the whole 24 hour period. The sensor was programmed to come on at the correct time by manipulating the depth of sensor and sink rate settings. The time that the pellets took to begin to exit the tank was measured, then the sink rate and sensor depth were set to reflect that amount of time. In this case the pellets took around 40 seconds to reach the sensor after being introduced by the feeder so the depth was set as 4 metres and the sink rate as 10 cm per second. In this case the sensor was set to be active for 40 seconds using the sense time setting. The sensitivity of the sensor (known as the gain) had levels from 1 to 5. This was regularly changed to match the level of suspended solids in the water column. For instance, if the suspended solids level increased then the gain was reduced and vice versa.

The feeder was produced by Imetronic and worked by rotating a disk with three small holes. When feed was to be released, the disk was spun so that one of the holes lined up with a corresponding hole underneath and a small measured amount of food fell through to the tank below. The amount of time for the disk to spin to release one hole of food was 6 seconds set in the Feeder menu under time. The minimum and maximum feed levels are the minimum and maximum amounts of time the feeder can spin in one actuation. In this case they were multiples of 6 seconds so the feeder would not stop between holes. The feeder delivered a known quantity of feed each time it spun. Every time the feed size was changed (as the fish grew) the amount of feed delivered by one spin changed. The feed rate was recalculated and entered into the software in order to keep an accurate record of feed introduced.

The fishes' hunger was tested every 15 minutes by adding a small amount of feed (one spin of the feeder). That time between feeds was set by entering 15 minutes in the Minimum and maximum sleep categories. 40 seconds after feed was added the sensor activated and counted the number of waste pellets exiting the tank. If the number of pellets counted was below the number set in "sense high" the feed was repeated. If the number was below "sense low" the feeding was repeated and increased. "Sense low" and "sense high" were often changed if changing the gain of the sensor was unsuccessful. If the sensor was counting too many pellets, the "sense low" and "high" could be raised and vice versa. As a safety feature, the "high repeat" was set, this was the maximum number of spins the feeder could perform before the system for that tank shut down and had to be reset manually.

Figure 2.8 shows an example of the computer screen seen during the trial. At the time of the screen shot, the system for that tank was working well and it shows a main morning meal and smaller feeding peaks throughout the day. There is a main meal time at 05:00 and this part of the graph is magnified in figure 9, so that the functioning of the AQ1 system can be observed clearly. The feeder is activated for a test spin of 6 seconds and the sensor counts less than the sense low minimum, so the level of feeding is increased for the second and third actuations, until it hits the set maximum of 30 seconds. This level is maintained until the pellet counts reach the sense high level and the feeder cuts to a lower level. This is then responded to by a lower count, so the feeder increases spin time again, until finally there are two counts over the sense high level and the system returns to 'sleep' for another 15 minutes.

The software interface that was used to control the AQ1 system was "AQPC". This program allowed the AQ1 unit settings to be maintained and showed the feed input and pellet counts of each unit. Screen shots from this program are reproduced in figures 2.8 – 2.12.

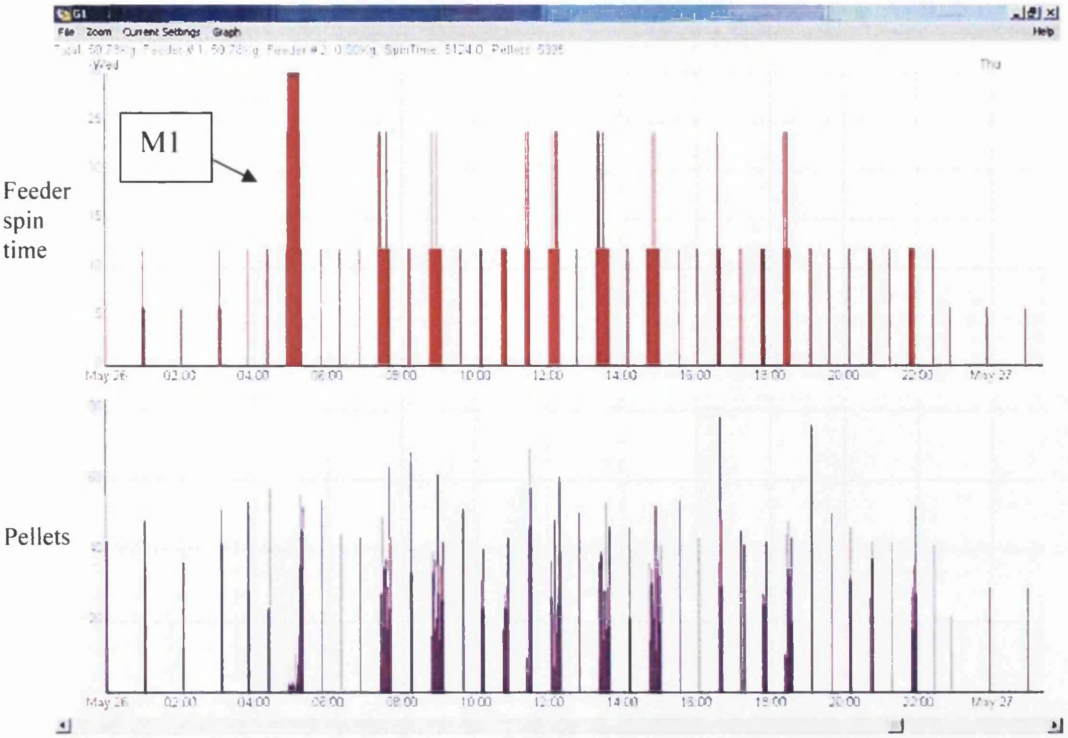
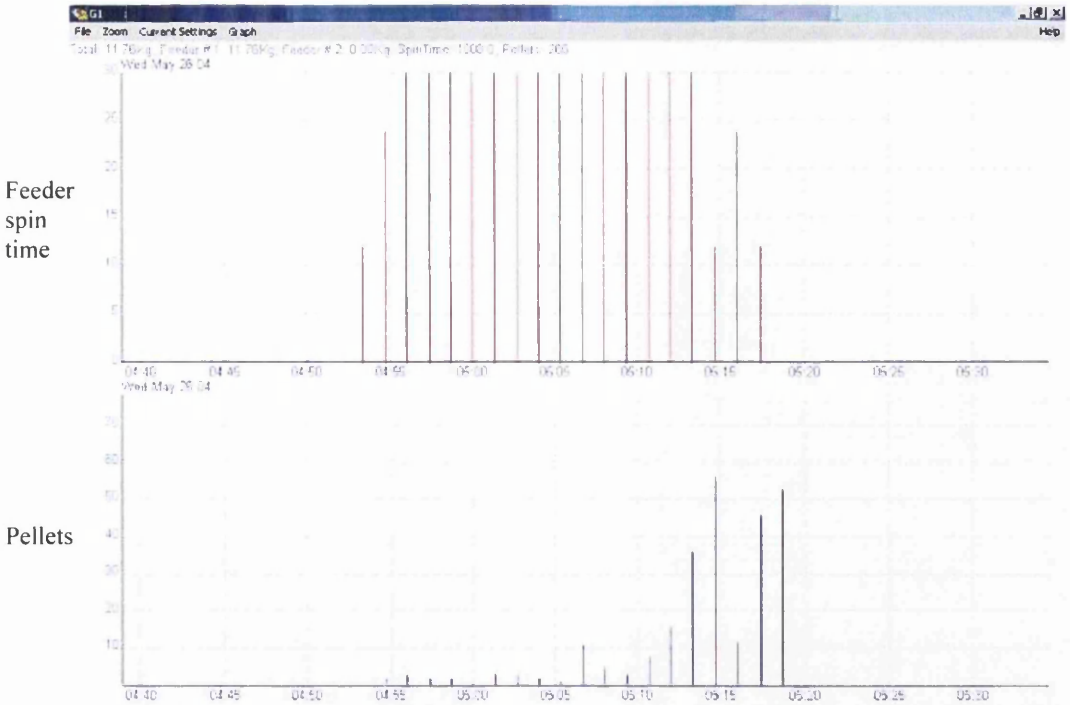


Figure 2.8. A screen shot of AQPC showing a detailed graph for one tank. The top graph represents the amount of time the feeder spent spinning, i.e., the amount of feed distributed into the tank. The bottom graph shows the number of pellets counted by the sensor 40 seconds after each feeder spin. M1 is the main morning meal, which is shown in greater detail in figure 9

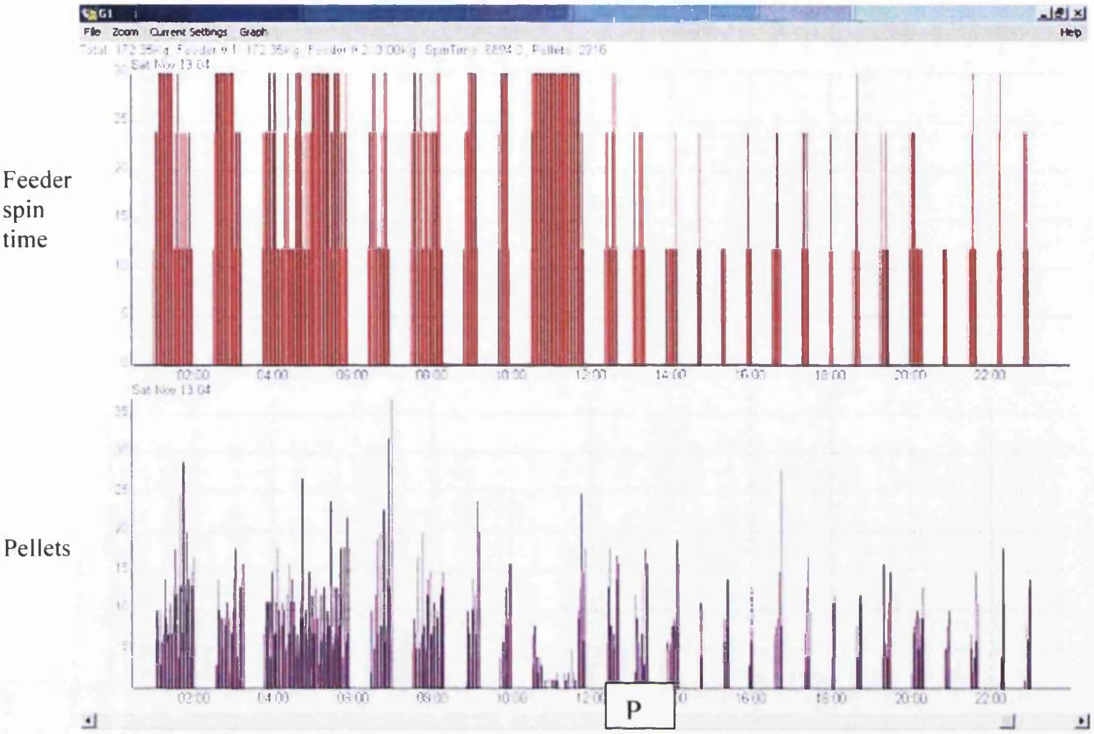


Total 11.76Kg, Feeder #1 11.76Kg, Feeder #2 0.00Kg, SpinTime 1000.0, Pellets 305

Figure 2.9 A screen shot of AQPC showing one meal. The top graph represents the amount of time the feeder spent spinning, i.e., the amount of feed distributed into the tank. The bottom graph shows the number of pellets counted by the sensor 40 seconds after each feeder spin.

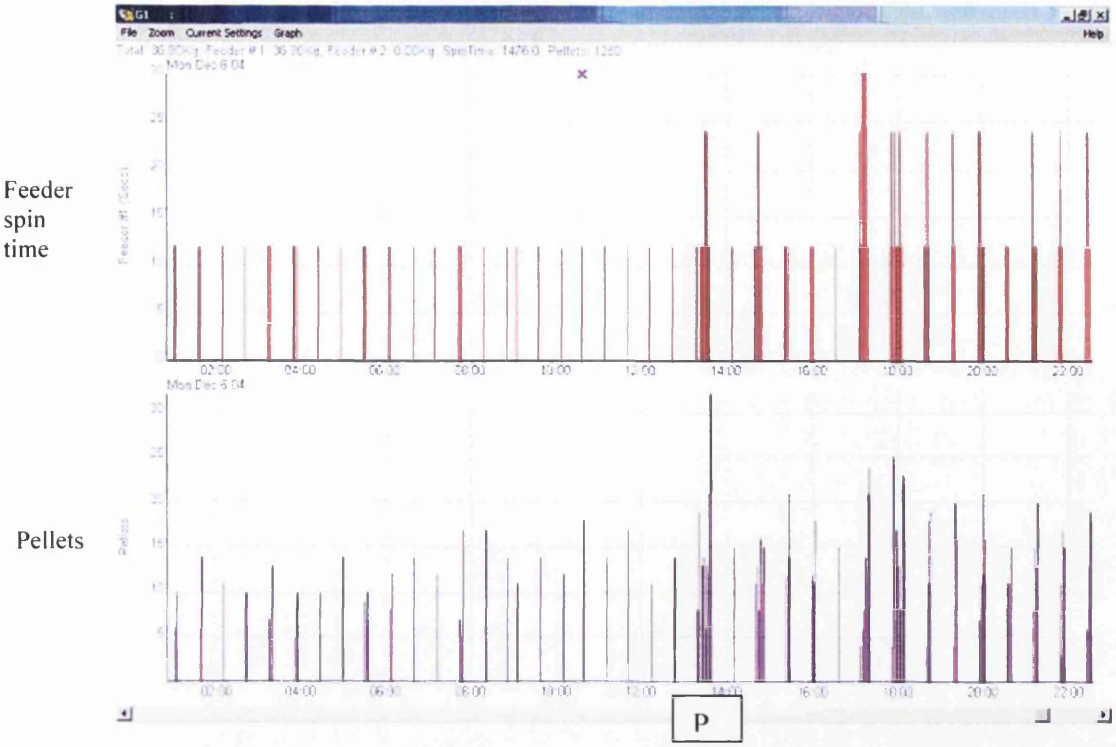
2.3.3 Problems

The AQ1 system is not usually used in tanks or for such small fish and so some problems were encountered during the trial. Steps were taken to attempt to keep the feed input as even as possible and the following screen shots give examples of these problems and how they were overcome.



Total: 172.35Kg; Feeder # 1: 172.35Kg; Feeder # 2: 0.00Kg; SpinTime: 8894.0; Pellets: 2316

Figure 2.10 A screen shot from AQPC graph readout of the daily feed input (top graph) and pellet counts (bottom graph). Until approx 13:00, over feeding due to sense low and high being set too high (8 and 15 respectively) and the pellet counts consistently being under these values. Point P marks a change in the program when the sense low was changed to 5 and the sense high to 10 leading to reduced feed input.



Total: 36.90Kg, Feeder #1: 36.90Kg, Feeder #2: 0.00Kg, SpinTime: 1476.0, Pellets: 1260

Figure 2.11 – Initially there is not enough feed being given because the pellet counts are exceeding the sense low and high levels. At point P, the sense low and high were changed from 5 and 10 to 10 and 15 respectively which resulted in higher feeding.

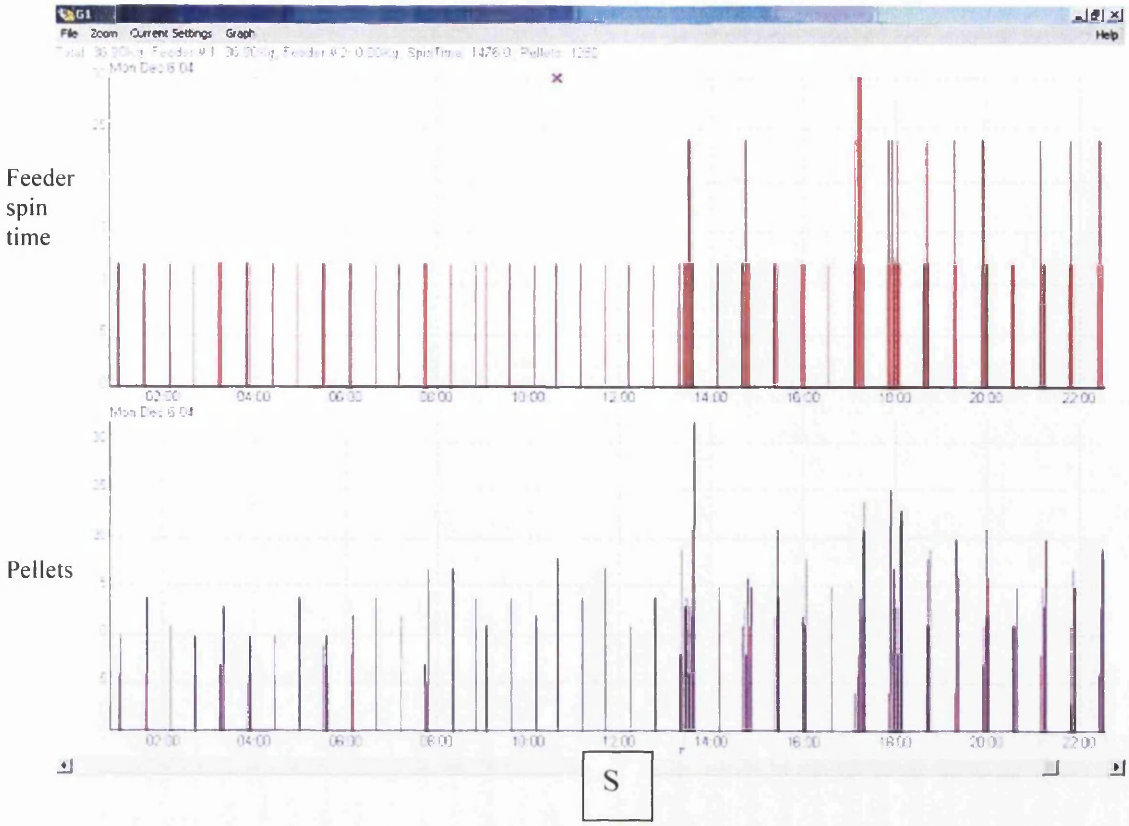


Figure 2.12 A screen shot from AQPC showing under feeding at the start of the day, at 10 am (point S) the gain of the sensor is decreased to exclude a wider range of debris sizes in order to decrease the pellet counts. This is successful and after the change feeding occurred at a higher level continues.

The AQ1 sensors were working at the limit of their ability because the pellets were smaller than the minimum recommended, though the sensors had been recalibrated. The sensors are designed to sense uneaten pellets in sea cages where the pellets sink slowly. However, as they were at the outlet of the tank with a fast flow, the pellets moved through at high speed making it difficult to differentiate between an uneaten feed pellet and a piece of faeces, seaweed or sediment. There were often peaks of suspended solids in the water when the sea was rough. This meant that the apparent number of pellets counted increased. As soon as this was noted the gain (sensitivity) of the AQ1 units was increased. Once the suspended solids in the tank had returned to a lower level the opposite problem occurred which caused over feeding.

Days such as those shown in figures 2.10, 2.11 and 2.12 were removed from the data set as well as days when the "high repeat" safety setting was implemented, as they do not give an accurate picture of feed input. Other days were removed if a “max high” was reached.

2.3.4 Monitoring growth

The fish were sampled every fortnight, alternating between batch and individual weighing. Batch weighing involved removing all the fish from each tank individually and weighing them together into a bucket of tank water. The fish were then counted back into the tank, so the mean weight of fish in that group could be calculated. Individual sample weighing involved weighing 30 randomly caught fish from each tank. Each fish was weighed in a beaker of tank water then their standard length measured using a fish ruler.

2.3.5 Monitoring water temperature and day length

Water temperature was measured daily by Machrihanish personnel to the nearest degree (Celsius) using a digital temperature probe. The photoperiod of the trial tanks was controlled by an automatic dimming unit connected to dimmable strip lights. The system was programmed to follow the ambient photoperiod with a 40 minute dawn and dusk sequence. The lighting system was updated weekly.

2.3.6 Data analysis

The feed conversion ratio (FCR) was calculated for all the data collected (including the removed days) to give a true picture of feed wastage through the trial using the equation over periods between weighing sessions.

$$FCR = \frac{Feedweight}{Weightgain}$$

Daily feed intake data in grams were retrieved from the AQ1 system at 1 minute intervals and divided by estimated biomass to give daily feed intake as a percentage of biomass per day. Changes with date were examined using polynomial regression. When assessing the data for each tank there were obvious differences in the data between tanks so they were considered separately for the regression analysis.

Time series analysis was then used to examine the occurrence of repeating patterns in the feed intake, using autocorrelation, with a lag period of up to 30 days and confidence limits of 95% using SPSS 14 for Windows.

Regression was used to investigate the relationship between temperature and photoperiod of both the daily feed intake and the FCR using Minitab 14.

2.4 Results

2.4.1 Temperature and photoperiod

Figure 2.13 shows the daily temperature for the year of March 2004 until March 2005 when the trial took place. The temperature ranges from 4°C in January 2005 to a high of 15°C between July and September 2004. Figure 2.14 shows the photoperiod for the trial period. The dawn and dusk times were updated weekly, so the curve is made of weekly "steps". The proportion of light over each 24 hour period ranges from 30% to above 70%. The rate of change of photoperiod is fastest at the equinoxes and slows at the solstices.

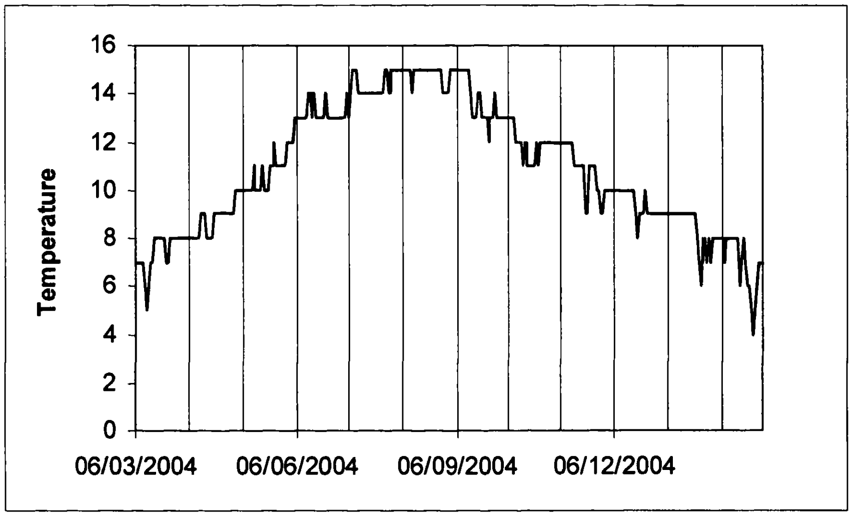


Figure 2.13 Water temperature (degrees Celsius) across the trial period

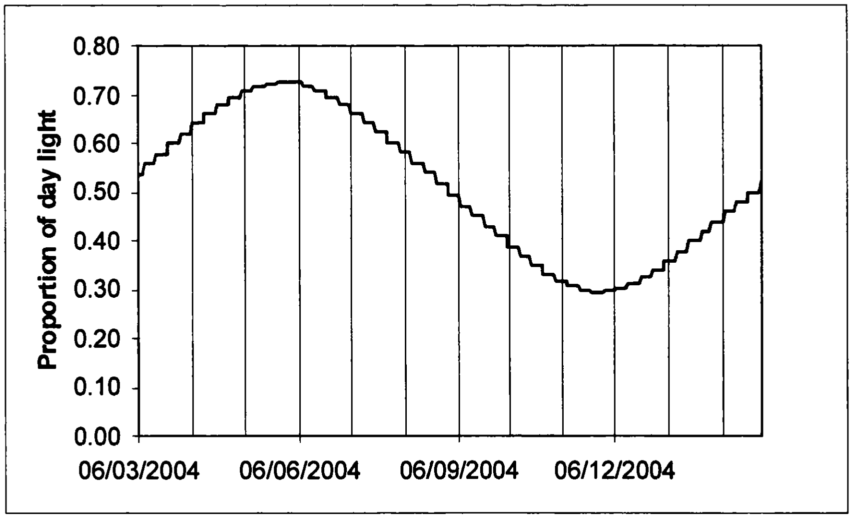


Figure 2.14 Proportion of light over 24 hours across the trial period

2.4.2 Production data

The first aim of the trial was to assess whether juvenile cod in tanks could be fed by the AQ1 system. This was successful and throughout the trial the fish adapted to using the AQ1 system and grew continuously. Gaining production data was not one of the aims of this project, however, since information was collected on biomass growth and feed delivery feed conversion ratios (FCR) were calculated.

2.4.2.1 Part 1

A one-way ANOVA shows that there was a highly significant increase in mean weight across the trial (F=83.08, $P<0.0001$).

Figure 2.15a shows the mean weight of the fish in the tanks with the main grades in during part 1 of the trial (denoted by an asterisk in figure 2.6) for the 4th March until the 30th September. The jumps in weight (marked by arrows) correspond with gradings and redistributions, see figure 2.6.

Figure 2.15b shows the mean weight of the small grade from 4th May until 12th August, an one-way ANOVA again shows a very highly significant increase in mean weight across the trial (F= 14.42 $P<0.0001$ df = 2).

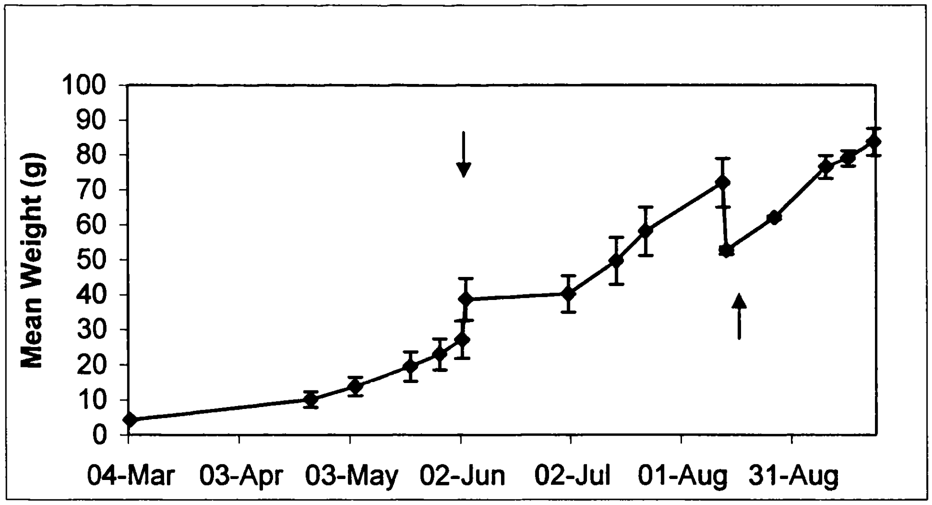


Figure 2.15a Mean weight of main grade of juvenile cod in grams across time with standard error of the mean. Arrows correspond with grades. For which tanks this graph refers to, see figure 2.6.

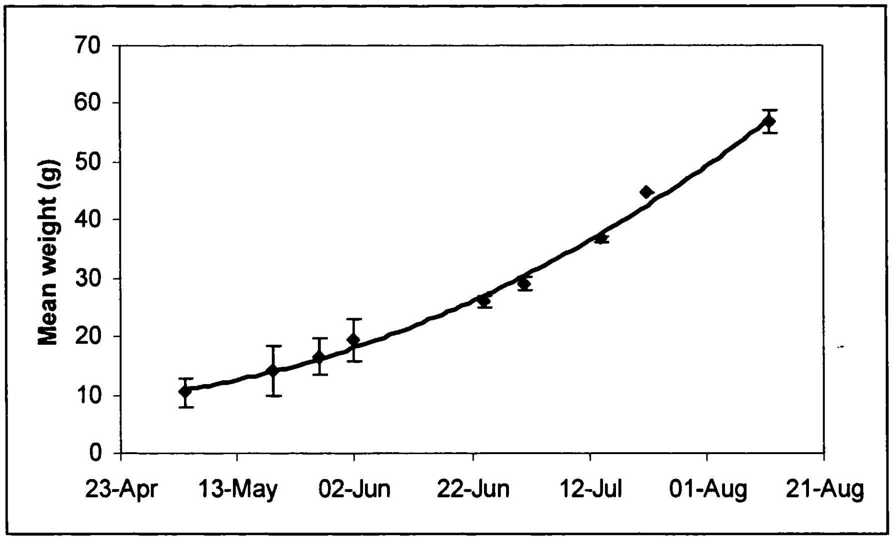


Figure 2.15b Mean weight of the small grade cod, tanks 1 and 4 (in grams) across time for the first phase of the trial from 4th May until 12 August.

2.4.2.2 Part 2

During part two of the trial, a different batch of cod were used, they adapted well to using the AQ1 system again. Figure 2.16 shows growth was steady with very little variance from the mean weight so no grading was carried out. The mean weight increased significantly throughout the trial (one way ANOVA, F= 181 $P<0.0001$ df= 3)

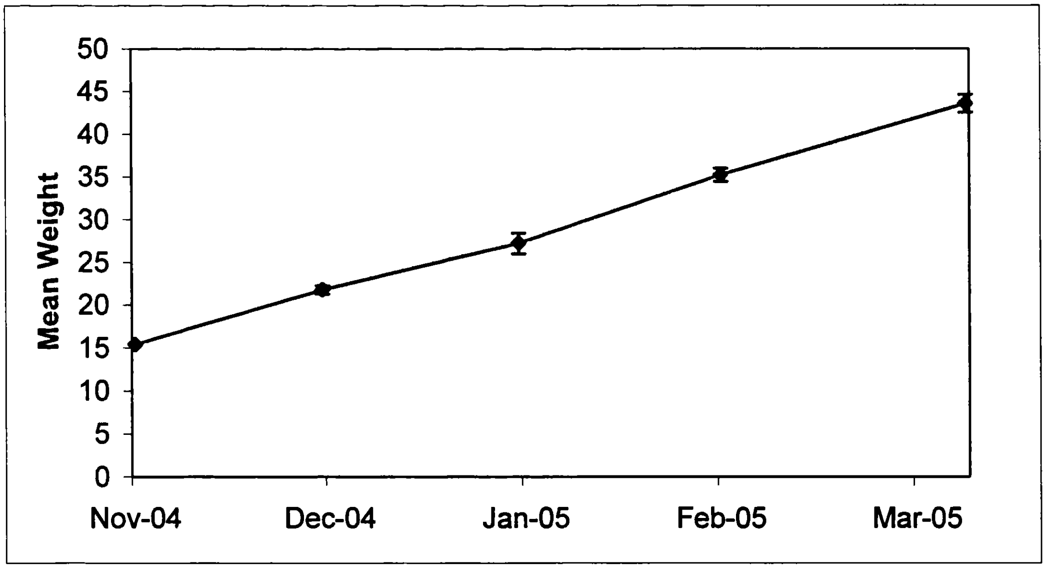


Figure 2.16 Mean weight of cod in grams with standard error across time for the second phase of the trial.

2.4.3 Feed conversion ratio

2.4.3.1 Part 1

Figure 2.17a shows the mean FCR of main grade during part 1. There were two dramatic peaks in FCR. The calculated FCR was not the biological feed conversion but simply the amount of feed introduced into the tank.

If the outlying peaks are removed, there is a significant rise in FCR across the 6 months of this trial as shown in Figure 2.17b (One way ANOVA, F= 4.65 P= 0.001 df=2). The two peaks have a high variation as they are caused by over feeding in one tank.

The FCR of the focused on grade increase across time. Towards the end of May, FCR dropped to an impossibly low level; this was caused by under reporting of the amount of feed being given; there were a high number of "high repeats" in this period and the days where this occurred were automatically removed from the data set (see section 2.3.3). The FCR peaked at the beginning of July, once more, caused by a sensor fault.

Figure 2.17c shows the FCR of the small size grade has two large peaks at around late May and mid to late June. If these are removed, there was a non-significant trend for feed conversion to increase over time. Even with the outliers removed, the FCR was generally much higher than predicted commercial FCR.

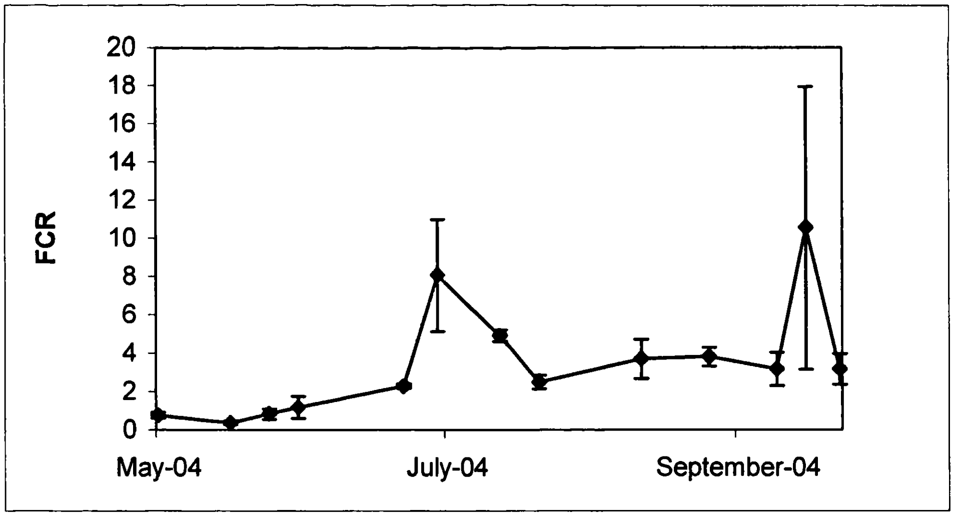


Figure 2.17a Mean FCR (feed conversion ratio) with standard error between tanks of the mean for the first phase of the trial for the main grade group.

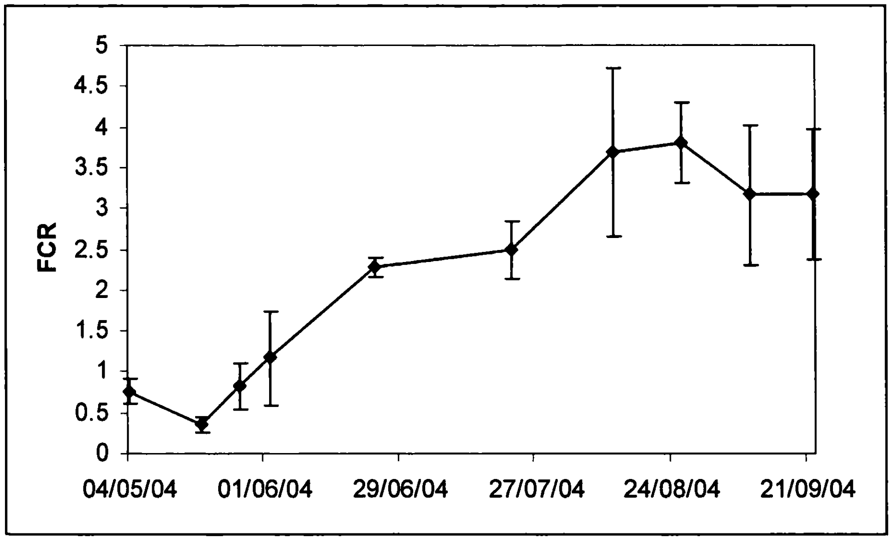


Figure 2.17b Mean FCR (feed conversion ratio) with standard error of the mean for the first phase of the trial for the main grade group with outliers removed.

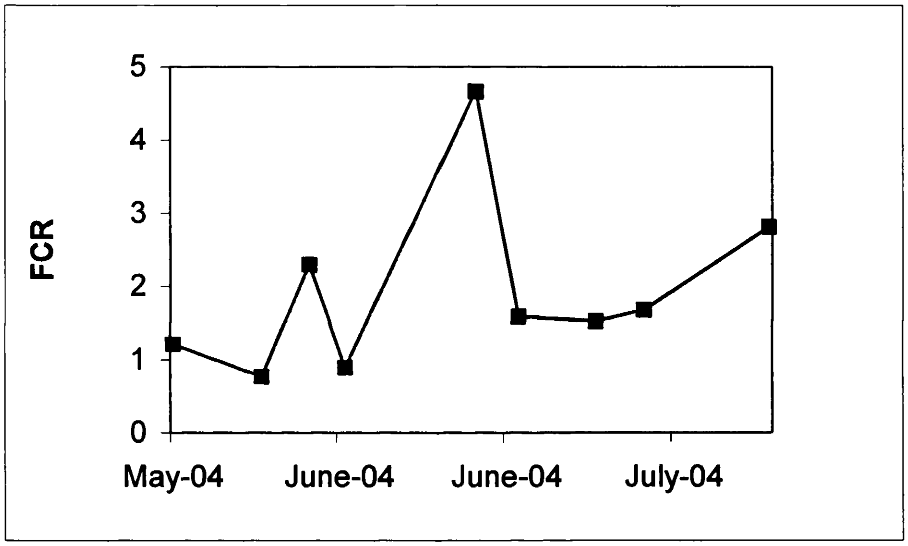


Figure 2.17c Mean FCR for the first phase of the trial for the small grade

2.4.3.2 Part 2

Figure 2.18 shows the mean FCR across all tanks in part 2. The FCR in part 2 varied between 1.5 and 2.75. There is a decreasing trend, although it is not significant (one way ANOVA F= 0.81 P =0.542 df= 3). There is less variation in the part 2 compared with the part 1.

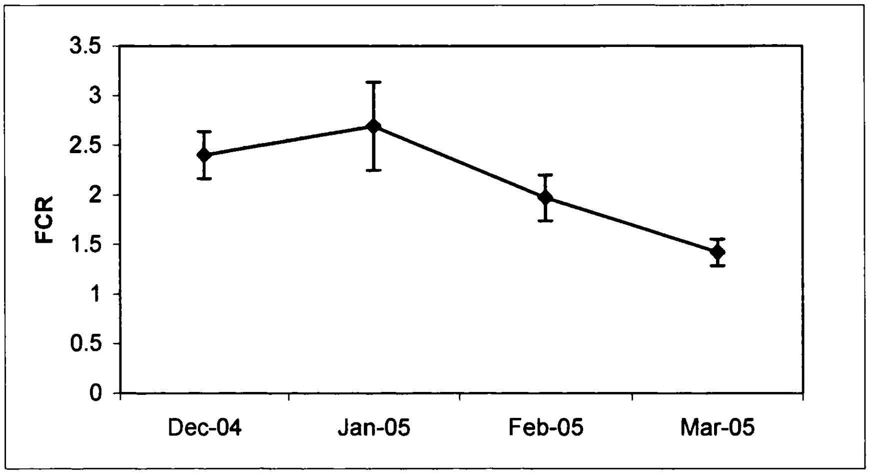


Figure 2.18 Mean FCR for the second phase of the trial with standard error of the mean for all tanks

Figure 2.19 shows the FCR at different day lengths for each tank. The photoperiod had no significant impact on the feed conversion ratio in any of the tanks. In tanks 2, 3 and 5 there was a non-significant trend for the FCR to rise as the day length increased while tanks 1 and 4 did not show any consistent pattern.

Figure 2.20 shows the effect of temperature on the FCR. In tanks 1, 2, and 3 there was a significant positive correlation showing that as temperature increased, so did FCR. Tanks 4 and 5 show a similar but non-significant pattern.

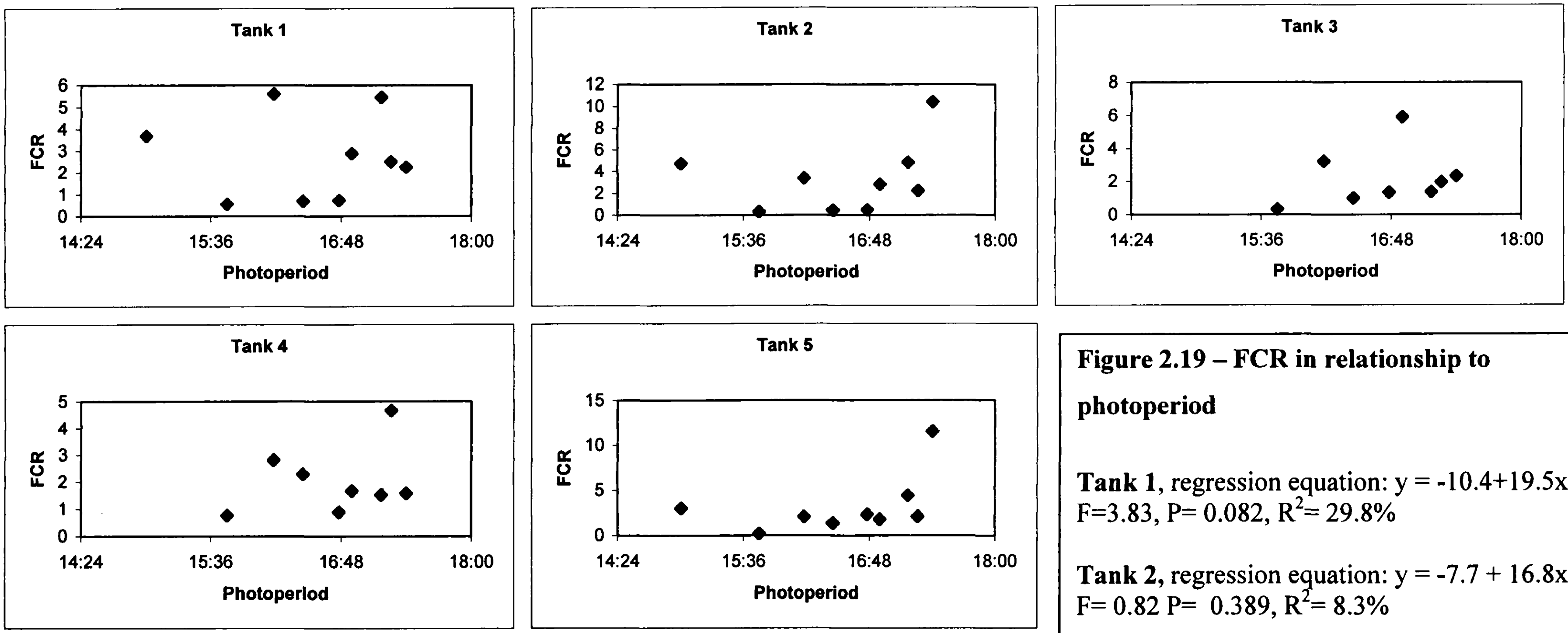


Figure 2.19 – FCR in relationship to photoperiod

Tank 1, regression equation: $y = -10.4+19.5x$
$F=3.83$, $P= 0.082$, $R^2= 29.8\%$

Tank 2, regression equation: $y = -7.7 + 16.8x$
$F= 0.82$ $P= 0.389$, $R^2= 8.3\%$

Tank 3, regression equation: $y = -12.7 +21.7x$
$F = 2.92$ $P = 0.138$, $R^2= 32.7\%$

Tank 4, regression equation: $y = -3.66 + 8.3x$
$F = 0.61$ $P = 0.466$, $R^2= 9.2\%$

Tank 5, regression equation: $y = 34.4 - 43.8x$
$F = 0.90$ $P = 0.368$, $R^2= 9.1\%$

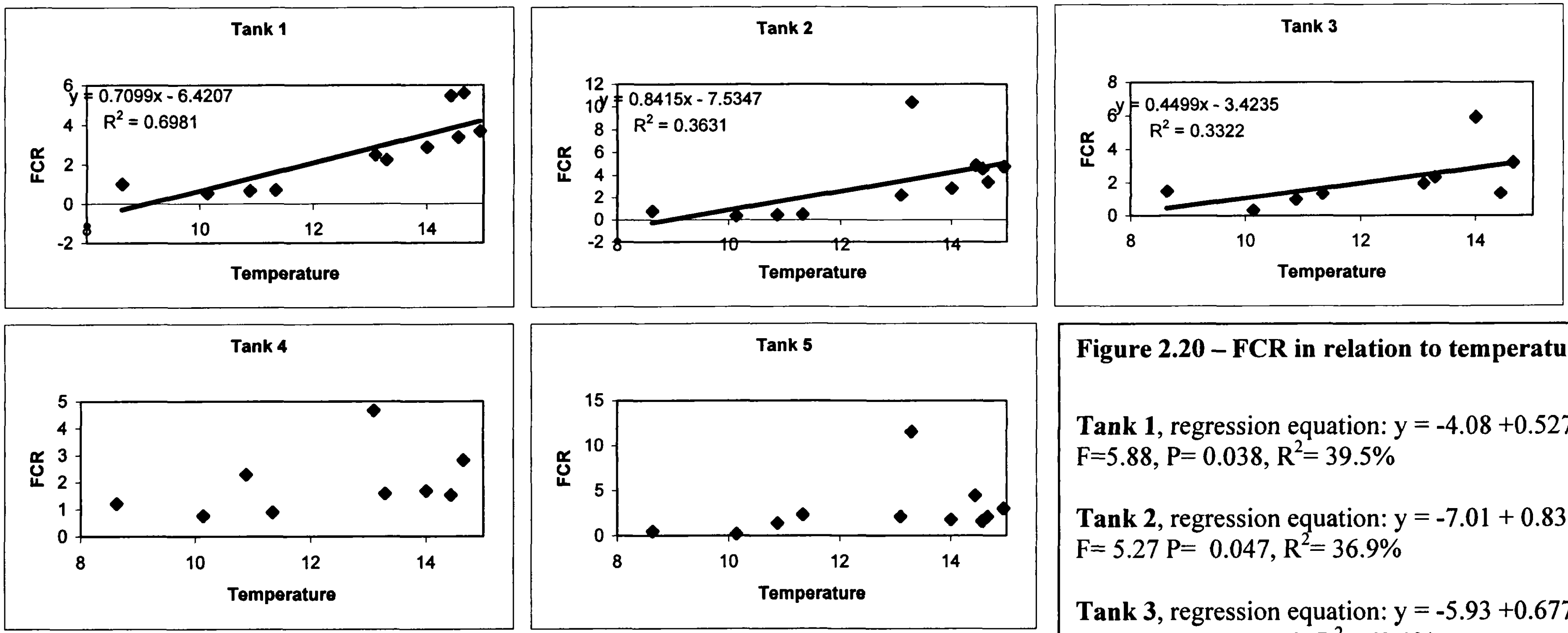


Figure 2.20 – FCR in relation to temperature

Tank 1, regression equation: $y = -4.08 + 0.527x$
$F=5.88$, $P= 0.038$, $R^2= 39.5\%$

Tank 2, regression equation: $y = -7.01 + 0.831x$
$F= 5.27$ $P= 0.047$, $R^2= 36.9\%$

Tank 3, regression equation: $y = -5.93 + 0.677x$
$F = 10.22$ $P = 0.019$, $R^2= 63.0\%$

Tank 4, regression equation: $y = 0.85 + 0.098x$
$F = 0.16$ $P = 0.705$, $R^2= 2.6\%$

Tank 5, regression equation: $y = -10.6 + 1.23x$
$F = 1.34$ $P = 0.277$, $R^2= 13\%$

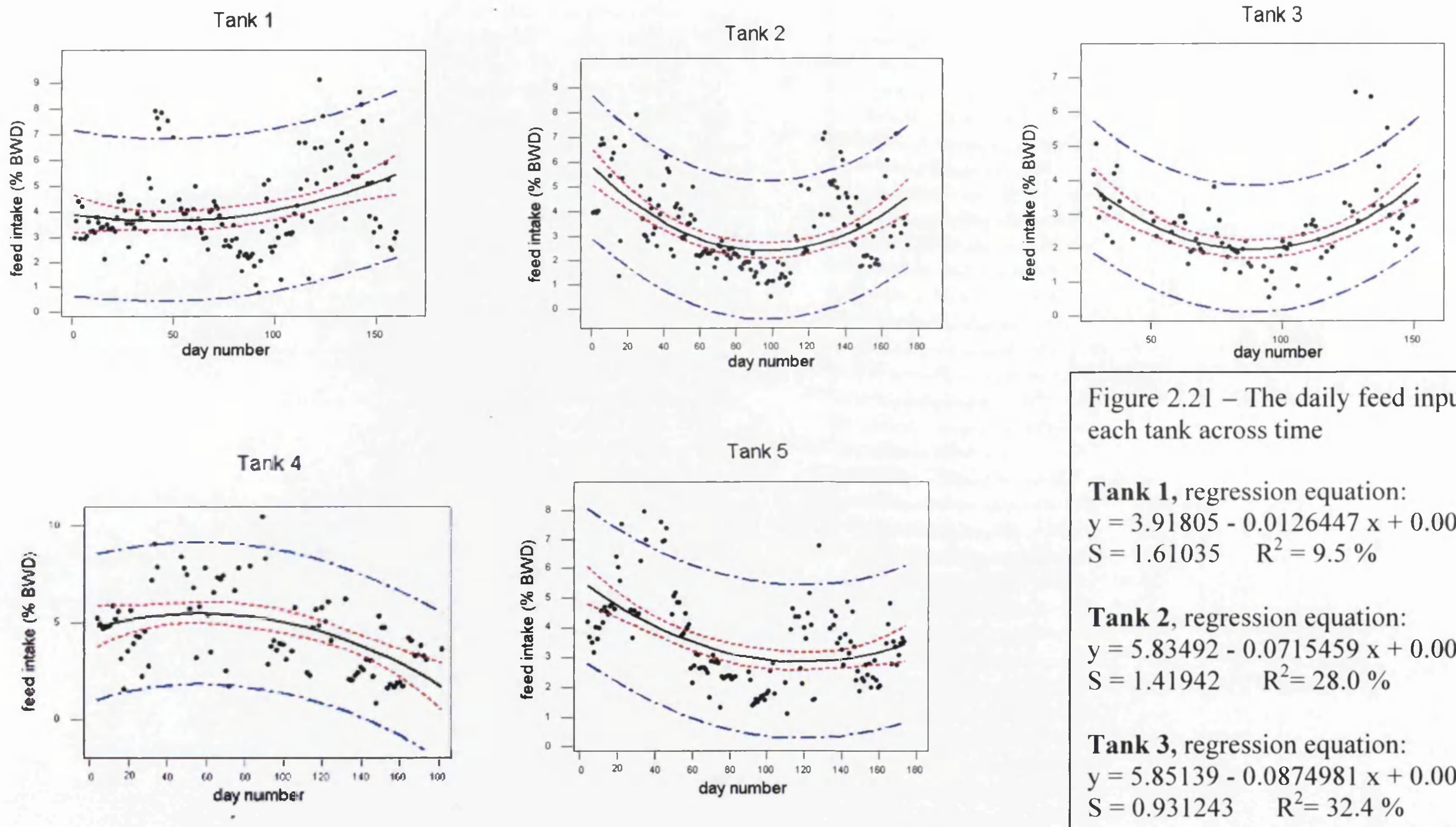


Figure 2.21 – The daily feed input (percent bwd) for each tank across time

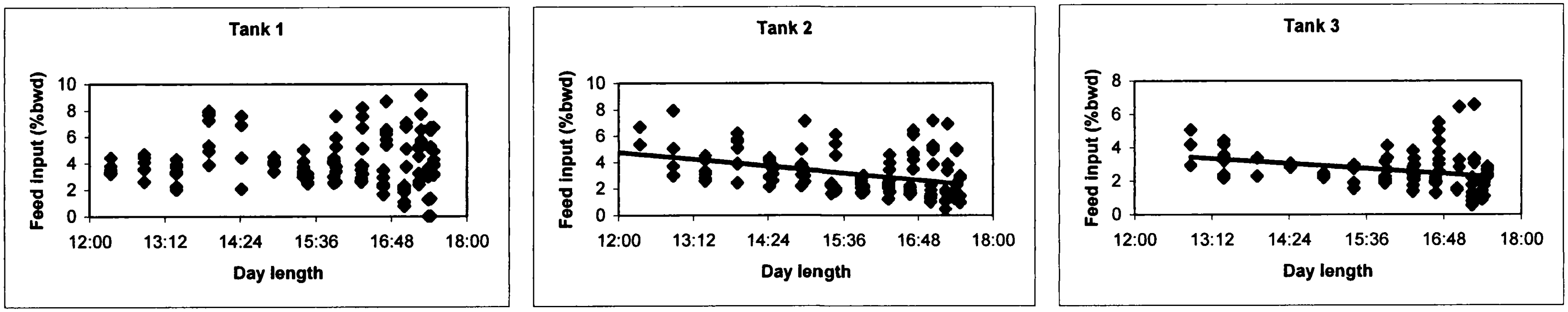
Tank 1, regression equation:
$y = 3.91805 - 0.0126447\ x + 0.0001385\ x^2$
$S = 1.61035 \quad R^2 = 9.5\ \%$

Tank 2, regression equation:
$y = 5.83492 - 0.0715459\ x + 0.0003696\ x^2$
$S = 1.41942 \quad R^2 = 28.0\ \%$

Tank 3, regression equation:
$y = 5.85139 - 0.0874981\ x + 0.0004929\ x^2$
$S = 0.931243 \quad R^2 = 32.4\ \%$

Tank 4, regression equation:
$y = 4.74745 + 0.0413677\ x - 0.0005845\ x^2$
$S = 1.82789 \quad R^2 = 24.5\ \%$

Tank 5, regression equation:
$y = 5.60470 - 0.0463850\ x + 0.0001951\ x^2$
$\mathbf{S = 1.29475 \quad R^2 = 24.6\ \%}$



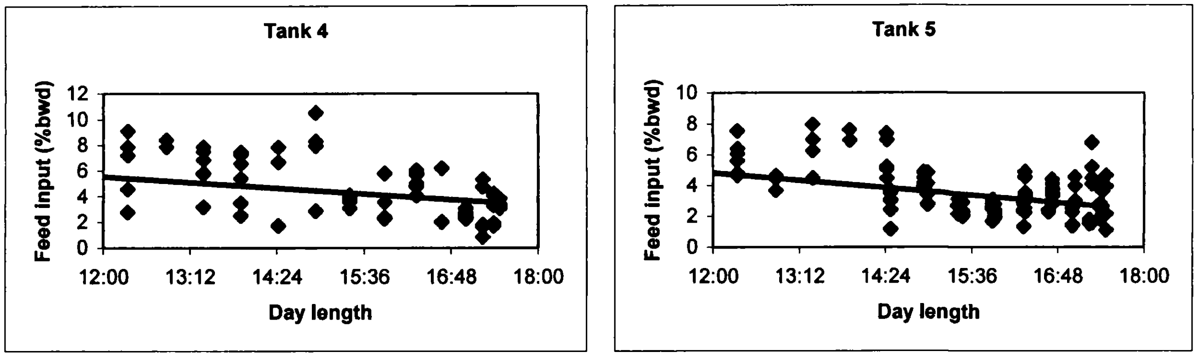


Figure 2.22 – The relationship between photoperiod and feed input (percent bwd) for each tank

Tank 1, regression equation: $y = 2.58 + 2.35x$
$F=1.99$, $P= 0.160$, $R^2= 1.3\%$

Tank 2, regression equation: $y = 9.97 - 10.4x$
$F= 40.34$, $P < 0.0001$, $R^2= 21.9\%$

Tank 3, regression equation: $y = 6.67 - 6.02x$
$F = 8.55$, $P = 0.004$, $R^2= 8.8\%$

Tank 4, regression equation: $y = 10 - 8.93x$
$F = 15.37$ $P < 0.0001$, $R^2= 15.2\%$

Tank 5, regression equation: $y = 9.89 - 9.99x$
$F = 49.09$ $P <0.0001$, $R^2= 26.8\%$

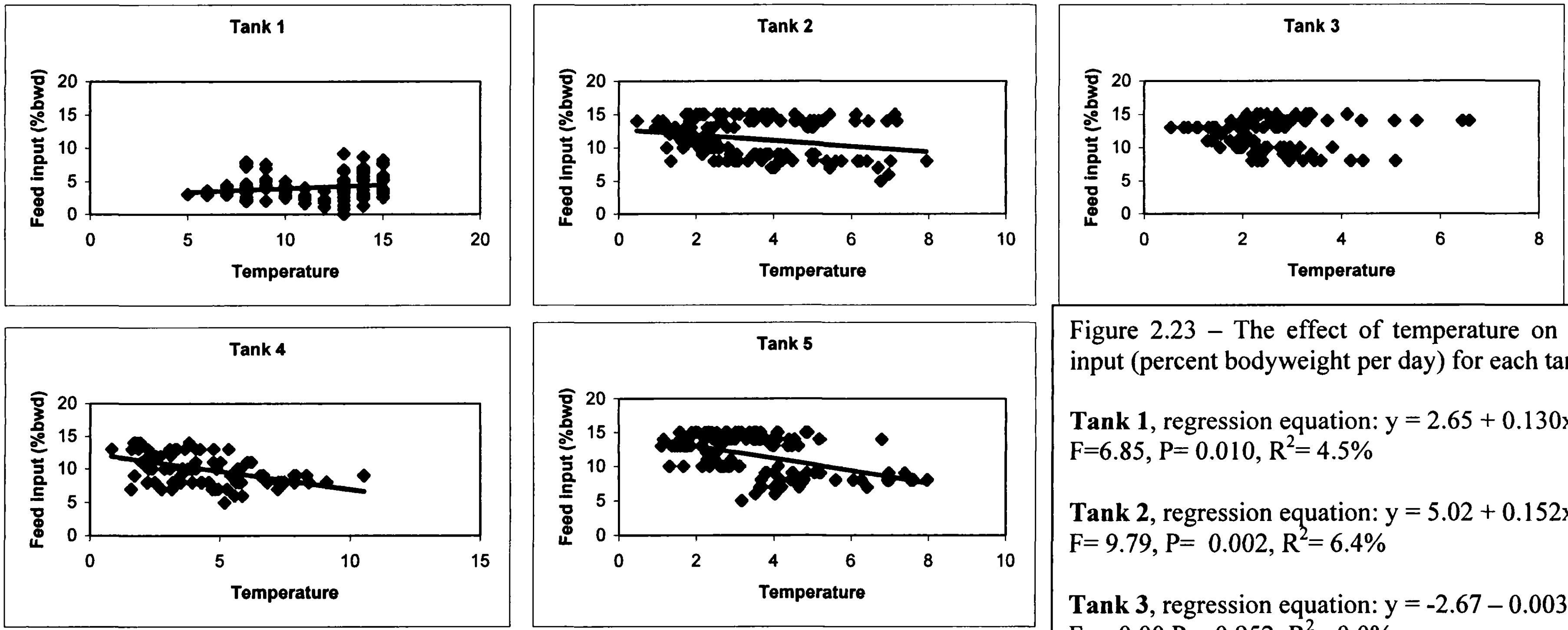


Figure 2.23 – The effect of temperature on feed input (percent bodyweight per day) for each tank

Tank 1, regression equation: $y = 2.65 + 0.130x$
$F=6.85$, $P= 0.010$, $R^2= 4.5\%$

Tank 2, regression equation: $y = 5.02 + 0.152x$
$F= 9.79$, $P= 0.002$, $R^2= 6.4\%$

Tank 3, regression equation: $y = -2.67 - 0.0031x$
$F = 0.00$ $P = 0.952$, $R^2= 0.0\%$

Tank 4, regression equation: $y = 8.81 - 0.435x$
$F = 26.78$, $P < 0.0001$, $R^2= 23.7\%$

Tank 5, regression equation: $y = 6.26 - 0.237x$
$F = 35.58$ $P < 0.0001$, $R^2= 21\%$

2.4.4 Daily feed intake

Figure 2.21 shows the daily feed input as a percentage of the total biomass for each tank over time. All tanks except tank 4 show a similar U shaped pattern with the percentage feed intake dropping to its lowest level around June and higher levels (between 6% and 10% bodyweight per day) in March and August. Tank 4 has a different pattern where there appears to be a negative polynomial correlation of percent feed intake and time. There is however, a lot of variation in all tanks.

Figure 2.22 shows the relationship between day length (hours of daylight) and the percentage body weight fed per day (bw.d^{-1}) for day lengths of between 12 and 17 hours of daylight. In all tanks except tank 1 there is a significant negative correlation. Tanks 2, 3 and 5 show a higher variation at the longer day lengths, possibly because there are more data points in this area.

Figure 2.23 shows the relationship between temperature and feed input. Tanks 2, 4 and 5 have similar patterns with a significant fall in feed input with increasing temperature, although the gradient of the graph of tank 2 is very shallow. Tanks 2, 3 and 5 show a bimodal distribution of feed inputs with the two peaks at around 8 and 15% bw.d^{-1}. Tank 1 shows a significant increase in feed input with increasing temperature but the feed input in this graph is lower than the other graphs.

Figure 2.24 shows the mean feed intake (%bw.d^{-1}) (across seven day periods) plotted against the rate of change of day length (as a fraction of the whole day). There is no significant pattern. There are eight outlying points at ±0.15, these points are due to the hour change going into and coming out of British summer time. The remaining points are evenly dispersed with no trend due to rate of change of photoperiod.

Figure 2.25 shows the mean feed intake (%bw.d^{-1}) (across seven day periods) plotted against the mean rate of change of temperature (over seven day periods). There is no significant trend. The majority of the points are positive or no changes in temperature, the points at the extremes of temperature change show a non-significant trend of having high feed intake, while the smaller changes are distributed across the whole range.

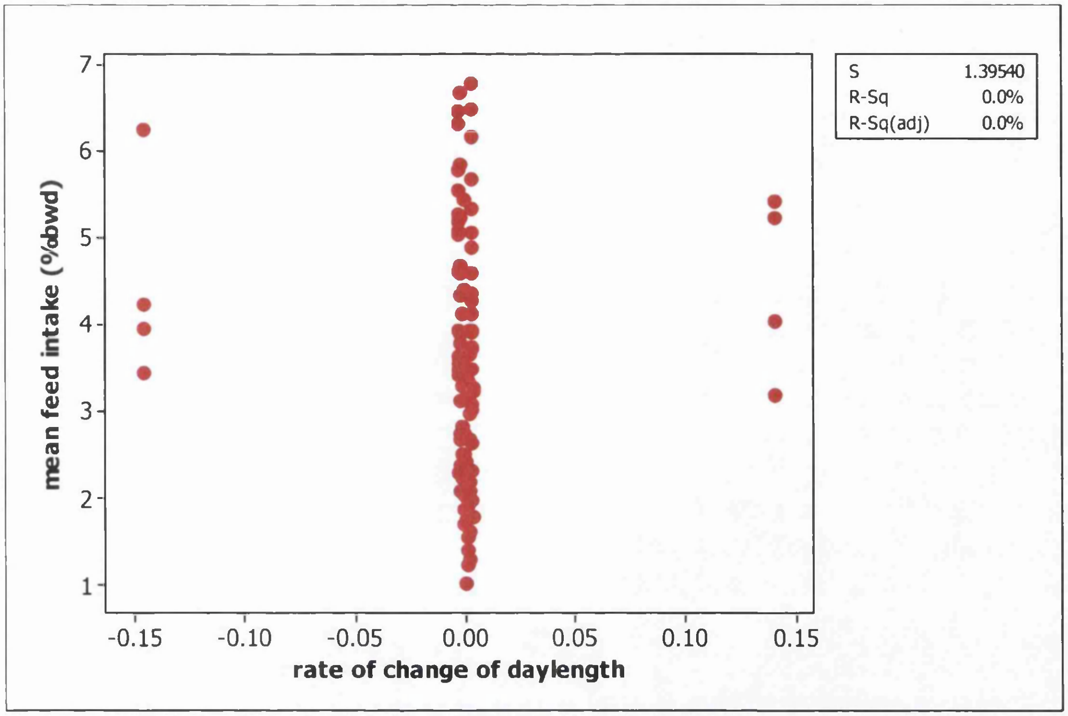


Figure 2.24 The effect of the rate of change of day length on mean feed input (percent bodyweight per day) for all tanks

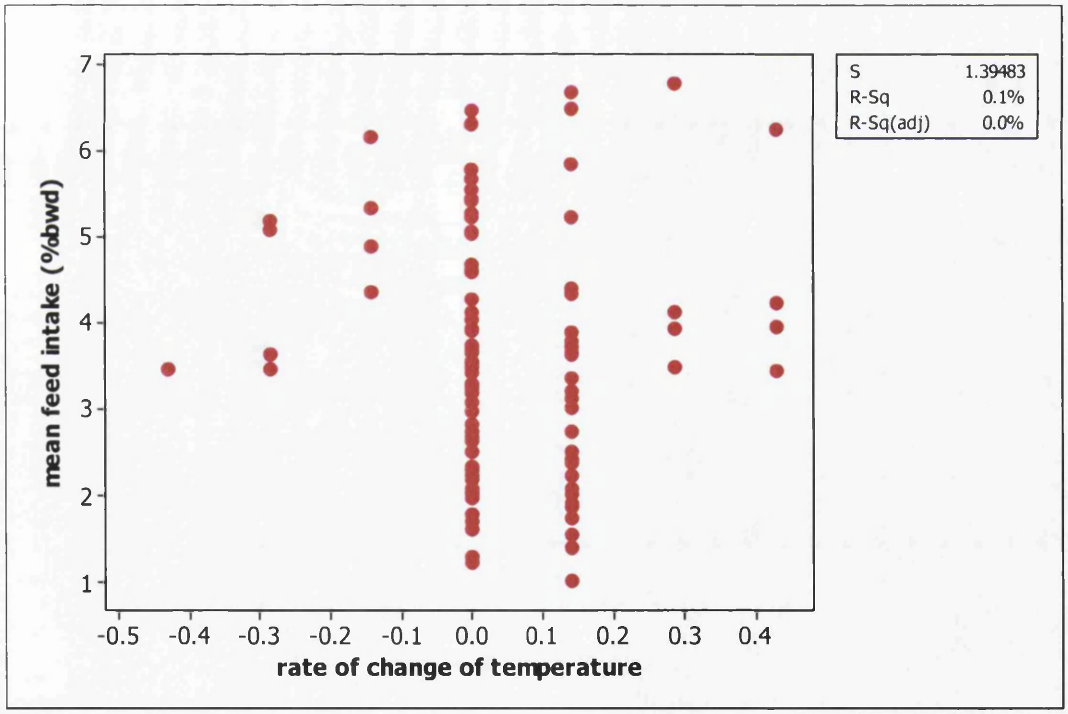


Figure 2.25 The effect of the mean rate of change of temperature on feed input (percent bodyweight per day) for all tanks

2.4.5 Medium term appetite rhythms

The daily feed input (percent body weight per day) was examined by time series analysis to investigate the level of autocorrelation in the data. (Figure 2.22). All tanks show a decreasing wave type pattern although with different levels of significance and periodicity. Tank 1 has a positive autocorrelation coefficient (ACF) from day 1 until day 14 with a wave of approximately 9 days. Tank 2 has a positive ACF from day 1 until 16, with a repeating pattern of 6 to 10 days. Tank 3 has a significant ACF from day 1 until 12 with a repeating pattern of 6 days. Tank 4 has significant ACFs on days 1, 5, 6 and 8 until 11 and tank 5 has a significant ACF at days 1, 3, 9, 16 and 26

The later significant lags are probably due to random noise as the data set is so large.

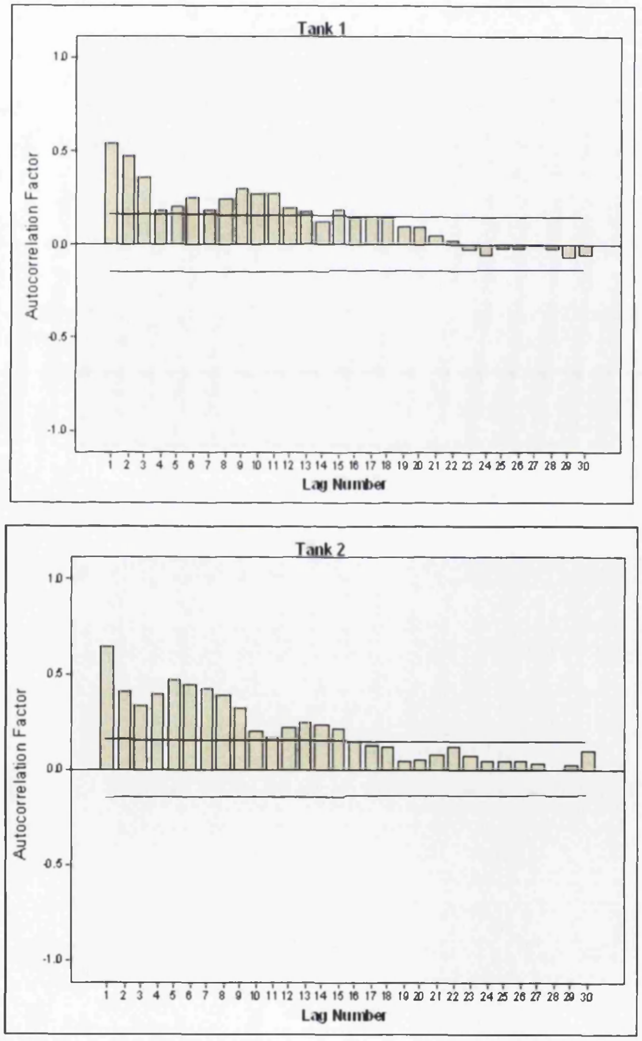


Figure 2.24 Autocorrelation coefficient for each tank with 95% confidence limits. There is a lag period of 30 days. (Continued over page)

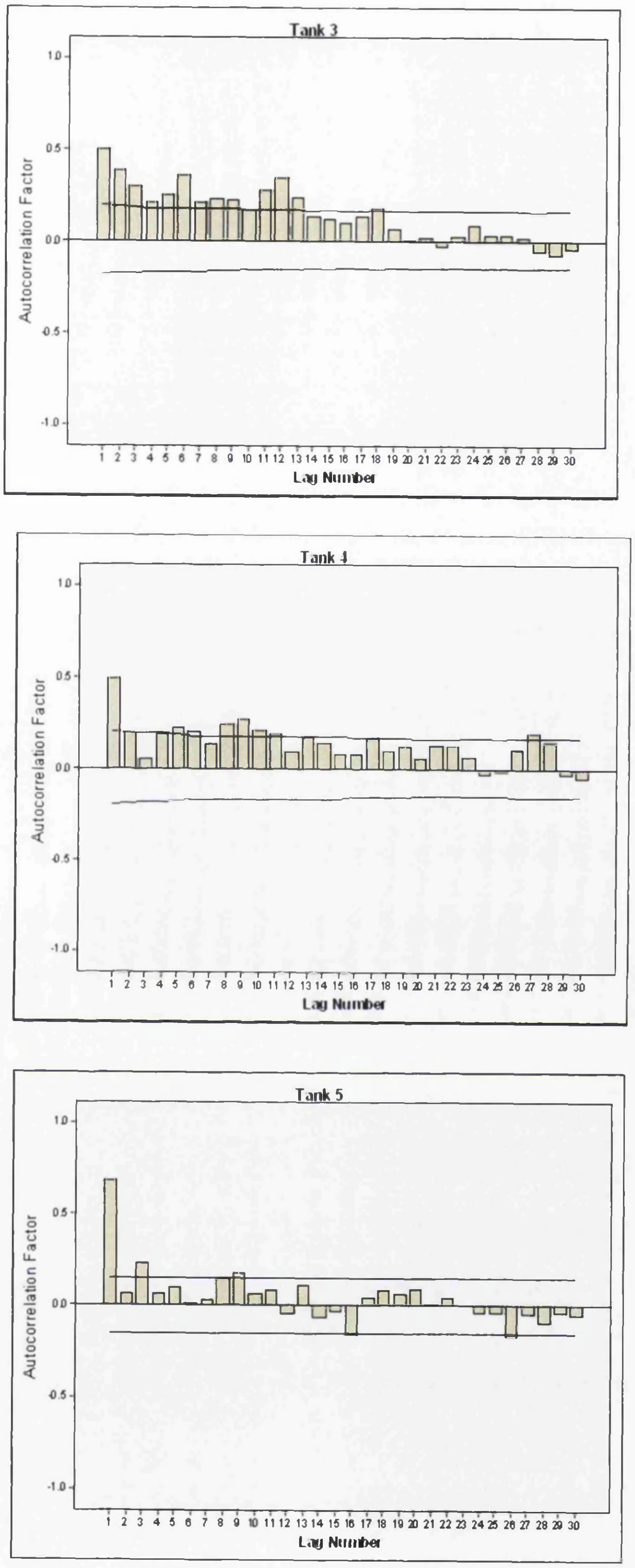


Figure 2.24 continued Autocorrelation coefficient for each tank with 95% confidence limits. There is a lag period of 30 days.

2.4 Discussion

The first aim of this trial was to set up and use the AQ1 system for small juvenile cod in tanks. This was successful but was labour intensive for the reasons mentioned in the methods section. The pellets were at the edge of the capability of the sensors and were often confused with faeces and suspended solids causing underfeeding. As the water was pumped in from the sea, suspended solids levels were constantly changing with tides and weather conditions so the algorithm and gain had to be changed in time with these factors. This would not have been a problem in larger fish as the pellet size would have been larger. At certain times the pellet counts were so high, even on the system's lowest sensitivity, that a particular tank was taken off adaptive feeding and it was used simply as an automatic feeder. At one of these points the tank was emptied and filled with tap water in order to test the sensors and the sensitivity returned to normal, even though the sea water that had prompted such high readings appeared clear. This suggests that if the water was filtered more thoroughly before reaching the tanks there may have been fewer problems.

2.5.1 Production

The FCR showed a slight trend, though not significant of increase with longer daylight hours and it also increased with temperature for temperatures up to 15°C. As temperature controls the rate of metabolism it might be expected that higher temperature should result in lower FCR because as the temperature rises, metabolism becomes more efficient. However, at the top temperature in the trial the fish may be beyond the optimum temperature for growth. However Kjartansson *et al.* (2007) found that juvenile cod from broodstock caught off western Norway had estimated optimum temperatures of 12.3°C when reared under 24 hour light. When reared under a simulated natural photoperiod for Bergen the estimated optimum temperature was 15.7°C. The fish used in that trial were native to Norwegian waters which are colder than the area that the southerly fish used for the present trial come from. Therefore it might be expected that the optimum temperature for the present fish may be higher.

Photoperiod did not appear to have any effect on FCR. This is in contrast to other fish species where the photoperiod can have significant effects. For example Nile tilapia (*O. niloticus*) have the best growth rate and FCR when kept at 18L:6D (El-Sayed and Kawanna, 2007). The mulloway, (Argyrosomus japonicus) a perciforme fish of the family Sciaenidae, shows best FCR at the photoperiod of 12L:12D (Ballagh *et al.*, 2008). The above studies assessed a number of unchanging specific photoperiods whereas this study had continuously shifting photoperiods as the seasons changed. This means that the results gained from the two papers cannot be compared directly. If a number of different continuous photoperiods were tested in this study there may have been more obvious differences.

Lighting is often used in aquaculture to promote faster growth and delay maturation. In an investigation of the effect of photoperiod on gonad development in cod, Hemre *et al.* (2002) found that 24 hour light gave the lowest FCR – this was not a direct effect of photoperiod, it was the photoperiod's effect of delaying maturation that was the cause.

It was found that in three out of the five tanks, a higher FCR was found at higher temperatures. This is opposite to the expected effect; generally higher temperature of an organism leads to an increased metabolic rate and therefore more efficient food processing. This is stated in a number of papers in a variety of species, for example, Nordgarden *et al.* (2003found this effect in Atlantic salmon (*S. salar*). A similar pattern is seen in Nile tilapia, but if the temperature is raised above the optimum for their growth FCR increases (Azaza *et al*, 2008). This may be part of the reason that the cod in the present trial showed a higher FCR at higher temperatures, the optimum temperature for this strain of the species may be approaching the higher temperatures in this trial. In the remaining two tanks there was no link found between temperature and FCR. This could have been because

Photoperiod and temperature were both changing weekly and daily respectively, meaning that there may be some level of interaction between them. This was difficult to determine as the temperature was uncontrolled and so the two factors could not be entirely separated.

2.5.2 Feed intake

In four out of the five tanks, the feed intake tended to decrease as the day length increased, though only slightly. This is opposite of the expected result – it is usually expected that fishes appetite grows as photoperiod increases (Kestemont and Barras, 2007), although this is not true for nocturnal fish. The following chapter investigates the daily feeding patterns found in this trial, and confirms that the cod are partly nocturnal, although not consistently.

It was found that the feed delivery varied between 0.5 and 11% bodyweight per day. Not all of the food expelled by the feeders would have been consumed, some of it was waste because of the AQ1 system testing the fishes' appetite every half hour and overfeeding by the system because of low pellet counts as discussed earlier in section 2.3.3. Other studies examining feeding patterns also report significant waste feed, for example Boujard and Leatherland, (1992b) and Landless, (1976). Attempts were made during the trial to collect waste pellets from each tank with the intention of adjusting the results to give the actual amount of feed ingested. However, it was not possible to collect waste pellets from each tank continuously and it was found from collecting individual samples that due to the fluctuating suspended solids level and its effect on the sensors, it was impossible to make any accurate estimations of the actual feed intake.

In this trial, there were three different effects of temperature found on the feed intake. One tank had a positive correlation, three tanks showed a negative correlation and one showed no significant correlation. In general, it is expected that higher temperature will lead to higher feed intake (up to the optimum temperature). The percentage feed intake of brown trout (*S. trutta*) virtually doubles with an increase in temperature from 10°C to 15°C (Elliot 1975). However, an investigation of seasonal growth and feed intake on wild cod carried out by Mello and Rose (2005) it was found that the temperature had both a positive and negative effect on growth, dependant on the age of the fish.

Gastric evacuation rates can control appetite and cod have a well defined stomach as they are carnivorous fish. In sole (*Solea solea* and *Solea senegalensis*) as in other species, a higher temperature leads to faster gastric evacuation, which means the fish will regain its appetite more quickly than at lower temperatures (Vinagre *et al*, 2007). This means that as temperature increases, the proportion of feed ingested by a fish should increase.

Therefore, only one tank out of the five showed the expected response to temperature. The negative relationships found may have been an artefact from the problems discussed in section 2.3.3 with the system set up. The fish were healthy and grew comparably with other cod at Machrihanish, so it is likely to be a system rather than biological fault.

In many other studies the effects of temperature and photoperiod on feed intake are investigated, however, they differ from the current study as they use a stable water temperature and photoperiod throughout the trial and test different temperatures as different treatments. These are preferable conditions for calculating a species' feed intake at differing temperatures, although in an aquaculture situation, the water temperature is usually ambient and fluctuates, so in this respect, the conditions more accurately match the current trial.

2.5.3 Medium term appetite rhythms

Time series analysis showed highly significant autocorrelation with a lag of up to 30 days. Tank 1 showed a positive lag of 31 days indicating that the fish in this tank continued to eat above the mean for all this time. In the other tanks there was a two week cycle. The fish were sampled around every two weeks which this may have had a bearing on this observation as this will have been stressful to the fish, which may have caused a dip in feed intake after each sampling. From these results it would appear the main predictor of feed input for juvenile cod is what the fish ate on the previous day. However, there were two patterns visible so it is not possible to draw absolute conclusions from this data set.

In commercial farms it is normal to feed twice per day, once in the morning and again in the evening with uneaten feed being monitored by underwater cameras (Bourhill, 2007, personal communication). The same percentage bodyweight of feed is given each day according to the feed tables unless there is a specific problem with a cage – eg disease, low oxygen etc. From the data of this trial it is hard to predict the fishes appetite as each tank had its own rhythm. In order for farmers to feed to the fishes appetite a self feeding or feedback based feeding system should be used.

Cages typically hold thousands of fish, and in comparison the tanks used in this trial were small, with very small groups of fish, so it would be interesting to carry out similar trials in cages to investigate whether the same patterns of autocorrelation are present.

Yamamoto *et al.* (2002) tested demand feeding against hand feeding twice a day to satiation in rainbow trout. It was found that there was no significant difference in feed intake, weight gain or whole body proximate composition between the two treatments. The feed intake was spread across the daylight day with a main feeding peak at dawn and another smaller peak at dusk.

2.5.4 Future work

There are still many unanswered questions that need further investigation. The fish used for this trial were sourced from coastal non-migratory broodstock local to the area. What would be the patterns for cod from other regions? Some cod populations are migratory while others are more stationary, does this influence feeding patterns?

Further investigation is required by running similar trials with stable temperatures and photoperiods to look in closer detail at whether they influence feed intake. 24 hour light is commonly used in the farming of cod, (although it is not usually carried out until the fish are older than those focused on in this trial) because of higher growth rates and the fact that it stalls maturation so it would be useful to investigate the effect of 24 hour lighting on seasonal feeding patterns.

The tanks used in this trial were relatively shallow and brightly lit. Cod are naturally deep water fish though juveniles do tend to swim close to the shore; however, they are not naturally exposed to such high light levels. It was found that FCR and feed input were negatively affected by longer day lengths, perhaps longer exposure to high light intensity was stressful for the fish and so negatively affected their eating. This could be tested by repeating the trial using larger tanks with lower lighting levels.

Chapter 3 Daily Feeding Patterns in Juvenile Cod

3.1 Introduction

In aquaculture, it is important to have a feeding regime that reflects the fishes' natural feeding rhythms. This means that fish should be fed at the height of their appetite for maximum intake, leading to lower feed wastage, lower pollution and less economic loss (Alanärä *et al.* 2001). Feeding rhythms may occur over relatively long periods, as in the seasonal patterns discussed in chapter 2. They may also involve shorter term changes in feed intake, for example over a single day. There is an extensive literature on daily rhythms in animals, including fish, and on the mechanisms that control such rhythms. These are discussed here concentrating on fish, using the same experiment as Chapter 2, but using different analysis.

3.1.1 Daily activity rhythms

Feeding and activity rhythms can be classified into three main groups; diurnal (feeding during daylight hours), crepuscular (feeding at dawn and / or dusk) and nocturnal (feeding during darkness hours).

3.1.2 Zeitgebers and entrainment

Biological rhythms are controlled by external synchronisers or zeitgebers (German for 'time giver'). The light / dark cycle is the most powerful synchroniser of biological rhythms (Sánchez Vázquez *et al.* 1995). Photosensitive receptor cells are found in both the retina and the pineal gland which then regulate the production of melatonin. Levels of melatonin during daylight are low and rise during darkness hours; this is represented for salmon parr in figure 3.1. Figure 3.2 gives an example of an activity pattern for another salmonid fish, the rainbow trout, measured by Sánchez-Vázquez and Tabata (1998).

Removing the pineal gland in fish has a significant effect on circadian rhythm. For example Kavaliers (1979) removed the pineal gland from lake chub (*Couesius plumbeus*). The fish were first entrained to a photoperiod of 12:12 LD then the lights were turned off allowing the fish to 'free run'. The fish settled into a 25.1 hour .activity cycle. After the pinealectomy, onset of activity at the beginning of the day was more variable and although activity cycles persisted, there was more variability of the period length.

Removal of the pineal gland of 1 year old salmon smolts had the effect of lowering melatonin level, but did not suppress production completely indicating that melatonin is produced in other areas. It is speculated that melatonin production may also take place in the retina (Porter *et al.* 1996). In Atlantic cod, as in salmonids, melatonin levels are low throughout the day and rise during night time. Smaller fish have higher melatonin levels and when submerged lights lit the cage over 24 hours melatonin secretions were unaffected (Porter *et al.* 2000). This result was opposed to Atlantic salmon whose melatonin production was interrupted by additional lighting.

Meal times can also act as zeitgebers when fish are fed at the same time each day. This is reflected in anticipatory behaviour which often takes the form of increased swimming speed before food is given. For example Reebs and Lague (2000) found that shoals of golden shiners (*Notemigonus crysoleucas*) showed anticipatory behaviour up to 4.5 hours before meal times. In a related paper it was found that golden shiners trained to expect feed at a particular time of day shifted their anticipatory behaviour with a sudden 6 hour shift in the light dark cycle, this is shown in the actinogram in figure 3.3 (Lague and Reebs, 2000). Rainbow trout (*O. mykiss*) can anticipate two meals per day and in this case the anticipatory behaviour is shorter, between 30 to 50 minutes before feeding is due (Chen and Tabata, 2002).

Experimenting with conflicting zeitgebers will show which one is most important to a species. Goldfish were given 15 minutes of light at hour 10 of a 24 hour cycle with food available in the last 2 minutes of light. After three weeks, the meal time was adjusted either 4 hours before the light pulse or 4 hours after. This was maintained for 3 weeks when the meal times jumped another 4 hours. Initially the fish entrained to the light pulse suggesting that it was the easiest zeitgeber to recognise. However, as the trial progressed the fish became entrained to feeding time supporting the hypothesis that feeding time can be a strong zeitgeber for goldfish (Aranda *et al.*, 2001).

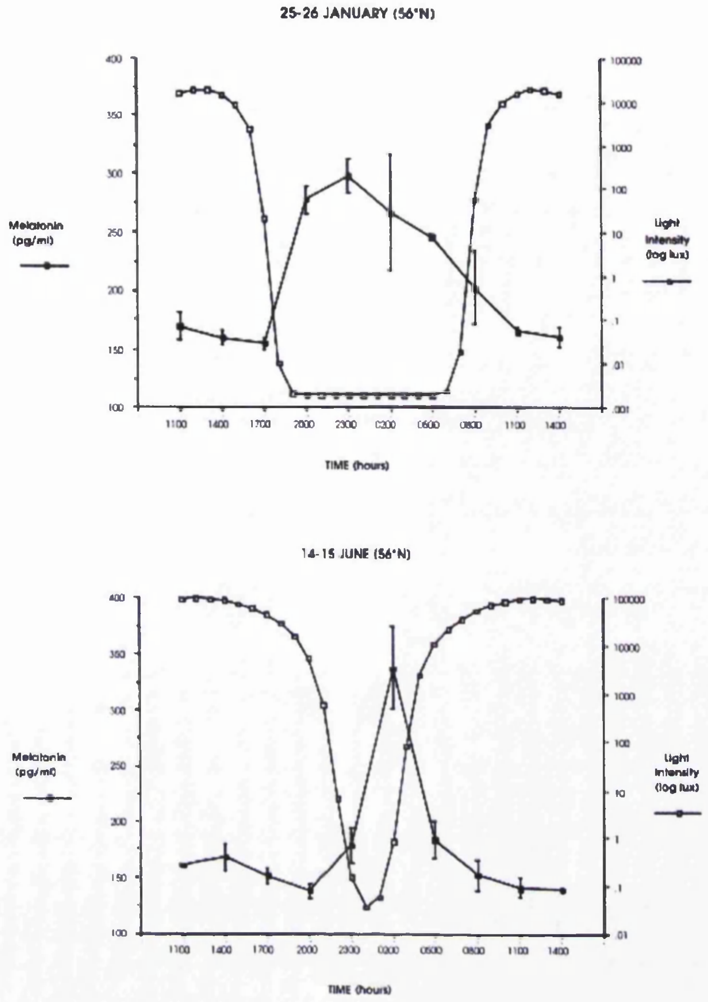


Figure 3.1 Diel plasma melatonin profiles from salmon parr maintained under a natural photoperiod; mean and standard error of the mean. Natural light intensity is shown as an indicator of day length. From Porter *et al.* 1998.

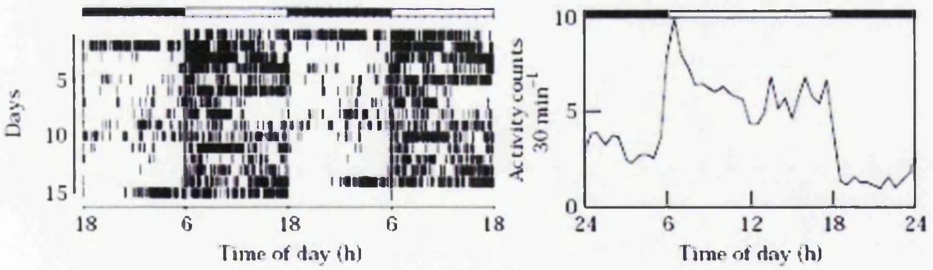


Figure 3.2 An example of an actogram (left) age diel activity profile (right) of an individual trout. Light and dark are represented by the black and white strips at the top of the diagrams (from Sánchez-Vázquez and Tabata, 1998)

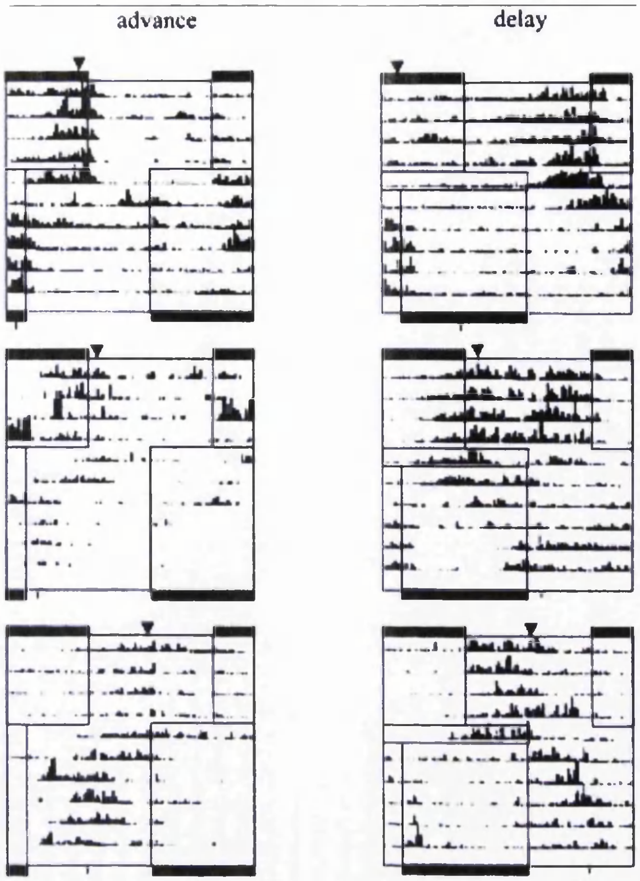


Figure 3.3 Actograms from golden shiner shoals fed at a fixed daily time (triangles) and then deprived of food and exposed to a 6-h advance (left panels) or delay (right panels) in the 12:12-h light–dark cycle. Filled bars at top and bottom of panels represent dark time (From Lague and Reebs, 2000)

3.1.3 Circadian rhythms and photoperiodism

Hillman (1976) wrote '*There is no more confusing literature than that on the relationship between circadian rhythmicity and photoperiodism*'.

Photoperiodism is the response of an organism to the timing and duration of light and dark (Koukkari and Sothern, 2006). In fishes an example is spawning and spermatogenesis. For instance the gonadal development of the Ayu (*Plecoglossus altivelis)* is controlled by changes to photoperiod. A shortening photoperiod stimulates gonadal growth while a lengthening photoperiod stops it (Masuda *et al*., 2005).

Some researchers believe that in order to use the term circadian a rhythm should be tested to ensure they are capable of free running at a rhythm of approximately 24 hours (endogenous) whilst others take a more liberal approach and use the term for any 24 hour rhythm (Koukkari and Sothern, 2006).

In 1977, the International Committee on Nomenclature of the International Society for Chronobiology published the following definition of circadian rhythms:
'Circadian: relating to the biologic variations or rhythms with a frequency of one cycle in 24 ± 4 hours; circa (about, approximately) and dies (day or 24 h). Note: term describes rhythms with an about 24-h cycle length, whether they are frequency-synchronised with (acceptable) or are desynchronised or free running from the local environmental time scale, with periods slightly yet consistently different from 24-h' (Halberg et al, 1977).

In addition to these endogenously generated rhythms, the cues that control daily patterns of activity and feeding are also influenced by the predictable changes in gut fullness and nutritional status that follow ingestion of a meal. A number of papers have concluded that daily meal timings coincide with the gastric evacuation times of fish. Grove et al. (1978) write that after a meal appetite return in rainbow trout coincided with the gastric emptying of the previous meal. Gastric emptying times vary depending on feed type, for example different species of prey organisms, pelleted or extruded feed (Jobling 1982).

Figure 3.4 shows a diagram of the processes involved in the control of a circadian rhythm. It shows the central pacemaker being synchronised by the pineal gland and retina; these are controlled by the ambient conditions, mainly light and temperature. The retina, temperature and other factors such as the diet quality and the animal's position in the hierarchy all control the satiation level of the fish which in turn influences the fishes feed intake.

Masking is the superficial change of rhythm characteristics by external environmental conditions (Koukkari and Sothern, 2006). For example, in a study carried out on an unclassified troglobiotic fish species whose natural environment is total darkness, exposure to a LD photoperiod caused a nocturnal locomotor rhythm to emerge. This rhythm disappeared when DD was restored. In this case the LD photoperiod masked the natural DD photoperiod (Trajano *et al.*, 2005).

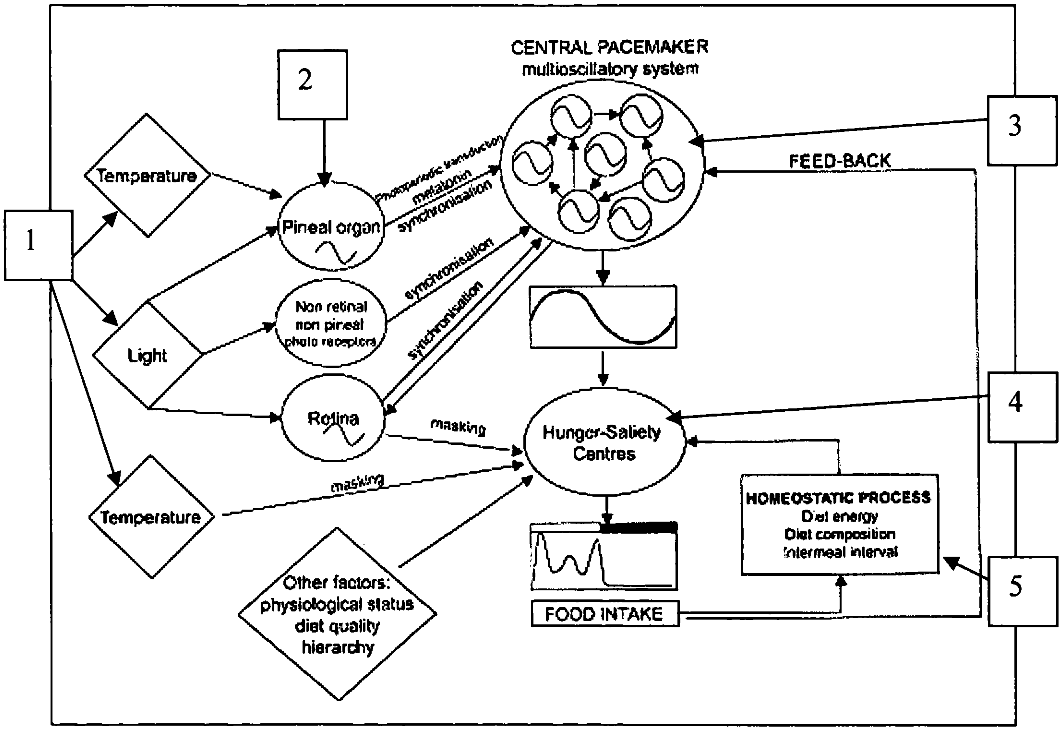


Figure 3.4. A schematic diagram of the circadian feeding system in fish showing entraining pathways and interactions. From Madrid *et al.*, (2001). 1 marks some of the external (temperature, light, diet quality, hierarchy) and internal (physiological status) zeitgebers that are the first stage in controlling feeding rhythms. 2 shows the areas of the fishes' body that produce melatonin 3 marks the central pacemaker or oscillator that houses the circadian clock. 4 is the food entrained oscillator which runs independently of the light entrained oscillators. 5 shows the other factors that impact on feeding patterns, for example, the energy levels required for homeostasis and the energy content in the feed.

The hourglass mechanism is a system that needs to be reset after each cycle by the controlling zeitgeber. This is opposed to a circadian rhythm which will continue to free run in the absence of any synchronisers. Hourglass clocks are usually used in development and aging and in the brain (Rensing *et al.* (2001). For example the induction of diapause in insects (a suspended animation type sleep state different to hibernation as the insect does not grow) is thought to be controlled by an hourglass mechanism.

Measuring a fishes' daily appetite can be done by using a demand feeder. This may involve use of a self feeder where fish learn to supply feed for themselves by pressing or biting a switch (Alanärä, 1996, Alanärä *et al.* 2001). Alternatively the pattern can be measured through using a feedback system where the number of waste feed pellets are counted and from this the level of hunger can be assessed (Fast *et al.* 1997).

3.1.4 Daily feeding patterns

Daily feeding patterns of many cultured fish species have been investigated and mapped, mostly over the past 20 years. Some examples of these are discussed below.

Atlantic salmon: The daily feeding patterns of the Atlantic salmon (*S. salar*) have been described in several papers. Kadri *et al*, (1991) noted that Atlantic salmon of approximately 1kg held in sea cages had distinct feeding and activity patterns, feeding in the early morning and then again 12 hours later in the evening. In a later study, Kadri *et al.* (1997a and b) found similar overall feeding patterns to those in the previous paper. They also investigated the split between different sized fish feeding at different times. It was found that small fish fed predominantly at first light, while feeding by the larger fish peak occurred an hour later. Blyth *et al.* (1999) studied the Atlantic salmon's daily feeding patterns throughout a whole year. Again the main feeding peak was consistently found to be at dawn, with a lower level of steady feeding throughout the rest of the day. However, Noble *et al.* (2007 b) found four different feeding patterns present, feeding peaks at dawn, mid day, dusk or arrhythmic depending on the time of year. Juvenile salmon switch to nocturnal feeding as the temperature falls below 10°C (Fraser *et al.* 1993). It is thought that the switch happens in order to reduce predation risks. It was also noted that aggressive interactions were significantly lower when the fish were foraging at night, in aquaculture, aggression between fish leads to fin damage and increased stress. Fraser *et al* (1995) conclude that juvenile Atlantic salmon become nocturnal at low temperature, irrespective of time of year.

Rainbow trout: Boujard and Leatherland (1992b) investigated the self feeding patterns of rainbow trout (*O. mykiss*) with different length photoperiods. They found that the majority of feed demands occurred during the photophase regardless of photoperiod. In all cases there was a morning feeding peak. At longer light photoperiods there was also a dusk peak, but in the shortest photoperiod the whole day's feeding is compressed into one extended 'meal', these patterns are shown in figure 3.5.

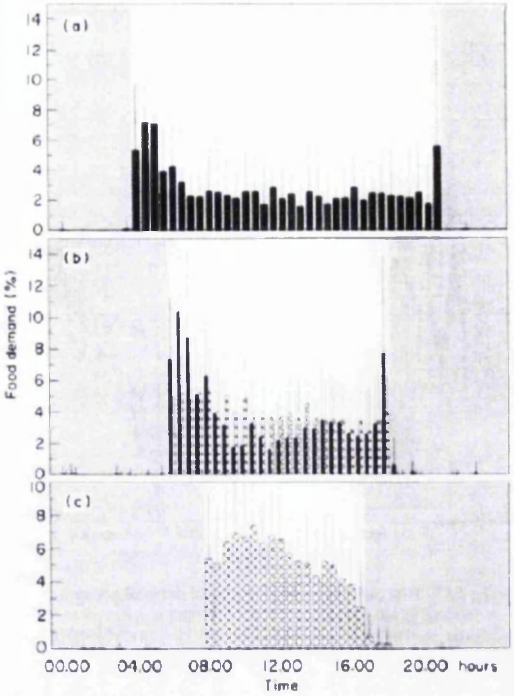


Figure 3.5 From Boujard and Leatherland, (1992b). Feeding patterns (% final live bodyweight per 30 minutes) observed during the last 8 days of the study period for (a) 16:8 LD (b) 12:12 LD and (c) 8:16 LD regimes. The shaded areas represent darkness hours

Similar results were found by Bailey and Alanärä (2006) who exposed rainbow trout to either 18:6 or 15:9 LD photoperiod. Again there was a significant morning peak and also a significant evening peak in both photoperiods.

Sea bass: (*Dicentrachus labrax*) is a widely farmed species across the Mediterranean regions. Begout Anras (1995) kept sea bass in sea water earthen ponds with access to demand feeders from June to October. The fish fed diurnally, first with an early feeding peak but from August with a feeding peak in the middle of the day. During the spring, summer and autumn, sea bass feed diurnally then during winter they switch to nocturnal feeding. Such switching and its causes are discussed in section 3.1.3 below.

Goldfish: The self-feeding patterns of the goldfish (*Carassius auratus*) were studied by Sánchez- Vázquez *et al.* (1996). The tendency was for fish to feed diurnally but the fish were able to switch their circadian rhythms from diurnal to nocturnal. Some fish were diurnal in their activity while being nocturnal in their feeding and vice versa.

The Florida pompano: (*Trachinotus carolinus*) is a perciform fish that feeds on other fish, benthic crustaceans and molluscs. Heilman and Spieler (1999) investigated this species daily feeding rhythm using demand feeders. It was found that the fish quickly learnt to use the demand feeders and settled into a feeding pattern with the main peak accounting for 20% of the day's feed intake, in the first 2 hours of daylight. The level of feeding subsequently fell throughout the rest of the day.

Yellowtail: The yellowtail (*Seriola quinqueradiata*) is another perciform fish from the north Pacific. It was found by Kohbara *et al.* (2000) that this species is capable of both nocturnal and diurnal feeding. Two experiments were run, one indoors with a 12:12 hour photoperiod and one outdoors with a natural photoperiod for July to September in Japan. The fish in the indoor trial settled into a diurnal feeding rhythm, sometimes with a peak occurring at the simulated dawn and at other times having stable feed intake throughout the daylight day. The outdoor fish fed exclusively at night. The authors suggest that the difference could be due to the higher light intensity in the outside tank during daylight hours. In a later paper, Kohbara *et al.* (2003) carried out a year long investigation of yellowtail self feeding in net cages. Diurnal and nocturnal feeding was found again, the main patterns being crepuscular with one or two main feeding peaks at dawn and dusk and nocturnal feeding sometimes with peaks around dusk and dawn.

Greater amberjack: Chen *et al.* (2007) investigated the circadian self feeding rhythms of amberjack (*Seriola dumerili*) under a 12:12 hour photoperiod. They were found to be diurnal with the main feeding peak occurring just after first light. When the fish were given constant light conditions, they continued free running in the established pattern for 43 days, showing they have very strong internal rhythms.

Yellow croaker: The yellow croaker (*Pseudosciaena polyactis*) is found in the Yellow and East China seas. Xue *et al.* (2005) studied its feeding patterns by analysing stomach contents of wild caught fish. It was found that the stomachs were fullest around 0800 hours and 2400 hours suggesting that in fish in the wild there were crepuscular and nocturnal feeding throughout the year.

In nearly all cases of diurnal feeding discussed above, a dawn feeding peak is one of the main features of the different species feeding peaks. This may be because the change from darkness to light is a strong zeitgeber.

3.1.5 General comments

In general, fish show species specific daily feeding patterns often related to availability of their natural food. However, in many cases these patterns change with season. A common pattern is a temperature dependant switch from diurnal to nocturnal feeding. In addition individuals within a species may vary even under constant conditions. So generally there is a degree of flexibility superimposed on species specific feeding patterns.

3.1.6 Fish that switch feeding patterns

Often a species of fish will switch from one feeding pattern to another. For example, sea bass (*D. labrax*) are diurnal throughout summer and autumn then switch to nocturnal feeding during winter and early spring (Azzaydi *et al.* 2000). It is thought that a change in prey type lies behind the switch (Madrid *et al.* 2001). There have been several papers exploring the zeitgebers that control this switch.

It was found that raising the temperature from 22°C to 28°C or lowering it to 16°C did not induce a switch to nocturnal feeding in 90 gram sea bass held in groups, suggesting that daily feeding patterns are controlled endogenously and do not change passively with temperature (Aranda *et al*, 1999a). In the same paper, changing the photoperiod from 12:12LD to 8:16LD also had no impact on feeding pattern when combined with the changing temperature. When fed diurnally instead of nocturnally during winter months, FCR was significantly lowered indicating that feeding at the wrong time can be detrimental to a fishes growth. This paper indicates that changes made in the lab do not induce nocturnal feeding, but in another study, Sanchez Vazquez *et al,* 1998 found that the switch did occur in sea bass held in inland tanks at ambient conditions suggesting that it is the combination of factors that cause the switch, rather than one individual factor.

Fraser *et al*., (1993) induced a switch from diurnal to nocturnal feeding in juvenile Atlantic salmon in the laboratory by lowering the water temperature to below 10°C. As the temperature fell more fish became nocturnal independently of photoperiod and season. The same pattern was also observed in wild salmon parr in three glacial rivers in Norway. There were no observations of the fish eating during daylight hours but 136 parr were spotted feeding during the night. Similar results were found in Amundsen *et al.* (2000). Stomach contents analysis was carried out on salmon parr from rivers in Finland and Norway. It was found that during early autumn the fullest stomachs were found at night, perhaps due to an increased availability of benthic prey nocturnally. In a later paper, about state dependant shifts between nocturnal and diurnal activity in Atlantic salmon. Metcalfe *et al.* (1998) suggest that nocturnal feeding in young salmon is preferable to diurnal feeding as it lowers the risk of predation. However, fish that are going to migrate to sea water and therefore need a fast growth rate will continue feeding during the daylight hours

3.1.7 Gastric evacuation and feeding patterns

The rate of gastric evacuation increases with temperature. This is represented in figure 10 below. The graph shows the rate of gastric evacuation for different prey items at different temperatures for cod. The curve starts at the lowest temperatures at a shallow gradient, then after approximately 6°C the gradient becomes steeper, until it shallows again at around 12°C as it reaches the fishes maximum tolerated temperature.

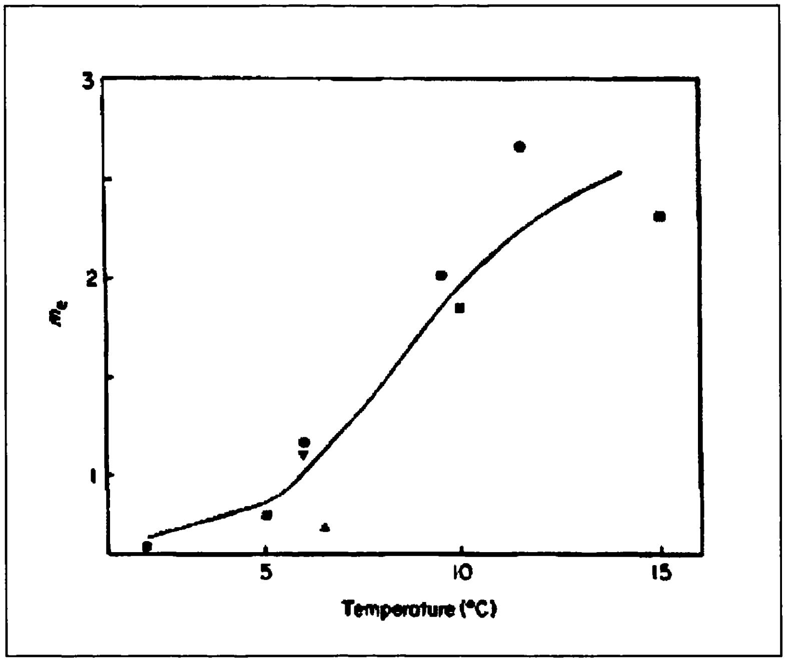


Figure 3.6 The relationship between gastric evacuation (m_e) and temperature for cod fed various food organisms ● fish (saithe) (Jones 1974) ■ Shrimp (Tyler 1970, 1977) ▼ fish (blenny) (Jones 1974) ▲ whelk (Jones 1974) From Jobling 1982

3.1.8 What is known about feeding and feeding patterns in cod?

Wild Atlantic cod tends to feed on benthic organisms such as polychaets, bivalves, isopods, decapods as well as fish such as capelin and gobies (Hop *et al.*1993, Klemetsen, 1982, dos Santos and Falk-Petersen, 1989 and Brawn 1969). The cod's stomach and oesophagus are strong and resilient and so they can eat heavily spined prey (Mattson, 1990). A discussion of the literature relating to cod's feeding patterns can be found in chapter 1. Meager *et al.* (2005) writes that cod are predatory fish that rely on sight and chemoreception to locate prey. Under low light conditions predation rates were significantly lower, suggesting that cod would feed predominantly during daylight hours. However, sea bass (*D. labrax*) are known to switch to nocturnal feeding and it was found by Rubio *et al* (2003) that feeding nocturnally reduces their pellet catching ability. This implies that even if a fishes' ability to feed is impaired, if there are strong enough cues nocturnal feeding will still take place.

In their paper based on seasonal growth of cod in Newfoundland Mello and Rose (2005) write that "amphipods, decapods and echinoderms dominated the Atlantic cod diet". They go on to state that cod increased consumption of fish (especially capelin) during the summer months of June and July.

3.2. Aims

The work described in this chapter is part of a wider study of natural feeding patterns of juvenile cod. Having examined seasonal feed intake in chapter 2, the aim here is to characterise daily feeding patterns in juvenile cod in tanks, fed to demand. This will be broken down into hourly feeding patterns, peak feeding hour and the proportion of feed consumed nocturnally. The impact of season, temperature and photoperiod will be examined.

3.3. Methods

The trial detailed in this chapter is the same as that in chapter 2. This study was carried out at the Marine Environmental Research Laboratory, Scotland. The husbandry was carried out in accordance with Stirling University's standard practices of vaccinations and grading. As described in chapter 2, this was carried out in two parts with different fish. Part 1 ran from 6th March 2004 until 24th August 2004. Part 2 started with a second batch of fish with a start weight of 5 grams on 12th November 2004 and continued until 14th February 2005. The housing conditions and methods are the same as in chapter 2.

3.3.1 The AQ1 System

Each tank had an AQ1 unit controlling their respective feeders. As described in chapter 2, this system delivers food at set intervals to test the fishes hunger. If the fish consume the test feed, more feed is offered until the fish are satiated. It therefore provides an accurate record of feed intake that can be used to examine the feeding pattern over a period of hours. Problems with the functioning of the system (discussed in chapter 2) meant that the feeders did not work in all tanks on all the days. Days when the AQ1 did not give an accurate record of feeding patterns were identified by the following criteria and omitted from the analysis.

1. No days where program sensitivity changes occurred
2. No days where the program was either turned off because 'max high' was reached or terminated manually.
3. No days where high levels of overfeeding or underfeeding take place.

3.3.2 Sampling

The fish were sampled every fortnight, alternating between batch weighing and individual sample weighing, where the fishes standard length was also measured so condition factor could be assessed..

3.3.3 Data Analysis

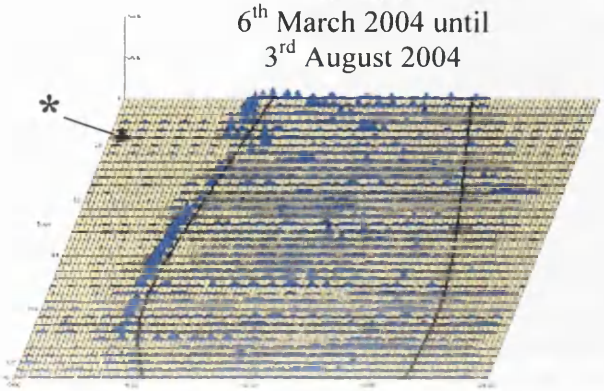
The raw data from the AQPC program gave a list of feed weight deposited in any given minute; this was sorted into hourly sections and ten day blocks for each tank using SAS for Windows, version 8. The data were plotted in a histogram to establish that it was normally distributed.

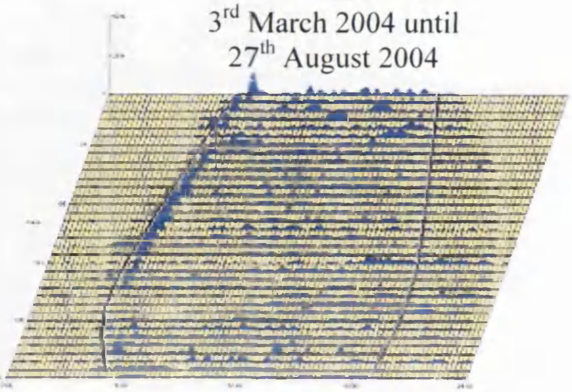
The data was divided into ten day blocks. The first analysis presents each mean daily feeding pattern in each ten day blocks for each tank. The patterns and tanks were compared using two-way ANOVA and it was found that there were no tank differences but there were significantly different mean feeding patterns across 24 hour periods, so these were analysed by one-way ANOVA followed by a Tukey test with 95% confidence limits (using Minitab 15). The data were then reanalysed looking at the mean peak feeding hour over ten day blocks for each tank. Tank differences were analysed by one-way ANOVA. The relationship between mean peak feeding hour and mean temperature and photoperiod for each ten day block was analysed using regression analysis. The data were then resorted into the mean percentage of feed consumed nocturnally. The relationships of these figures with temperature and photoperiod were then investigated using regression analysis.

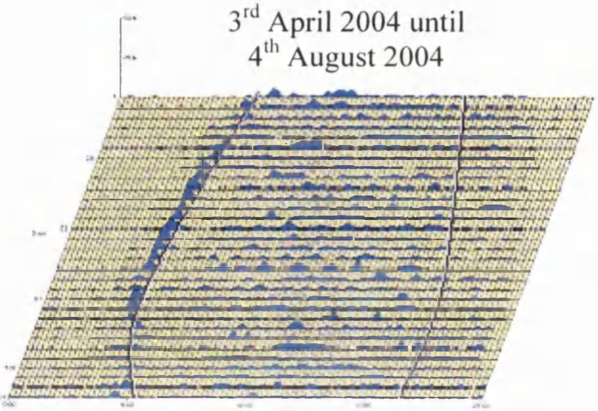
3.4 Results

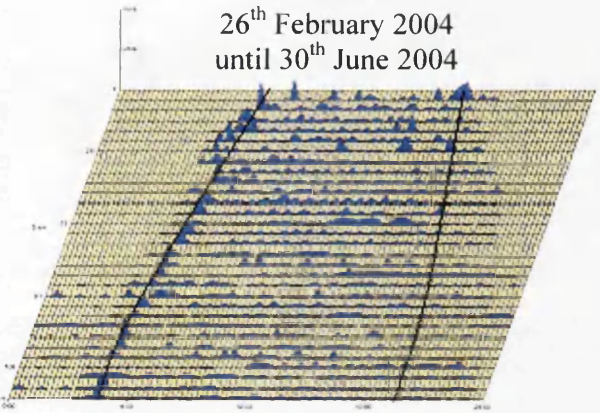
3.4.1 The raw AQ1 data

Figure 3.7 shows the raw, uncut data produced by the AQPC program for part 1 of the trial for all tanks (6th March 2004 until 27th August 2004) The graphs are a simple output from the AQPC program and contain all data including the 'untidy' data that were removed from the data set before analysis. Therefore, all the feeding malfunctions are very visible, especially in the second half of the study for tanks 1 and 2 the data from which has largely been removed from any further analysis. The graphs show that there are strong feeding patterns in each tank throughout the first part of the trial. In each tank there is a strong feeding peak early in the morning which changes across time coinciding with dawn. This main pattern is repeated across all five tanks. While there was persistently a dawn peak, there was seldom any dusk peak with the feeding tailing off gradually in the evenings. There was also continual low level feeding through out daylight and lower levels of night time feeding. The rhythmic addition of feed every 30 minutes throughout each 24 hour period can be seen throughout the night as small repeating peaks, for example point * in figure 3.7. Figure 3.8 shows the equivalent data for the second part of the trial. There is less definable pattern of feeding in these graphs and less consistency between tanks. For example, tank 1 shows continuous browsing where there were no distinctive patterns. In some cases in Figure 3.7 there was no dawn feeding peak, this is because the graphs are produced from raw data before 'corrupted' days were removed according to the criteria outlined in section 2.3.3 in the previous chapter.


Tank 1
6th March 2004 until
3rd August 2004


Tank 2
3rd March 2004 until
27th August 2004


Tank 3
3rd April 2004 until
4th August 2004


Tank 4
26th February 2004
until 30th June 2004

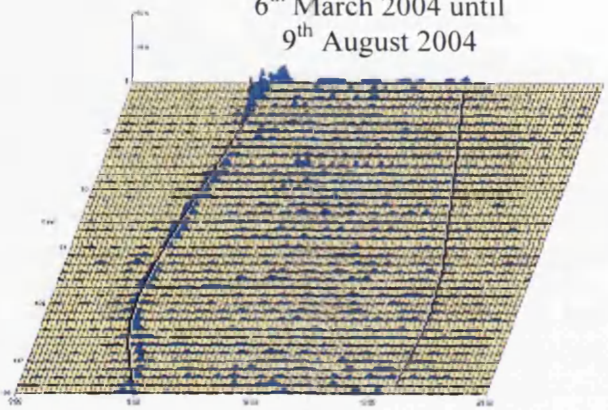
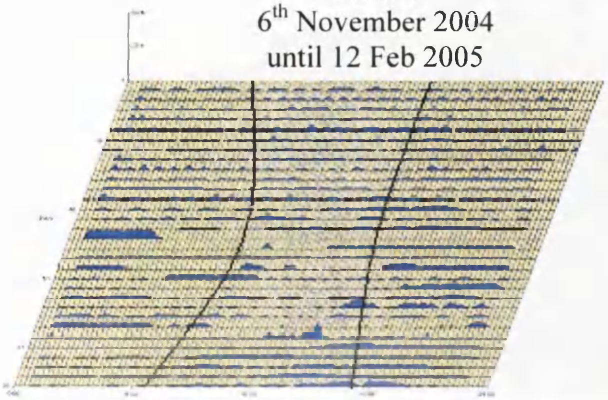

Tank 5
6th March 2004 until
9th August 2004

Figure 3.7 Feeding patterns of tanks 1-5. Part 1 of the trial. Output from AQPC, version 3.5.

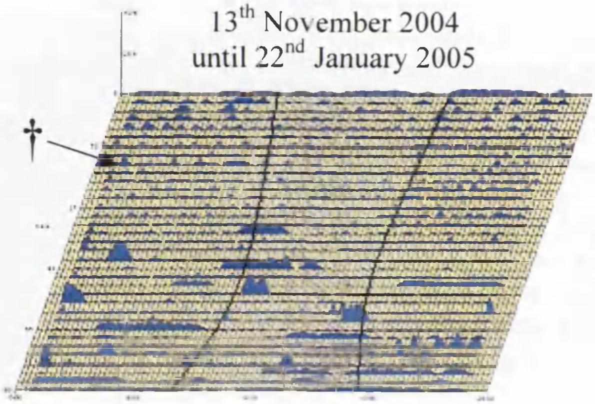
The x-axis represents time of day, The y-axis is the day number and the z-axis is the relative feed intake as a percent of total daily feed input. Dawn and dusk are marked by black lines

*** illustrates an example of the rhythmic introduction of feed into the tank each half hour to test appetite.**

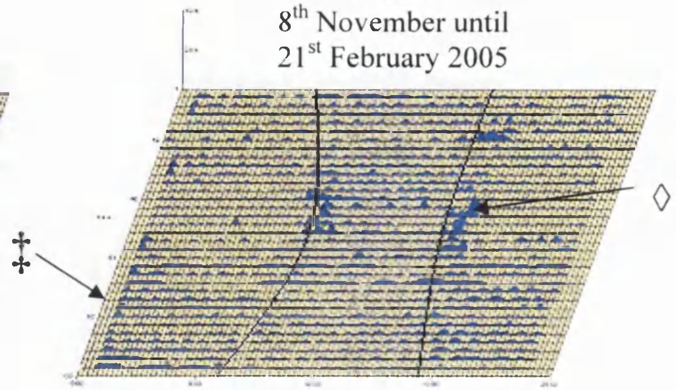
Tank 1
6th November 2004
until 12 Feb 2005



Tank 2
13th November 2004
until 22nd January 2005



Tank 4
8th November until
21st February 2005



Tank 5
8th November 2004 until
16th February 2005

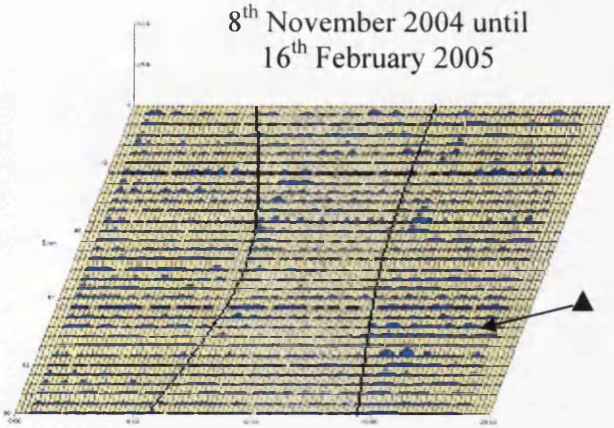


Figure 3.8 Feeding patterns of tanks 1,2, 4 and 5. Part 2 of the trial.

The x-axis represents time of day, The y-axis is the day number and the z-axis is the relative feed intake as a percent of total daily feed input. Dawn and dusk are marked by black lines.

†

‡ All three marked points show times of nocturnal

browsing which is further discussed on page 19
▲

◊

3.4.2 Analysis of 24 hour feeding patterns

Figure 9 show the mean hourly feed intake (as a percentage of daily feed intake) of all active tanks for each ten day block. A two way ANOVA showed there was no significant difference across tanks, but there were highly significant differences across the day. ANOVA results can be seen in table 1 and the significant differences determined by the Tukey test are located in appendix 1.

Part 1

The first main pattern to be seen is a large feeding peak at dawn, accounting for up to 25% of the days feeding. This pattern is seen in blocks 1, 2, 4, 5, 6, 7, 8, 9, 10, 11 and 13. There were occasional significant peaks at around the middle of the daylight day, these can be found in sections 1, 2, 4 5, 6, 7, 8, 9, 10, 12 and 13.

Part 2

In some blocks, there was no significance across the day due to high variance and no discernable trend or variation throughout the 24 hour period, this was found in sections 29, 31, 33 and 35.

Block 34 has an afternoon peak with increased variability at around 15:00 with little change in pattern or variability over the remainder of the day.

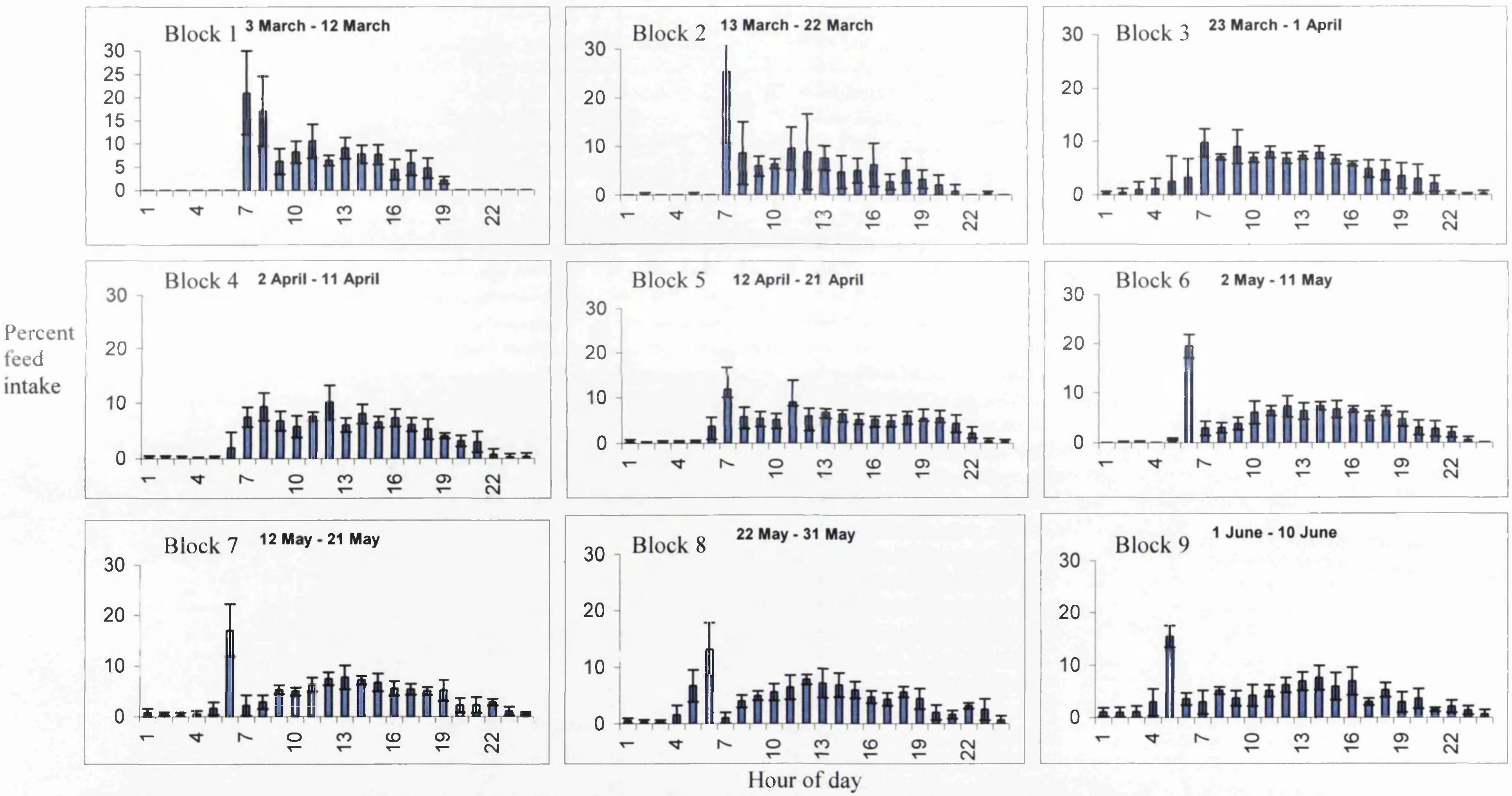


Figure 3.9 Mean and standard error of feed intake in each hour of the day for all tanks in 10 day blocks

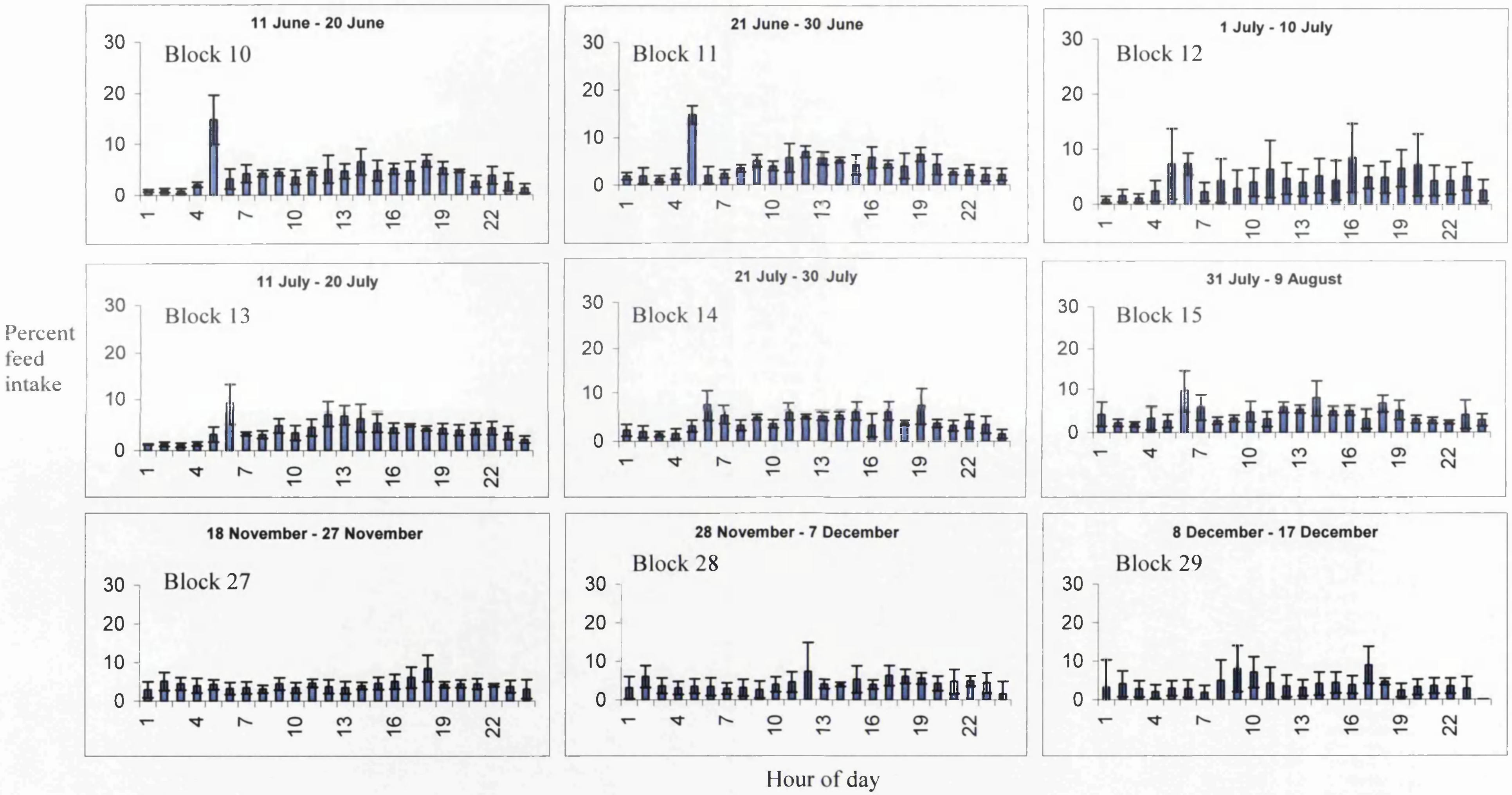


Figure 3.9 Mean and standard error of feed intake in each hour of the day for all tanks in 10 day blocks (continued)

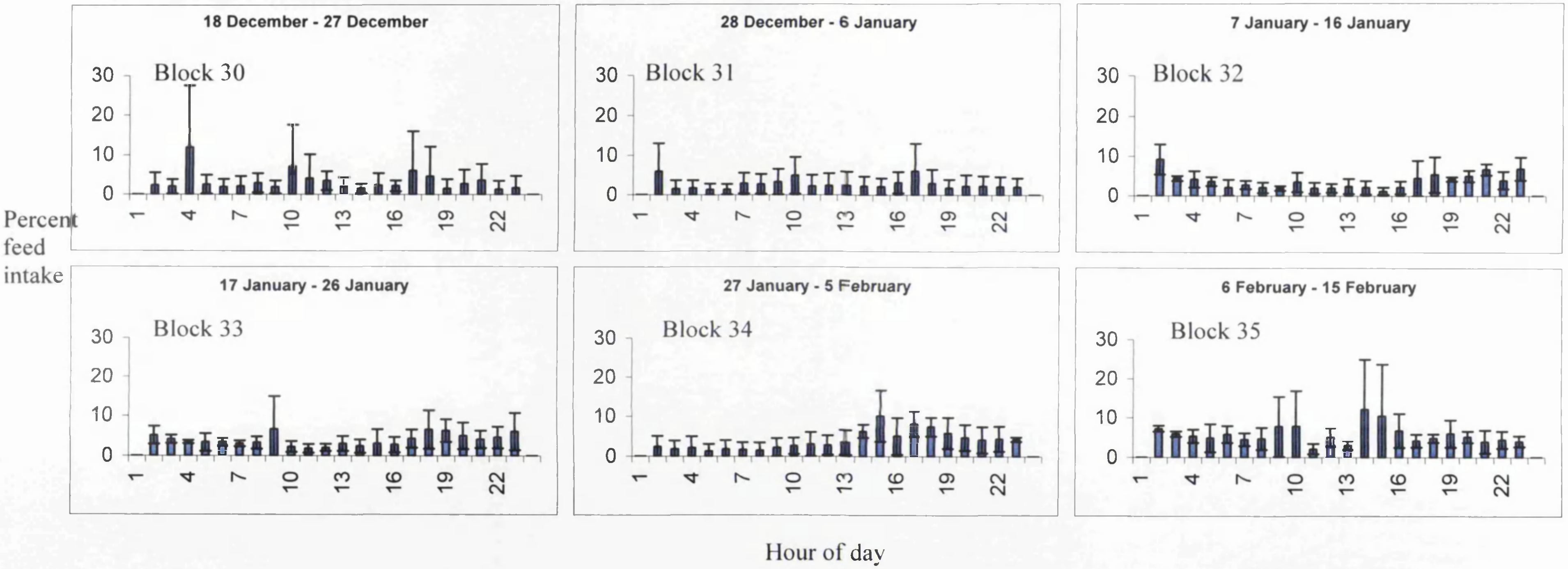


Figure 3.9 Mean and standard error of feed intake in each hour of the day for all tanks in 10 day blocks (continued)

Block number	F	p	df
1	16.05	≤0.0001	23,72
2	7.29	≤0.0001	23,72
3	11.13	≤0.0001	23,72
4	24.95	≤0.0001	23,96
5	13.74	≤0.0001	23,96
6	22.60	≤0.0001	23,96
7	55.65	≤0.0001	23,96
8	25.49	≤0.0001	23,96
9	15.84	≤0.0001	23,96
10	18.63	≤0.0001	23,96
11	10.80	≤0.0001	23,72
12	12.54	≤0.0001	23,72
13	1.49	0.103	23,72
14	7.18	≤0.0001	23,72
15	4.97	≤0.0001	23,72
16	3.25	≤0.0001	23,72
27	2.02	0.013	23,72
28	1.92	0.019	23,72
29	1.10	0.368	23,67
30	2.21	0.013	21,44
31	1.18	0.301	21,58
32	3.27	≤0.0001	23,66
33	1.07	0.4	23,66
34	2.51	0.002	22,66
35	1.20	0.299	21,44

Table 3.1 ANOVA results of mean food intake per hour for each block giving f, p and the degrees of freedom (between, within)

3.4.3 Analysis of peak feeding hour

Figure 3.10a gives the peak feeding hour for each 10 day block in the first part of the trial. For the first 120 days there was very little variation and the hour with the highest feed consumption was consistently around first light. From block 13, there is a sudden increase in variance. Block 13 is the block following the summer solstice (June 21st). If blocks 13 – 19 are omitted a one-way ANOVA (F=7.61, df=13 p<0.0001) followed by a Tukey test shows block 4 is significantly different from blocks 3, 5, 6, 7, 8, 9, 10, 11 and 12. In block 4, tanks 1 and 5 follow the early morning peak trend shown through blocks 1- 12. However, tanks 2, 3 and 4 all show the peak feeding hour between 12 and 1pm.

Figure 3.10b shows the peak feeding hour for the second part of the trial. There is high variation across the whole time period. There is no visible pattern and a one-way

ANOVA shows there are no significant differences (F = 1.26 p = 0.304). The means vary from 07:00 to 18:00 hours.

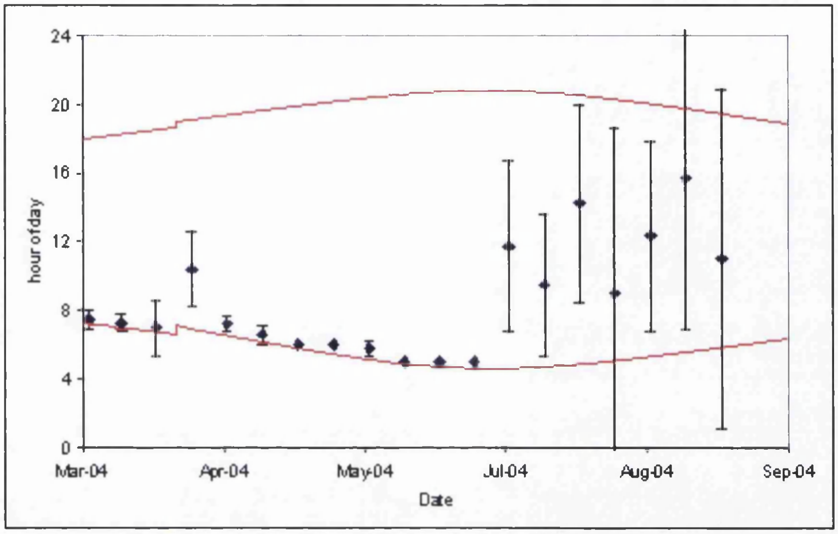


Figure 3.10a The mean peak feeding hour (with standard error of the mean) of fish against time in part 1 of the trial. Dawn and dusk are shown in red lines.

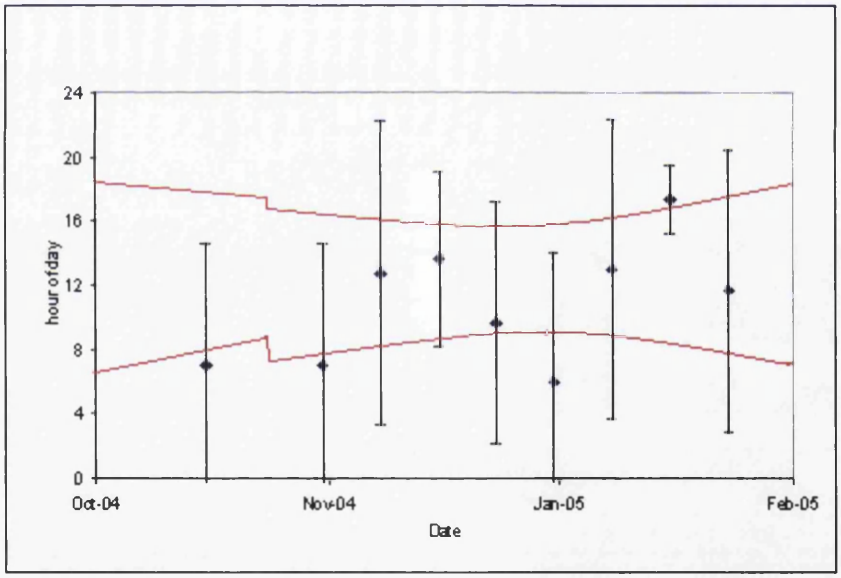


Figure 3.10b The mean peak feeding hour (with standard error of the mean) of fish against time in part 2 of the trial. Dawn and dusk are shown in red lines.

Figure 3.11 shows the regression of the mean peak feeding hour and day length. A significant positive relationship was found with the peak feeding hour.

hour falling as day length increases. There are two groups of points, the first under the regression line follows the approximate ambient dawn times, while points above the regression line are distributed across the remainder of the 24 hour period and reduce with increasing day length. This lower group of points are mainly comprised of part one of the trial, while the higher group are made up of the second part.

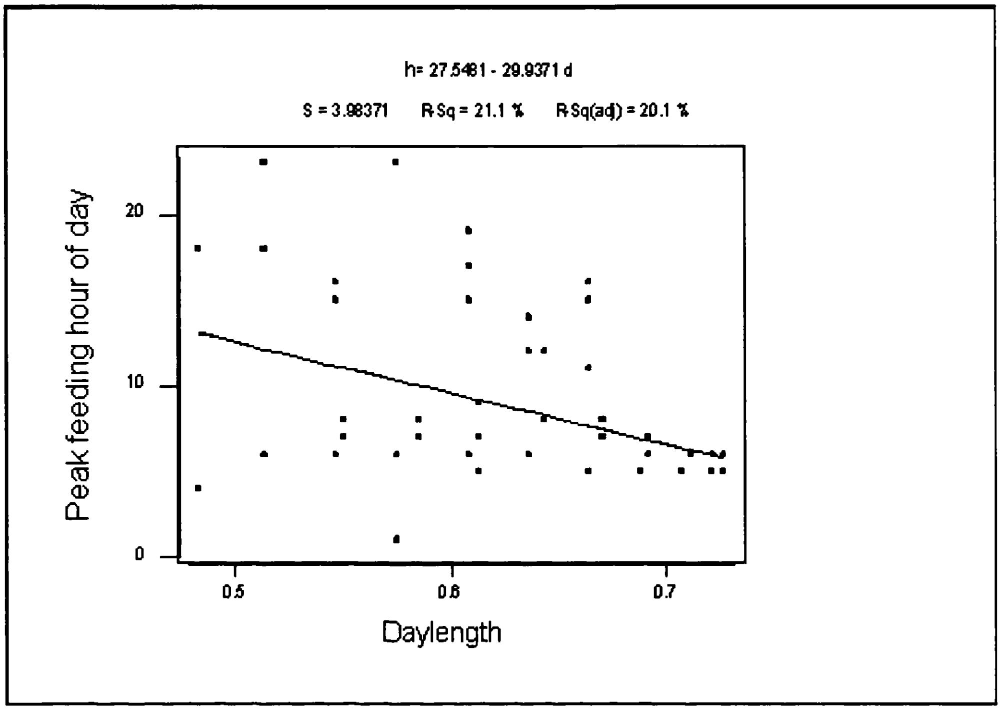


Figure 3.11 Regression of peak hour of feeding against day length (as a fraction of daylight in the 24 hours) for both parts of the trial h = peak hour d = day length.

Figure 3.12 shows the relationship between peak feeding hour and temperature for both parts of the study. At low temperature, the peak feeding hour is only early, while at the higher temperatures, there are two groups of points, the first cluster in the early hours and the second later in the day. If the points circled are disregarded, there is an obvious negative trend between peak feeding hour and temperature. Figure 3.13 also shows a negative relationship between peak hour and mean temperature for the second part of the trial, with the regression line following dawn.

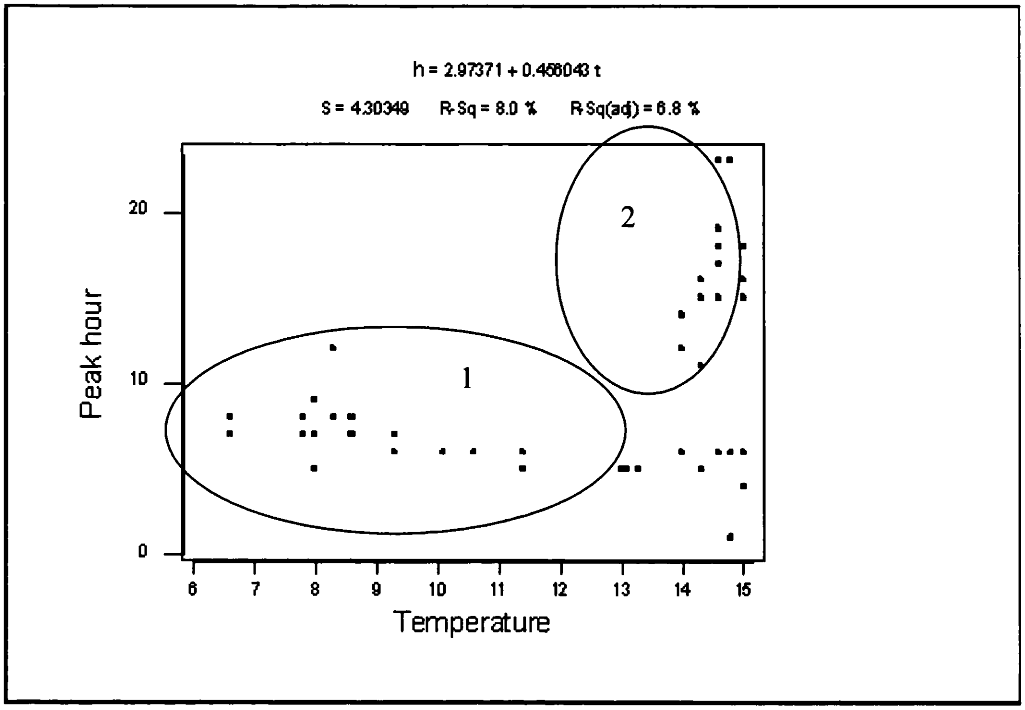


Figure 3.12 Regression of peak hour of feeding against temperature for part 1 and 2 h = peak hour t = temperature The points in area 1 are from part 1 and the remaining points are from part 2

3.4.4 Nocturnal consumption

Figure 14 shows the percentage of feed taken during night time hours over the full year. There is an obvious split between parts 1 and 2 with part 2 having significantly more feed consumed at night than the first part. Table 2 shows the differences between parts 1 and 2. There were twice as many sample days in part 1 as in part 2 and the variability is much higher in part 2 A one way ANOVA showes that part 1 and Part 2 are very highly significantly different (f= 2635, p<0.0001, df-=867.

	Part 1	Part 2
Mean	14	64
Standard deviation	10.2	18.6
n	584	279

Table 3.2 Mean nocturnal consumption in the two parts of the trial.

When the data is corrected to allow for the changing photoperiod, a one way ANOVA shows that the parts are still very highly significantly different (F= 2590, p<0.0001, df- =867.

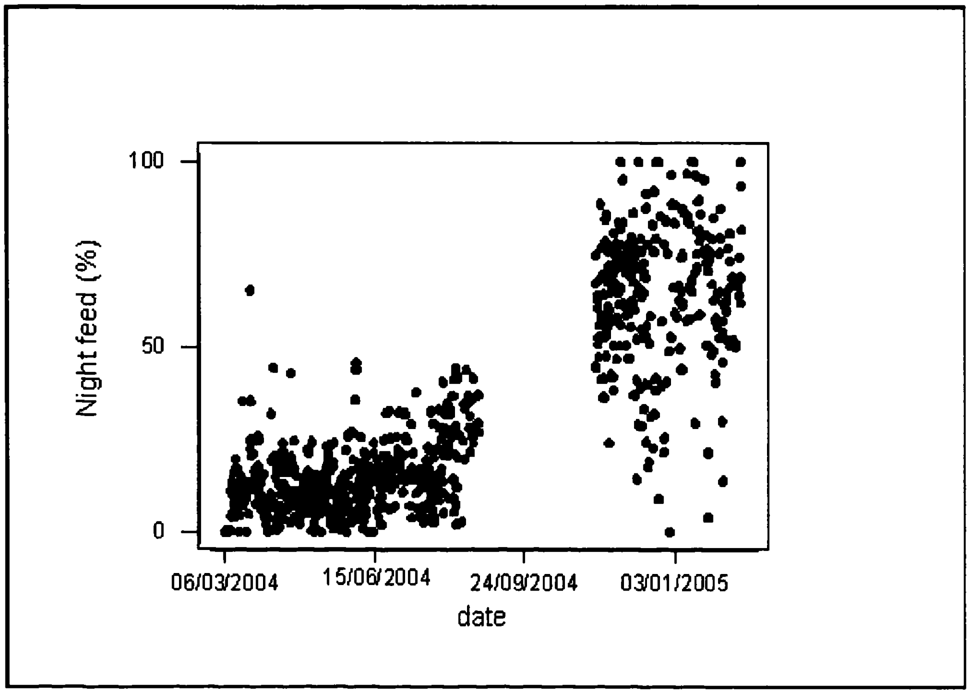


Figure 3.14 Scatter plot of the percentage nocturnal feed across time for both parts 1 and 2.

Figure 3.15a and b show the relationships between day length and percentage of feed consumed at night. Figure 3.15a shows a significant negative relationship (p>0.001 F=52.29 df = 604. Figure 3.15b shows a slightly positive trend that is just significant (p = 0.037, F= 4.38. df = 262) The main range of nocturnal feeding in part one is from 0 to 40% with some outliers up to 70%. The second part has a range of 40% to 100% with some low outliers spanning from 0 to 40%.

Figure 3.16a and 3.16b show the relationship between the mean percent of nocturnal feeding and temperature in both parts of the trial. In the first part, there is a significant positive correlation between pattern nocturnal feeding and increasing temperature (p>0.0001, F=79.68 df=583, while in the second part, there is no definable relationship (p=0.58 F=0.31 df=278).

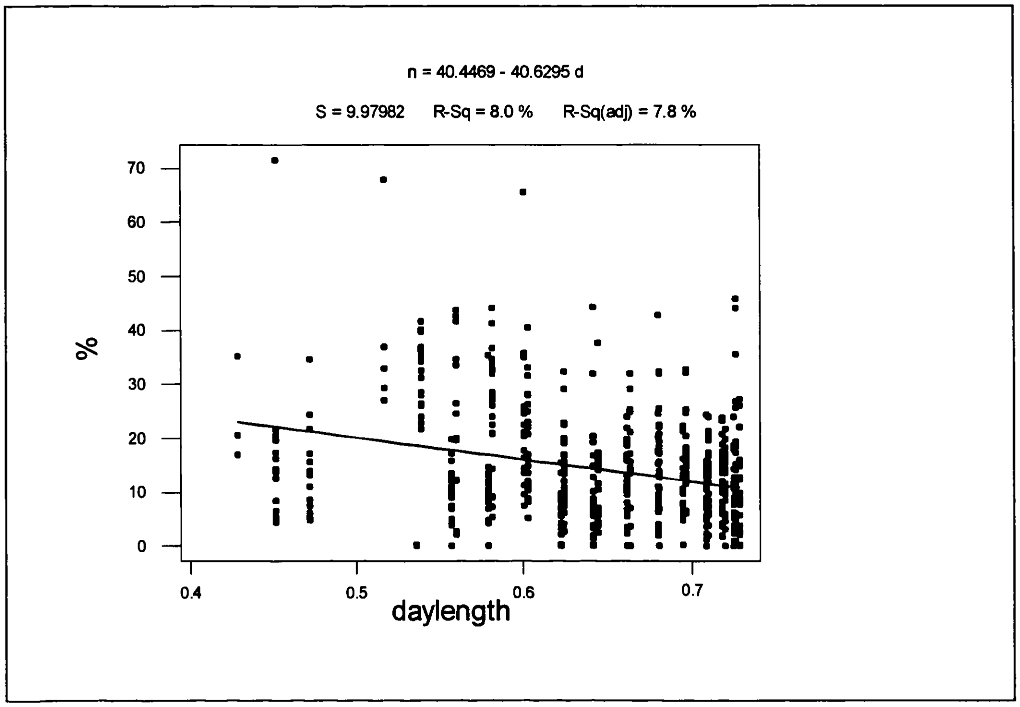


Figure 3.15a Regression of percentage feed consumed at night against day length, Part 1 n = night time feed input d = day length

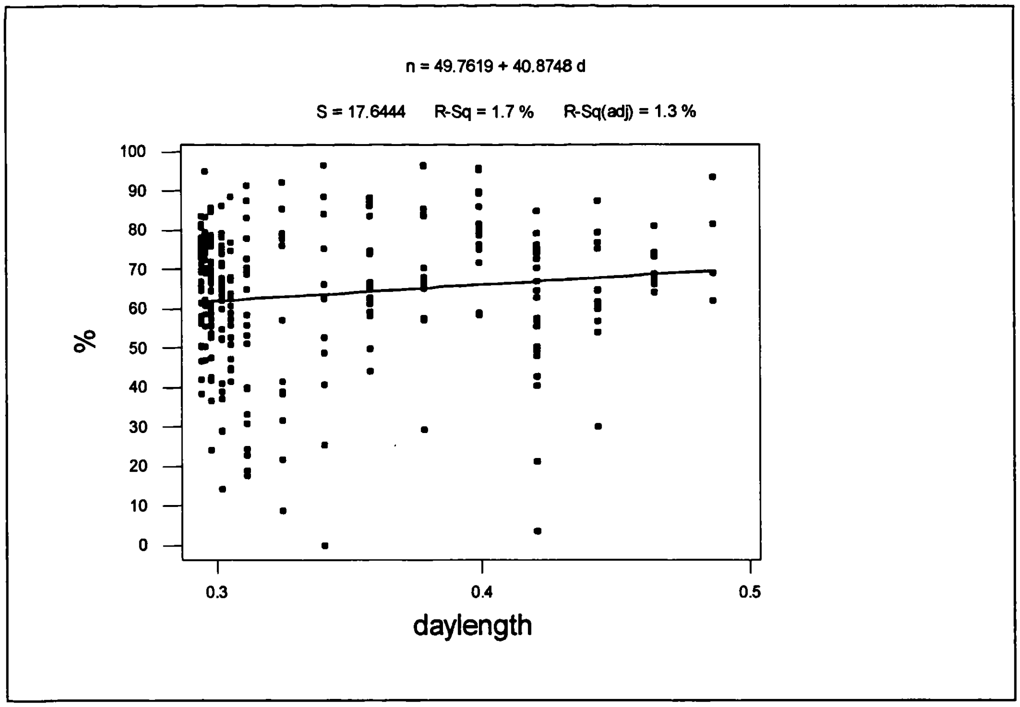


Figure 3.15b Regression of percentage feed consumed at night against day length Part 2 n = night time feed input d=day length

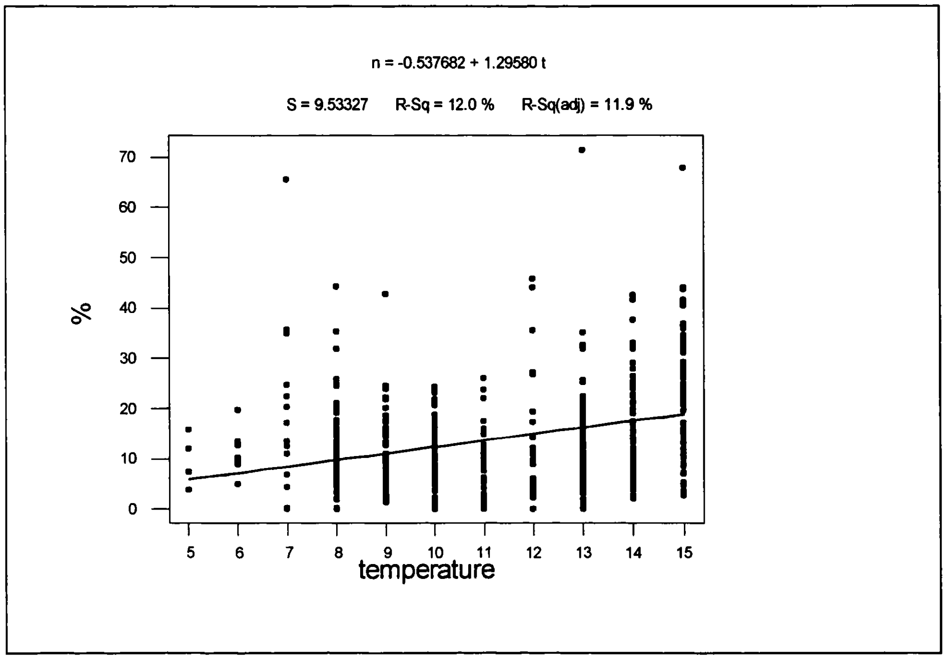


Figure 3.16a Percentage feed consumed at night at different temperatures Part 1 n = night time feed input, t = temperature

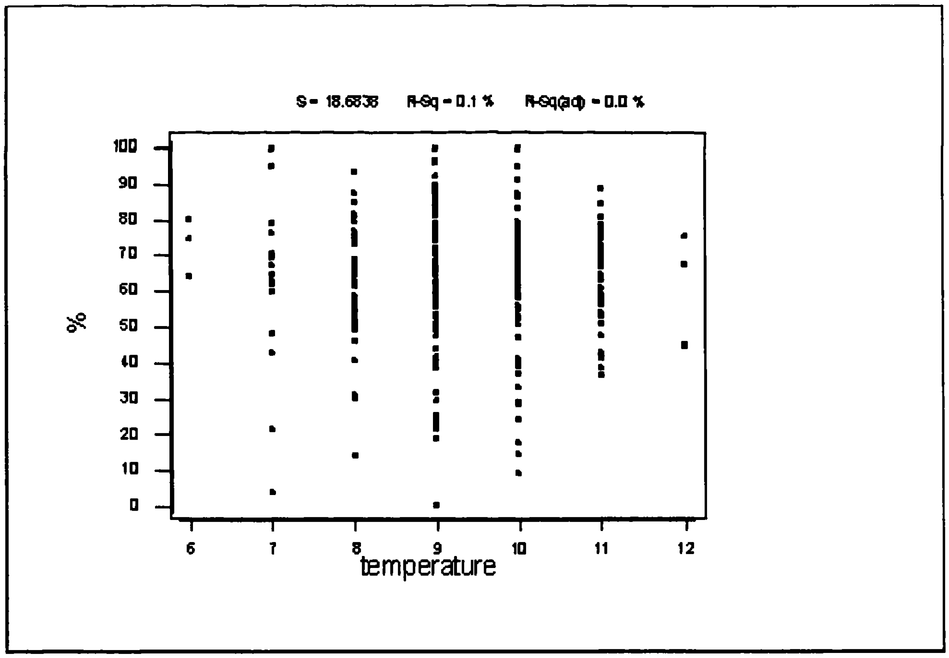


Figure 3.16b Percentage feed consumed at night at different temperatures Part 2 n = night time feed input t = temperature

A multiple regression on the effect of temperature and day length on the proportion of feed taken at night (standardised for the changing photoperiod) for part 1 shows that day length has a highly significant influence ($p<0.0001$) while temperature does not ($p=0.624$) R^2 =72% F= 217.73.

In part 2, the same trend is seen, day length has a highly significant effect ($p<0.0001$) and temperature has no effect ($p=0.439$) $R^2=68\%$ $F= 177.82$.

3.5 Discussion

In aquaculture, it is important to feed when the fishes' appetite is at its peak, in order to avoid feed wastage. The aim of this study was to investigate the natural feeding patterns of juvenile cod in tank conditions using an interactive feeding system.

3.5.1 Daily feeding patterns throughout the year

The fish showed significant feeding patterns from the beginning of the trial. Throughout the trial, there was very little variation in feeding patterns between tanks, but the feeding patterns did change over time. There was one main trend found in 15 out of the 24 ten day blocks analysed. That was the presence of a significant feeding peak at dawn. On some days, this could account for up to 30% of the total daily intake. In some ten day blocks, there was a dusk feeding peak. These results suggest that that the main feeding pattern found in juvenile Atlantic cod is crepuscular. This is in common with a large number of other fish. For example, Kadri *et al.* 1991 found a crepuscular feeding rhythm in caged Atlantic salmon (*S. salar*). The same pattern was found by Paspatis and Boujard, (1996), also in Atlantic salmon and by Blyth *et al.* (1999). Kohbara *et al.* (2003) also found crepuscular feeding in yellowtail (*S. quinqueradita*). In some of the remaining 9 blocks, there are non significant small feeding peaks at dawn, these blocks generally have higher variation, leading to less chance of a peak being significant.

A second feeding pattern, nocturnal browsing, emerged later in the trial. This occurred in combination with daytime browsing in some cases, but in others there was little daytime food consumption. For example, in block 32 there are feeding peaks at 02:00 and 23:00. This may be caused by a fault in the program whereby it stops delivering feed at 24:00 and resumed at 02:00, this fault started at block 30 and continued until the end of the trial. The peak seen at block 32 at 02:00 could be due to the fishes hunger building during the two foodless hours. The small peak at 23:00 could be in anticipation of the foodless period. It has been found that meal timing can act as a strong zeitberger both in mammals such as rats and mice and fish (Chen and Tabata, 2002). These points have been left in the data set as they make up a large proportion of the data in part 2.

3.5.2 Proportion of feed input at night

The two parts of the trial produced two distinctly separate clusters of night time feeding. The first part showed a mean nocturnal feed intake of 10% while the second showed a much wider variance with a mean nocturnal intake of over 50%. Although there were significant patterns found, there does not appear to be a consistent yearly pattern. The two different parts did not show patterns that could be joined up. The difference between the two parts could be down a number of factors; the two sets of fish were the result of two different matings at different times of the year. The first group were produced by photoperiod manipulation of the parents and hatched 'out of season', while the second group were as a result of spawning at the natural time of year. In addition to this, there were different program conditions at the start of the trial, which may have influenced the fishes' feeding patterns; this is discussed in the paragraph below. The fish in group two may have been more sensitive to disturbance or there may have been a higher level of disturbance during the day that could have also caused the fish to feed preferentially at night. Discussion with personnel on a cod farm concluded that one of the main features of cod feeding in a commercial setting was the fishes reluctance to feed if disturbed by human activity. (Personal communication, Bourhill, 2007).

When the trial was set up for part 1, for the first ten days, a highly restricted feeding program was instated during the night, this was in order to assure that the feeders did not empty and overfeed during the night. A possible impact of this could have been to set the fish on a crepuscular feeding pattern, as the program was suddenly unrestricted at dawn, the reappearance of a larger quantity of feed may have stimulated feeding. Although the program was altered to provide unrestricted access over 24 hours, the fish may have become imprinted with that feeding pattern. Other studies have found that fish do respond to feed availability as a zeitgeber and can often continue taking food at times artificially set even during free running conditions, for example, Sea bass in Azzaydi *et al.* 1998 However, dawn feeding is a very widespread feeding pattern in fish, for example it is found in a proportion of self feeding rainbow trout (*O. mykiss*) (Chen *et al.*, 2002), self feeding yellowtail (*S. quinqueradiata*) (Kohbara *et al.*, 2008) and small Atlantic salmon (S. salar) (Kadri *et al.,* 1997)

When part 2 began with a new set of fish, there was no restriction on feed access, and much less of a definable pattern was evident. However, as stated, the fish were produced at a different time of year, from different broodstock. The conditions were also different – in part 1, the temperature was low and rising with a short moderate but lengthening photoperiod, while in part two, the opposite was true the temperature was decreasing steadily while the beginning of part 2 occurred just before the winter solstice, then the photoperiod increased. This means that it is difficult to compare the two parts accurately.

In part one; there was a significant negative relationship between the percentage of feed taken at night with day length. To an extent this can be explained by the longer night length when the day length is shortened, so if feeding was continuous there would automatically be more consumed during darkness hours, however, when day length is corrected for, the relationship is still significant and negative. The opposite is true in the second part, although the gradient of the relationship is low. The differences in effect of photoperiod between the two parts may be explained in part by the reasons stated in the above paragraphs in relation to the differences in nocturnal feeding.

Throughout part 1, the temperature rises from a low of 5°C to a peak of 14 to15°C, which is maintained until the trial ended. In this part the proportion of feed taken nocturnally increases with increasing temperature. However, a multiple regression with photoperiod and temperature suggests that it is photoperiod and not temperature that is the significant factor.

In the second part of the trial. There was no significant patterns to the nocturnal feeding when analysed with temperature and again, a multiple regression showed photoperiod to be the significant factor.

The results in this trial were unusual in comparison with other studies of the kind as more often, a species is found to either be always nocturnal or diurnal, or make total switches to change between the two depending on life history stage or season, for example European catfish (*Silurus glanis*) and Senegal sole (*Solea senegalensis*) were found to be nocturnal by Boujard (1995) and Bayarri *et al.*, (2004) respectively. A study that found different feeding patterns within the same species was Kohbara *et al.* (2000), which found that yellowtail (*Seriola quinqueradiata*) kept outdoors were exclusively nocturnal while those kept indoors under a 12:12 light dark regime were diurnal. The current study found the cod's feeding patterns appeared much more freely changeable, in some cases switching from nocturnal browsing to mainly crepuscular feeding and back again in a matter of 30 days and in some instances, there appeared to be 24 hour browsing with no significant differences in feeding level across the day.

3.5.3 Peak feeding hour

For the first 11 ten day blocks there was very little variation between tanks, apart from blocks 3 and 4. The peak feeding hour is consistently falling as dawn becomes earlier. After block 12 (June 2004) there is a sudden dramatic increase in variance, this change coincides with the summer solstice. The results for the second part of the trial also show no obvious pattern and a huge variation between the tanks. When plotted against mean water temperature, the points form two clusters. The first shows that a low temperature, there is an early peak feeding hour, corresponding to the dawn time. The second cluster shows at high temperatures, mean feeding peaks occurred later. This could be because during some warmer days, there were two crepuscular feeding peaks, instead of one main peak at dawn. If the dusk feeding peak was greater than the one at dawn, in this analysis, it would show up as the main peak. As the temperature rises, gastric evacuation time decreases, which would lead to the fish being ready for another meal more quickly than during colder conditions. This may account for the later feeding peaks occurring as well as the morning peaks. In addition, as temperature rises, amount of feed needed by a fish goes up (for example, Jobling, 1982), so at higher temperatures a second feeding peak may be needed to satiate the fish.

When the peak hour is correlated with the day length, there is a significant negative correlation. Again, this is due to the strong feeding peak at dawn, as the day length increases, dawn becomes earlier, and so the feeding peak becomes earlier. In part 2, there is a significant negative correlation between peak feeding hour and temperature. This can be explained by increasing temperatures being present when dawn is earlier and so as the temperature increases, the dawn feeding peak gets earlier.

The literature describing cod feeding is limited, the main conclusion being that large cod should be fed at most once every day, or even once every two days (Jobling 1988). This suggestion does not apply to small juvenile fish, and generally, the industry is not happy to feed less than twice per day (Bourhill, personal communication, 2007). Some farms provide feed by automatic feeding periodically adding feed throughout the day, as well as a large hand feed.

Farmed cod do have a different feeding strategy compared with captive salmon, which feed vigorously at the surface which causes the water to appear to 'boil' at feeding times. Cod tend to feed below the surface and are less visible; they do not appear to get as 'excited' at food as salmon do. This means that there is less visual feedback for the farmers of cod, making feeding more difficult. Therefore, an accurate feeding strategy is even more important to avoid feed wastage and under feeding.

The most important points from this study for farmers to take into consideration when making their feeding plans are outlined below:
This trial has shown a number of significant daily feeding patterns for juvenile cod, and although there have been some correlations with temperature and photoperiod, not enough was found to accurately predict the feeding patterns at any particular temperature, photoperiod or time of year. It appears that cod, like other species are able to adapt to feed availability which is useful from a farmer's point of view, however, there is also evidence that cod choose to feed throughout the day so feeding large distinct meals with long gaps may not be the optimum feeding strategy.

The feeding patterns of cod can be disrupted by human activity as well as unfavourable environmental conditions. Consider use of either self feeders or an interactive feedback system such as the AQ1 system to allow fish to adapt their feeding patterns to when they are content enough to feed. Additional use of submerged cameras can help monitor uneaten feed levels and feeding levels can be updated accordingly.

Future work should be carried out looking at cod's feeding patterns in commercial aquaculture conditions. This trial was based on a relatively small number of fish in small, shallow brightly lit tanks; fish in cages may have different reactions to changing environmental conditions.

The instance of nocturnal feeding should be explored further; it may be that like Atlantic salmon, feeding at night may not be efficient (Alanärä, 1992). Alternatively, they may be more like sea bass which grow best when their feeding strategy is inverted to nocturnal feeding during the winter, which gives better feed efficiency (Azzaydi *et al.* 2000). Although as the factor that causes switching feeding pattern has not been discovered in this study, feeding using demand or adaptive feeders will allow the fish to feed to a changing pattern.

This study only investigated the feeding patterns of juvenile cod up to a weight of 100 grams. It is possible that as they become larger, the feeding patterns become more consistent, especially as younger fish tend to feed more regularly than their older counterparts (Burrows 1972). There are also marked differences between the environment used in this trial to hold the cod and commercial (and wild) situations. The tanks were small, shallow and brightly lit whereas in commercial settings, fish are generally held in larger, deeper tanks with a lower light intensity. The flow rate and feed type are however, similar to those found in farm situations.

Chapter 4 Development and deployment of a demand feeding system for juvenile cod

4.1 Introduction

Chapters 2 and 3 looked at the appetite of juvenile cod using the AQ1 interactive feeding system which, although successful, had some problems associated with it. An alternative method of mapping the feeding patterns of fish is the use of a demand feeder. This method has rarely been used for cod in research and is investigated in this chapter. I aim to investigate the possibility of using a demand feeding system for juvenile cod. I also investigate possible sources of individual differences in response to the demand feeder, including size and coping strategy.

4.1.1 Possible advantages of demand feeding

Demand feeding is a useful tool, both in the aquaculture industry and for scientific investigation of feeding patterns and diet choice. It allows fish to choose when and how much to feed, by training them to press a trigger or sensor that rewards the fish with food. Fishes feeding patterns and appetite can vary depending on numerous factors such as dissolved oxygen content, temperature, water quality, light intensity and perceived dangers. This makes it hard to predict how much they are likely to eat on any given occasion. Providing a demand feeding system overcomes this problem by giving fish the opportunity to control their own feed and thus potentially reduce feed wastage. This reduces environmental impact and also reduces labour costs and costs of wasted food.

In some cases, fish growth is lower on a demand feeding system in comparison with hand-feeding to satiation (Gélineau et al. 1998). However, Yamamoto et al. (2002) found no difference in growth of juvenile rainbow trout using demand feeders or hand feeding. In some studies, self feeding can improve feed conversion, for example Sánchez-Muros et al. (2003) fed gilthead sea bream (*Sparus aurata*) either by hand or through a demand feeder; the demand feeder improved FCR and protein efficiency by up to 60%. Atlantic salmon parr fed either automatically every 10 minutes or on demand by an interactive feedback system (both from dawn until dusk) did not show any difference in growth between the two systems. Additionally there was significantly less fin damage in the demand fed fish suggesting reduced stress and aggression and, due to lower feed wastage, the FCR was improved also (Noble et al., 2007). However, Wagner at al. (1995) found no difference in growth, FCR or fin damage between cutthroat trout (*Oncorhynchus clarki*) that were hand-fed four times a day and demand feeding fish. However, four feeds per day is very labour-intensive and not all farmers can feed to this level; the fish may not be fully satiated, so with fewer feeds, aggression and therefore fin damage may increase. The growth of rainbow trout (*Oncorhynchus mykiss*) was significantly higher in fish using demand feeding than restricted automatic feeders (Alanärä, 1992).

It is known that cod can use a demand feeder. Franco et al. (1991) trained cod to use a demand feeder by touching a feeding cup. This was used to investigate their preference for different feed stimulants, with the aim of developing baits for long line fishing, the feed stimulants in current use were attracting fish, but ingestion did not follow. The experiment investigated the palatability of different compositions of synthetic squid mixture. The self feeding system is outlined in figure 4.1.

4.1.2 Types of demand feeders

The main elements of a demand feeder are the trigger and the feed delivery system. There are a number of types of demand feeders, distinguished mainly by the nature of the trigger.

The simplest form of demand feeder is a feed container with a mechanical trigger, connected to a gate, hanging at the water surface. When the trigger is moved, the gate opens, allowing a known amount of feed to fall into the water (Alanärä et al., 2001). An example is shown in Figure 4.2; the feeder is suspended over the water with the pendulum just into the water, fish can then release feed by moving the pendulum.

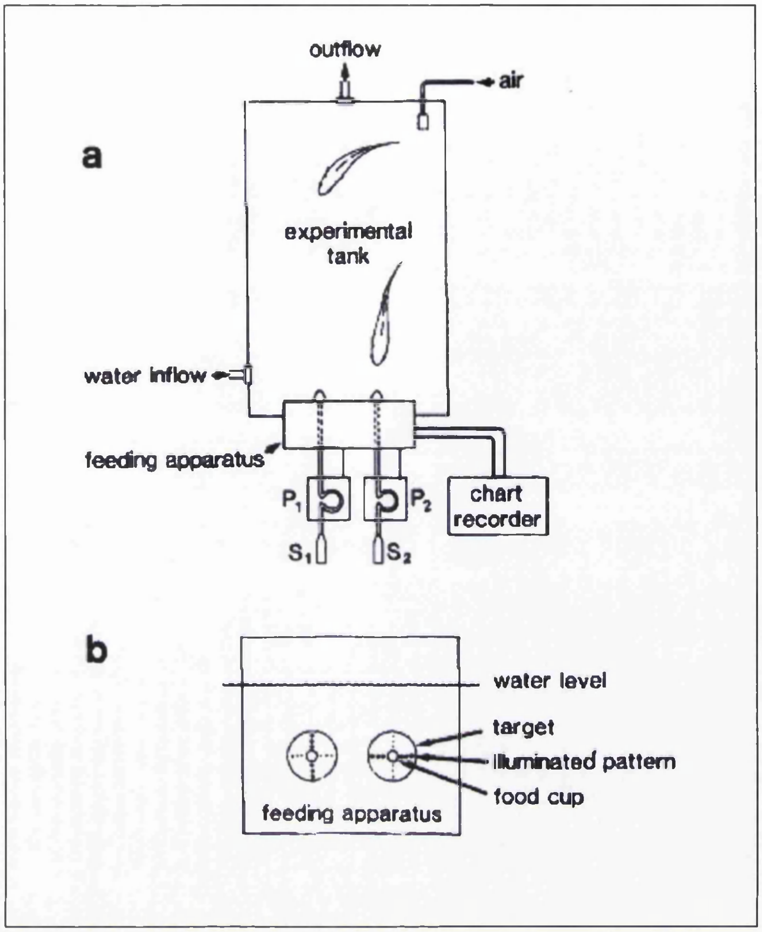


Figure 4.1 a) view of experimental equipment showing the feeding apparatus in place at one end of the tank. P peristaltic pump, S food delivery syringe b) Front view of the feeding apparatus showing the location of food cups and LED pattern from Franco et al. (1991).

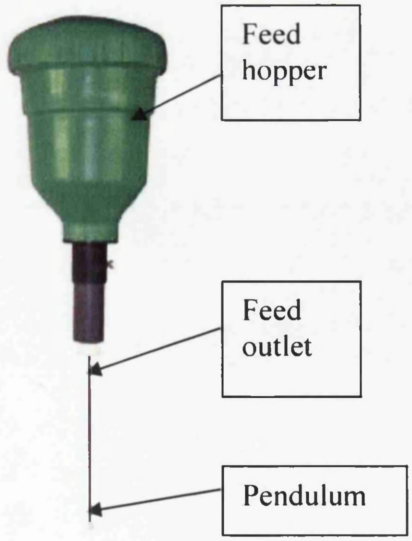


Figure 4.2 Pendulum feeders (from Dynamicaqua.com)

More sophisticated electrical demand feeding systems usually consist of a trigger attached to a control box and an electrical feeder. These are more useful for research than a mechanical trigger as they can be connected to a computer and each activation can be logged, building a picture of the subject fishes feeding patterns. This type of system was used with cod by Franco *et al.* 1991 as outlined in section 4.1.1, whose apparatus is outlined in figure 4.1. The most basic trigger is a lever made from metal or plastic, usually with a bead on the end that hangs in or just above the water surface. The main drawback of this type of sensor is accidental triggers by fish swimming past. This can be avoided by positioning the trigger just above the water surface. Another method of protecting the trigger from accidental actuations is to place a screen around it (Coves at al. 1998). This means that fish do not accidentally collide with it during normal swimming activity.

Rubio et al. (2004) working with European sea bass used a steel string sensor with a rubber bead attached, that fish had to bite and pull in order to release feed. This style of trigger is advantageous over simpler push triggers, as it stops accidental feedings caused by fish swimming close to the sensor, or by wind or wave action. Sea bass learned to use this method within 20 days and retained this knowledge for over a year, whilst being fed by other means. It was also found that fish presented with the bite sensor were more likely to feed at night, as opposed to fish using a screened push trigger.

Amano et al. (2007) uses a photosensitive sensor in a demand feeding system for ayu. Fish had to swim within one centimetre of the tip (which hung 0.5cm below the water surface) to actuate the feeder. This type of sensor is particularly useful for small fish, as they may not have the strength to actuate the other types, although there may be occasional accidental triggers due to fish swimming through the sensor area.

4.1.3 Reward level and response interval

An important factor that influences fishes' ability to fulfil their daily appetite whilst demand feeding is the reward level given at each trigger of the sensor (Alanärä, 1994; Alanärä, 1996; Gélineau et al. 1998; Brännäs and Alanärä, 1994). Reward levels should be set to the amount of pellets the fish can catch before they fall out of reach (Alanärä et al. 2001). One sensor actuation provides feed for more than one fish. Reward level should be adjusted according to stocking density (Alanärä, 1996).

Rainbow trout respond to low reward level by increasing bite activity when given access to a low energy feed, however, Arctic charr failed to increase their demands to take in more feed, and therefore grew more slowly than their counterparts being fed on a higher energy feed. However, in another experiment detailed in the same paper, the charr were able to compensate for smaller reward sizes and so consume enough feed to fill them to satiation (Alanärä and Kiessling 1996).

Gilthead sea bream, (*Sparus aurata*) increase the number of triggers when the reward size is decreased but when the rewards are restricted to a certain time, there is no modification (Velázquez et al., 2006).

Response interval (the amount of time a feeder takes to dispatch the feed after the trigger is pressed) is also an important factor for the success of demand feeders. In a trial using rainbow trout, it was found that a response interval of over 15 seconds restricts the ability of fish to increase their feeder actuations to satisfy their energy requirements (Shima et al., 2003).

4.1.4 Learning to use the demand feeder

Naïve Arctic charr began triggering the sensor of a self feeding system on day 1 of an experiment by Brännäs and Alanärä, (1993). Chen et al. 2007 found that Amberjack (*Seriola dumerili*) held individually also learnt to use a demand feeder quickly. In contrast juvenile turbot (*Scophthalmus maximus*) took 20 days to learn to use the feeder (Burel et al.,1997). Ayu (*Plecoglossus altivelis altivelis*) held individually took between one and 52 days, and around half the fish failed to learn to use the feeder through the whole trial period which ranged from August to December 1999 (Amano et al., 2006). This is a large variation and supports the theory that if fish are held in a group, it is likely that due to the length of time some individuals take to learn to use a demand feeder, not all of the fish in a group will be responsible for triggering feeds.

4.1.5 Coping strategies

Animals often show striking differences in behavioural and physiological responses to challenge (or "coping strategies") (Korte et al., 2005). Some individuals (designated "proactive") typically show an active (fright and flight), adrenaline-based response to various challenges, are aggressive towards competitors, take risks in the face of potential danger and are relatively inflexible, tending to form behavioural routines. They also have an energy-expensive life style, based on a high resting metabolic rate. In contrast, so called "reactive individuals" show a passive (freeze and hide), cortisol–based response to challenge, avoid risk (including fights), but are responsive to, and flexible about, environmental change. They generally have a low-energy life style, with a low resting metabolic rate (ibid). Reactive and proactive mice differ in the fine structure of their hippocampus and also in expression of genes related to neurogenesis in this region (Feldker et al., 2003). The two coping strategies may be clearly distinct, or they may represent two extremes of a continuum.

Understanding this theory has implications in aquaculture, as robust, bold fish are likely to recover more quickly from handling and be more likely to thrive in aquaculture conditions when compared with more timid fish, however, boldness and aggression are usually linked, and in crowded aquaculture settings, aggression in fish is a negative factor, and can cause fin and body damage to the surrounding fish which leads to lower market price (Huntingford, 2007).

Frost et al. (2007) writes that these traits can be modified by manipulating the experiences of individual fish, in this case, rainbow trout. For example, if a bold fish is made to watch a submissive fish's reactions, it becomes less bold. Conversely, if a timid fish is introduced to a smaller fish, it will become the dominant fish, and become less timid.

Brännäs and Alanärä, 1994 report that in groups of demand feeding rainbow trout and arctic charr, there were only one or two fish responsible for triggering the feeder. The trigger fish tended to be a high size rank.

As in all types of feeding, in order to prevent the dominant fish monopolising the feeder, it is important to spread the feed over a large area. The reward level should also be set at a level where the maximum number of fish will be fed, whilst not adding waste feed.

4.1.6 Cortisol and whole body corticosteroid

Cortisol is a corticosteroid hormone produced in response to a stressor. Measuring plasma levels of cortisol can give an indication of how stressed a fish is. In small fish and larvae, where it is difficult to remove enough blood, the whole body can be homogenised and immuno-reactive corticosteroid extracted for assay. King and Berlinsky (2006) compared plasma cortisol with whole body immunoreactive corticosteroid in juvenile cod after standard stressors. It was found that the corticosteroid concentration remained raised for longer than plasma levels, suggesting that there are other steroids reacting with the immunoassay.

Serum cortisol levels fluctuate across the day, lowest levels are during the night and the level rises in the photophase (Pavlidiset al., 1999). Concentration of cortisol is also affected by photoperiod. Fro example, in the common dentex (*Dentex dentex*) the highest levels were found when the photophase was longest (Pavlidiset al., 1999).

4.2 Aims

The main aim of the study described in this chapter is to design a demand feeding system for juvenile cod in a closed recirculation system and to investigate the process by which cod learn to use it. To examine possible effects of coping strategy (assessed by novel object test and whole body immunoreactive corticosteroid level on this process.

Methods

4.3.1 Fish and husbandry

35 juvenile cod were supplied by Machrihanish Marine Farm and the Marine Environmental Research Laboratory (Machrihanish). They had a mean start weight of 5.5 grams. These fish were split into seven groups. The fish were marked using white elastomer in a number of patterns on the dorsal side of each fish in order to aid individual recognition. They were housed at Glasgow University in the Graham Kerr Building Experimental aquariums in a recirculation system consisting of four 100 litre round tanks, mounted above waste feed collectors (made by Per Holland). The water flowed through the collectors into a 200 litre sump tank containing the biofiltration media, Kaldnes K1.

The water was returned to the tanks by two Eheim pumps via two ultra violet sterilisers (TMC Vecton 25W). Water in the sump was also filtered by a protein skimmer (Aquamedic Turboflotor 1000 Multi). The flow rate in each tank was approximately 4 litres per minute to each tank.

The water was made using Instant Ocean salt to a salinity of 32‰. The water quality (ammonia, nitrite, nitrate and pH) was monitored daily while the filter media contained in the sump was maturing, becoming weekly when water conditions were more stable. 20% water changes were carried out at least weekly. The water temperature was maintained between 8°C and 10°C by air conditioning the room to that temperature. The photoperiod was 10:14 (L:D) hours using the room's strip lighting on a 24 hour timer.

4.3.2 The demand feeding system

One of the key features of the feeding system was the ability to withdraw the sensor from the tank if a preset number of actuations occurred over a given time. This was implemented because the tanks and filter system were enclosed and so over feeding needed to be avoided.

The demand feeding system comprised a platform over each tank, holding a sensor attached to a motor and a feeder. Each of these were attached to a control box, which in turn was attached to a computer. The platforms, sensor and motor were constructed by the Bioelectronics Department of the University of Glasgow. Pictures of the hardware can be seen in appendix 2

The feeders were made by Imetronic. They consisted of an internal disk with three holes that, when activated, rotated to allow a measured amount of around 0.17grams of feed fell through a delivery tube and into the tank below.

The software and display allowed a bite limit (how many activations the sensor can have in each period before extracting the sensor) to be set. This could be different for each tank, depending on fish behaviour and experimental setup. A representation of a feed hopper for each tank was present to give a visual cue for how many actuations were left for each tank. On the right hand side of the screen shot were three time dials. They were set to the time when the system switches of the time to switch on, and the accumulation time (the amount of time that the number of bites should be grouped together) this was usually set at one minute. Figure 4.3 shows a simplified flow diagram of the processes controlling the feeding system.

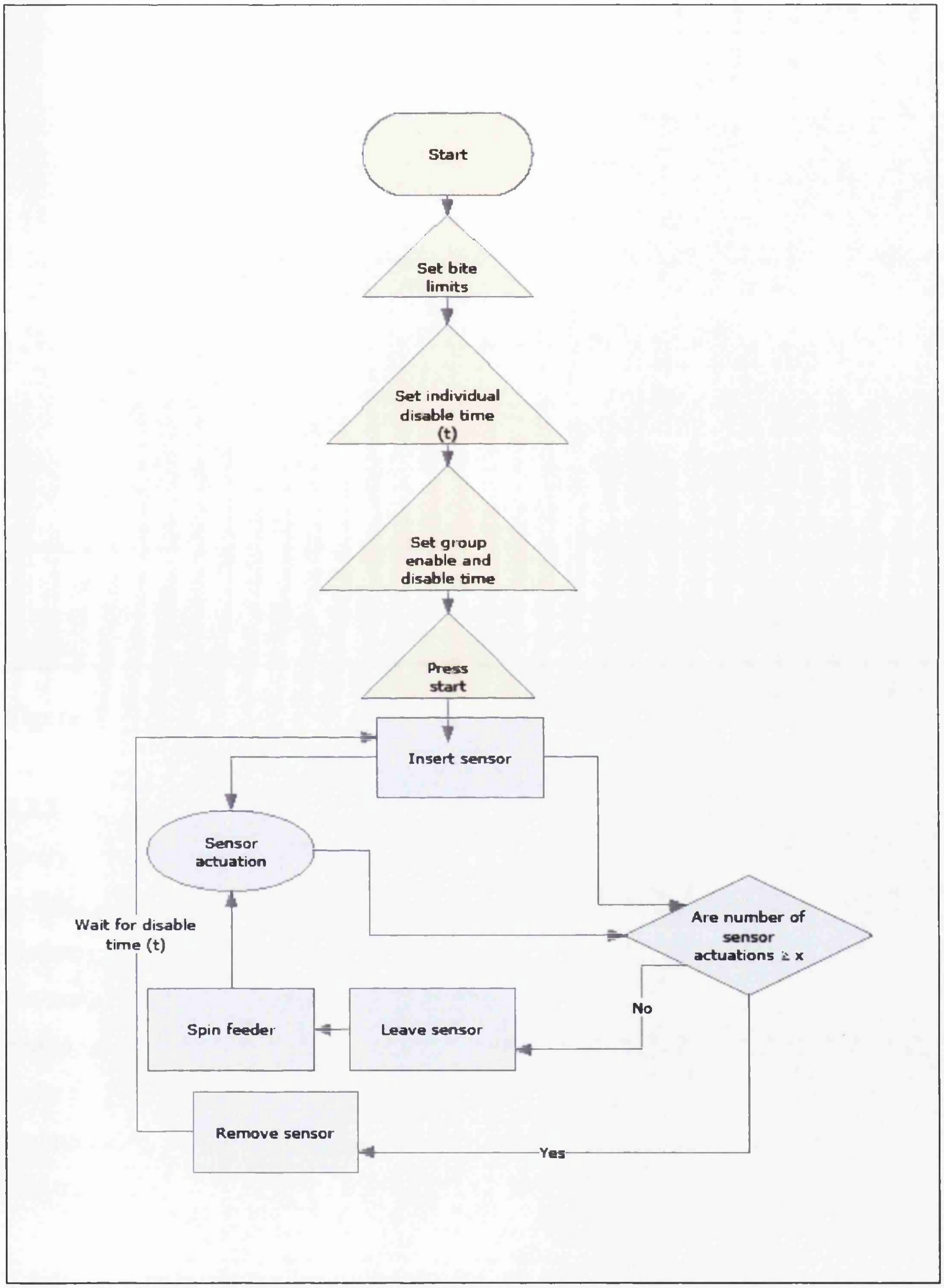
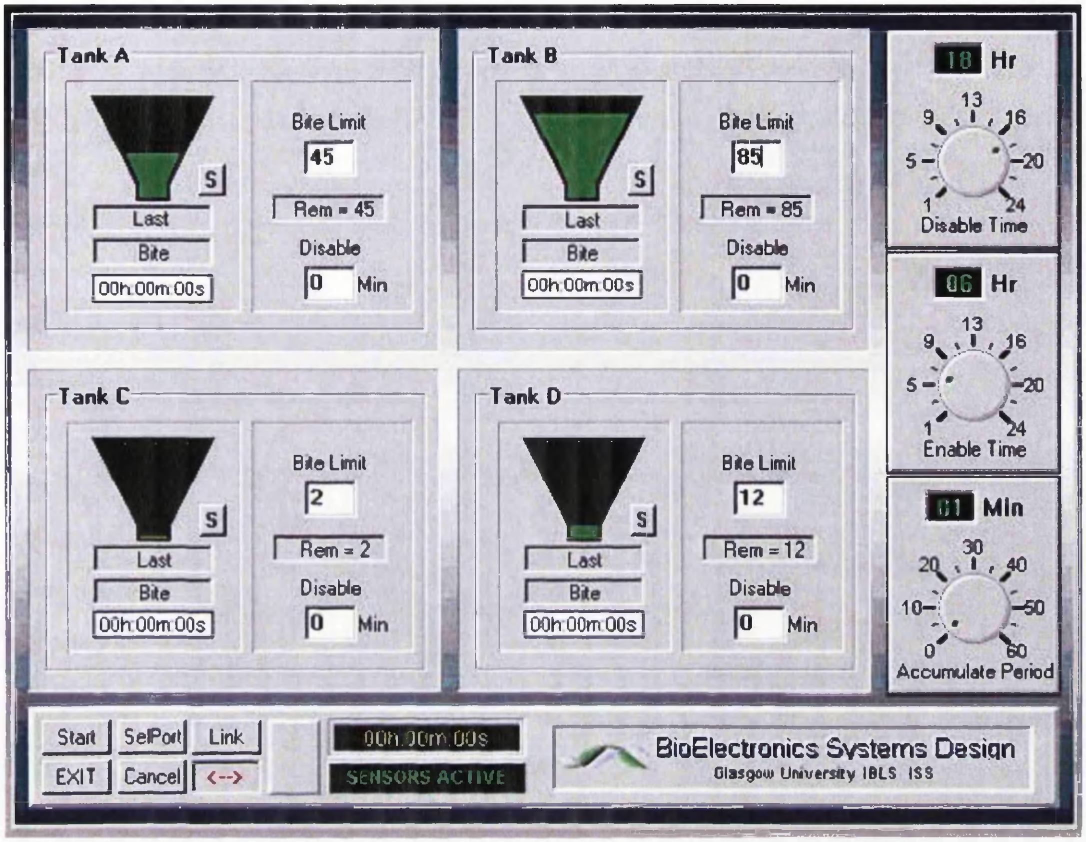


Figure 4.3 Flow diagram of the processes involved in the running of the demand feeding system. The yellow boxes represent stages where an operator input is required and the grey boxes are those that are run by the processor.


Tank A
Bite Limit 45
Rem = 45
Disable 0 Min
Last Bite 00h:00m:00s

Tank B
Bite Limit 85
Rem = 85
Disable 0 Min
Last Bite 00h:00m:00s

Tank C
Bite Limit 2
Rem = 2
Disable 0 Min
Last Bite 00h:00m:00s

Tank D
Bite Limit 12
Rem = 12
Disable 0 Min
Last Bite 00h:00m:00s

18 Hr Disable Time
06 Hr Enable Time
01 Min Accumulate Period

00h:00m:00s
SENSORS ACTIVE

Figure 4.4 A screen shot of the software

4.3.3 Training to use the sensor

Every day, a sensor was manually introduced into each tank for 30 minutes and each time a fish interacted with this sensor, feed was added (approximately 4 pellets) a short distance away from the sensor. At first, interaction was specified to be a fish approaching the sensor, but not necessarily coming into contact. As the training progressed, the fish had to come closer and eventually make contact which resulted in the sensor activating in order to get a reward. In tanks where there was no activity in the vicinity of the sensor, a maintenance amount of feed was fed to avoid starvation of the fish. Each group of fish was trained individually at the speed at which the group progressed.

4.3.4 Novel object test

Individual risk taking was assessed by recording the measurements of each fish in each group to a novel object. This is a standard way of screening risk taking behaviour in fish

(Sundströme et al., 2004) and other animals such as great tits (*Parus major*) (Fidler et al., 2007). The test was carried out on 3 tanks, each containing 5 fish. Three objects of approximately 10x10x10 cm, in random patterns of red, yellow, blue and green were constructed out of Lego, two of them were weighted with aluminium sheets. The objects were added to each tank in turn, the first, un-weighted object floated on the surface close to the edge of the tank and the two weighted objects sank to the bottom of the tank at around the central point. Each object was attached to a piece of string tied to the edge of the tank to hold it in the correct position. Each tank had one object a day for three consecutive days. The fishes' response was recorded by an infra red camera mounted above the tank. An infra red led light illuminated the area. Each test was 40 minutes long beginning when the novel object was introduced into the tank. The resulting video was analysed by classifying the fishes behaviour into three categories; Swimming within one body length of the object (S), swimming within half a body length of the object (CS) or coming into contact with the object / interacting (I). The time of each action was noted. Body length was represented by placing an acetate film over the screen with circles of the appropriate measurements (i.e. one body length and one half a body length) marked.

4.3.5 Whole body immunoreactive corticosteroid assay

The fish were removed from the tanks and killed individually by overdose of benzocaine followed by destruction of the brain. Disruption to the fish was kept to the minimum to avoid influencing the corticosteroid level, so the water level in each tank was dropped slowly to make catching the fish easier. The fish were then homogenised and processed to test for whole body corticosteroid as in an updated version of King and Berlinsky (2006).

4.3.5 The experimental setup

Seven groups of cod were trained to use the demand feeder, using the protocol outlined above in section 4.3.3, the first four groups were in tanks 1 to 4, after 30 days, the process started again with three new groups of cod in tanks 1, 2 and 3. These final groups were given that novel object test and whole body corticosteroid outlined in the previous two sections.

4.4 Data analysis

During the training to use the feeder, the number of actuations during the daily period of each fish were recorded. For each group, the daily total number of actuations were grouped into 5 day periods. The data were bimodal and therefore the non-parametric Kruskal-Wallis test was used to determine the presence of any significant differences.

4.4.1 Feeder training

In the final three groups, each fish was categorised into three groups; NO (never interacts with the sensor), OC (occasional sensor interaction) and TF (the trigger fish that was responsible for the majority of interactions). The morphometrics of each fish (start weight, end weight, after the 30 days and percent weight change) put in its corresponding category were investigated by one way ANOVA and differences highlighted by Tukey post-hoc test.

4.4.2 Novel object test

Initially, three categories of interactions were measured; S (swim within one body length of the novel object), CS (swim within half a body length of the object) and I (physical contact is made with the object). The three categories were compared using Pearsons correlation to find if the categories were significantly distinct from each other.

Once the fish had been classified by feeder interaction, these results were plotted against the novel object interactions of each individual to investigate the level of correlation between the two factors.

The relationship between the corticosteroid level and end weight of each fish measured (in groups 5, 6, and 7 was plotted and against both physical and swim interactions in the novel object test and their regression equation calculated.

4.5 Results

4.5.1 Development of an attractive trigger

In order to train the fish to operate a trigger initially, a plain sensor was used (figure 4.5a). When this was introduced into the inhabited tank, the fish showed no interest or recognition that it was present. A literature search revealed that the maximum retinal light absorption of the cod is in the blue-green area of the spectrum (Bowmaker 1990). Therefore, a blue bead on a piece of blue airline was fitted over the silicone area of the sensor as seen in figure 4.5b. This modification did not lead to any increase in the fishes' actions towards the sensor. Then green craft glitter was mixed with aquarium silicone and spread over the bead and tip of the sensor. This was attractive to a number of fish luring them to approach the sensor, which meant the training could begin.

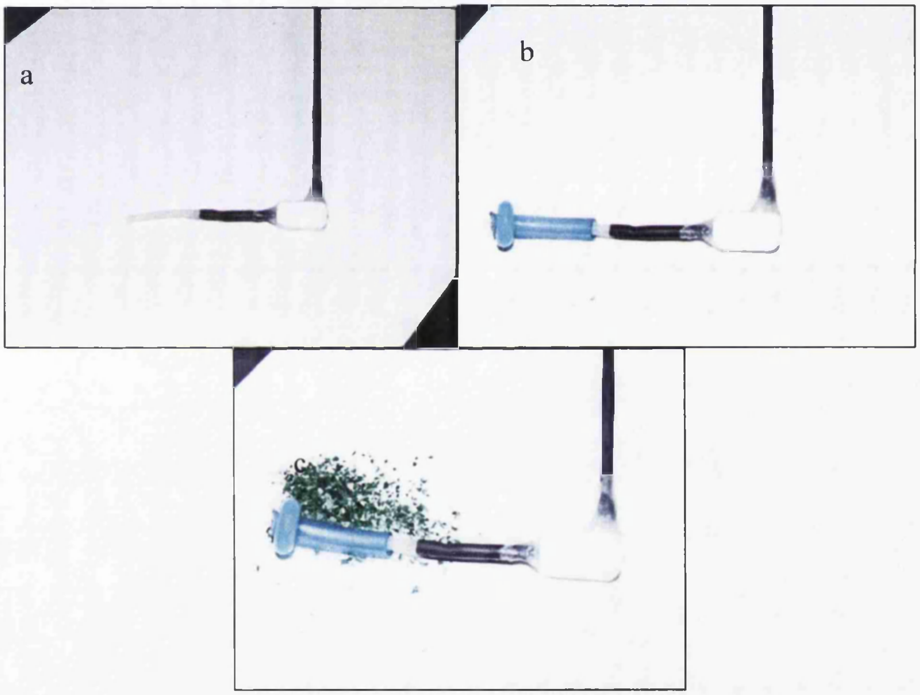


Figure 4.5 Different stages of sensor development to make it attractive to juvenile cod. a) the bare sensor b) with blue airline tubing and a blue bead attached c) the sensor with glitter that was mixed with silicon and painted on the end of the airline and bead.

4.5.2 Level of response to the trigger

The trigger was introduced into the water of each tank for thirty minutes once a day, in order to teach the fish to associate touching the sensor with receiving a food reward. Figure 4.6 shows the distribution of the frequency of the interactions across the whole experiment (groups 1 to 7), there is a clear bimodal distribution with the highest peak being at 0 and the secondary peak at around 7 interactions per 30 minute training period.

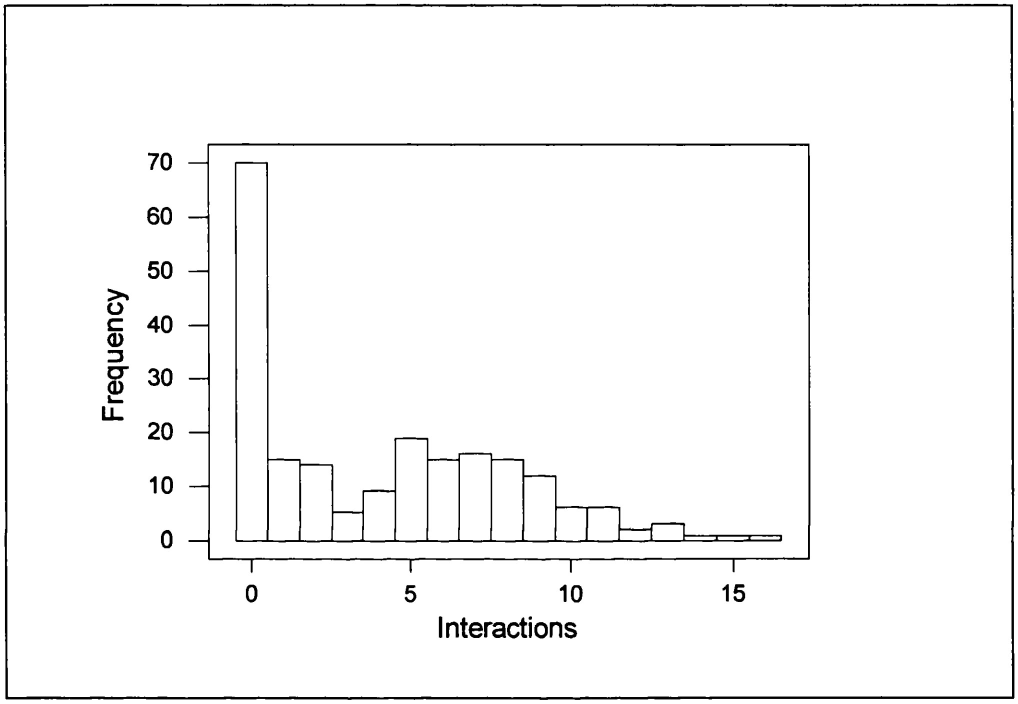


Figure 4.6 Frequency distribution of interactions made with the sensor per 30 minute training period across all 7 groups of cod, showing bimodal distribution of results.

The progression of the median number of sensor interactions per training session in each group is shown in figure 4.7. Each group appears to have quite individual learning patterns. Groups 1,3,4,6 and 7 all appear to reach their peak interaction rate by day 25, some of these groups reach their peak quicker. Group 5 was slow in showing interest and interacting with the sensor, while group 2 were initially slow to start off, but started to increase their demands from day 20. On day 25, the fish responsible for the interactions in group 2 jumped out and died, the remaining fish only began to start interactions again 5 days later after the trial ended.

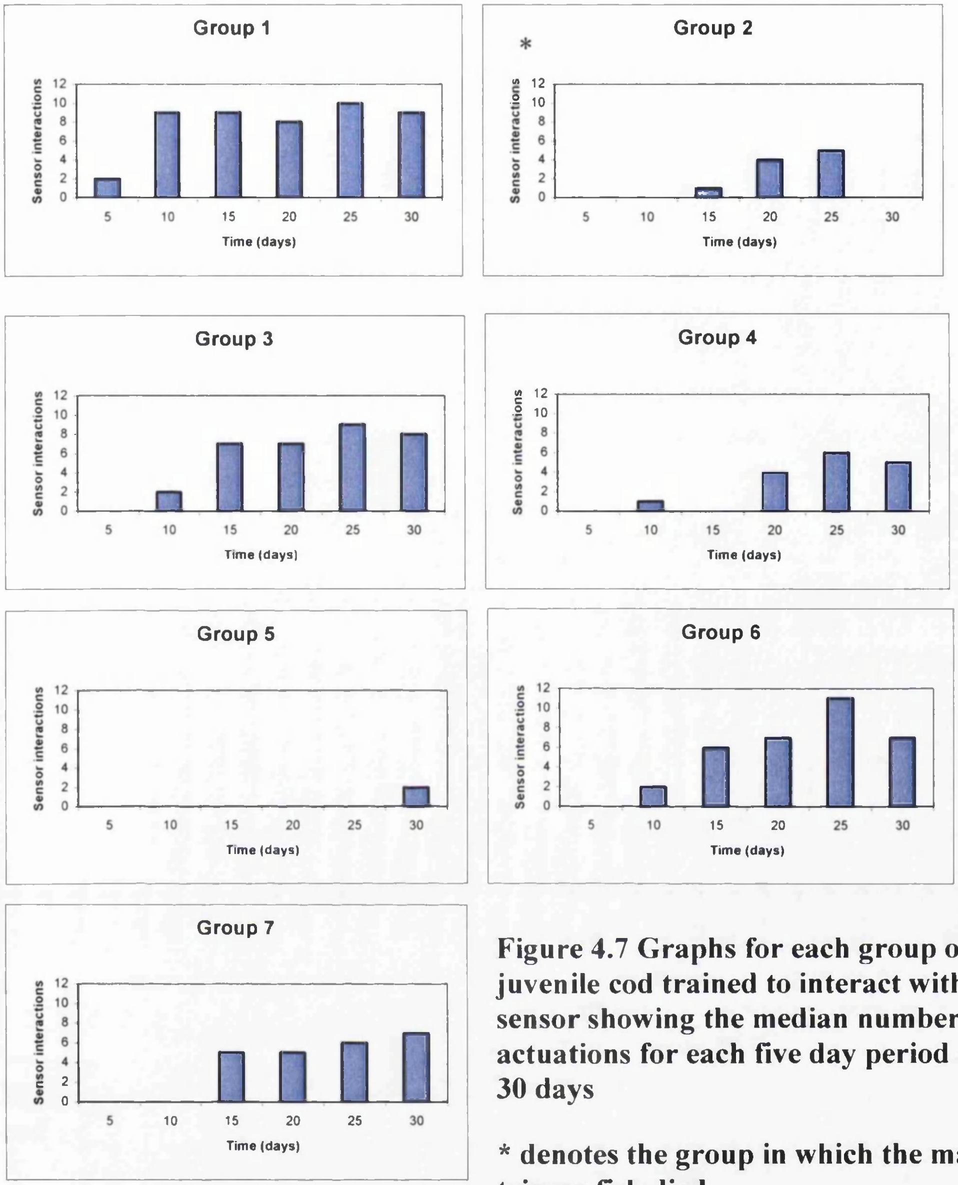


Figure 4.7 Graphs for each group of juvenile cod trained to interact with the sensor showing the median number of actuations for each five day period over 30 days

*** denotes the group in which the main trigger fish died**

Group	H	df	P
1	9.02	5	0.108
2	12.62	5	0.027
3	19.38	5	0.002
4	21.33	5	0.001
5	7.55	5	0.183
6	22.49	5	≤0.0001
7	20.64	5	0.001

Table 4.1 Kruskal-Wallis results showing level of significance for differences across the 30 day period for each group in the number of sensor interactions.

Figure 4.8 shows the frequency of the total number of interactions of individual fish during the full 30 day entraining period. By far the most common number of interactions was 0-10. This category was further broken down in Figure 4.9, which shows the majority of fish made no interactions.

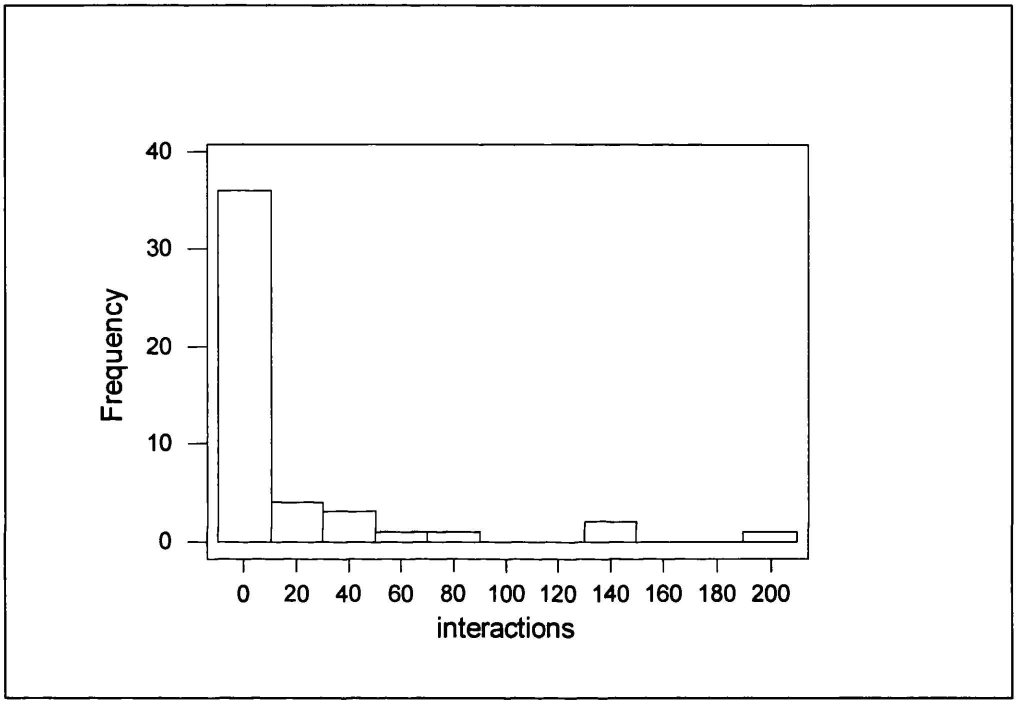


Figure 4.8 Frequency distribution of the sensor interaction classification of juvenile cod across all groups

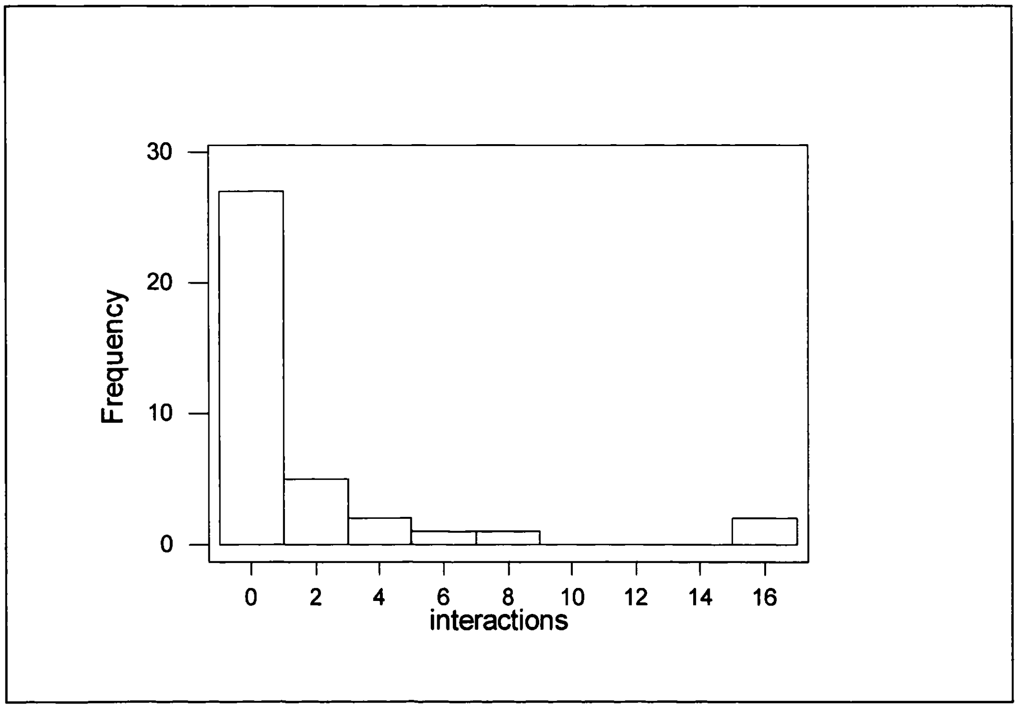


Figure 4.9 Frequency distribution of the sensor interaction classification of juvenile cod that make between 0 and 20 interactions, across all groups

In groups 5, 6 and 7, each fish was given a rating based on its interactions with the sensor, the ratings and number of fish in each group can be seen in Table 4.2.

Classification	Meaning	Group 5	Group 6	Group 7
NO	Has no recorded interaction with the sensor	0	2	2
OC	Has occasional interaction with the sensor	4	3	2
TF	Is the main trigger fish with the most sensor interaction	1	0	1

Table 4.2 Classifications of sensor use of individual juvenile cod in three groups

Figures 4.10a, b and c show the start and end weights and percentage change in weight of the fish in relationship to their sensor use; table 4.3 gives the associated statistics. Fish that showed no trigger action were significantly smaller than those that occasionally had interactions, but not significantly different to the trigger fish. There was no significant difference between the end weight of the trigger fish and the fish that occasionally activate the feeders but those that had no interactions were significantly smaller. The trigger fish showed the greatest growth rate (at almost 30%). There was no significant difference between the growth of occasional and no interaction fish, but the fish that had no interactions seemed to show greater variability of growth.

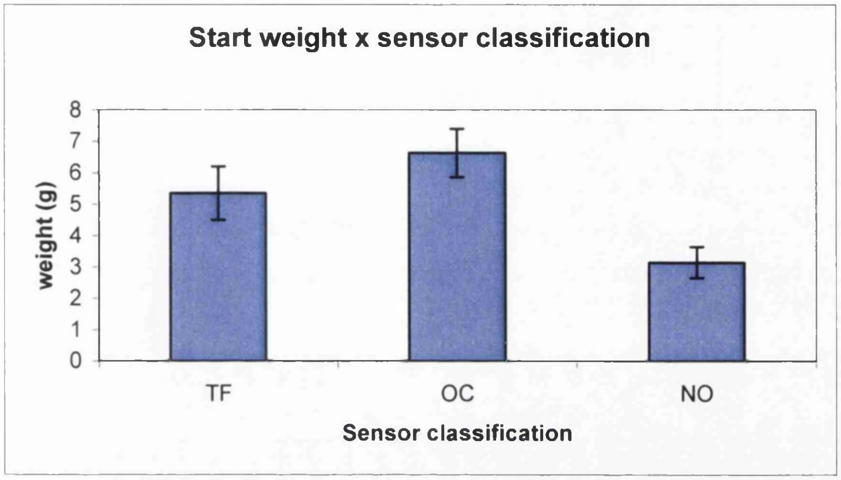


Figure 4.10a Mean (and standard error) start weight of individual fish with their sensor classification. NO = no sensor interaction OC = occasional sensor interaction and TF = the main trigger fish.

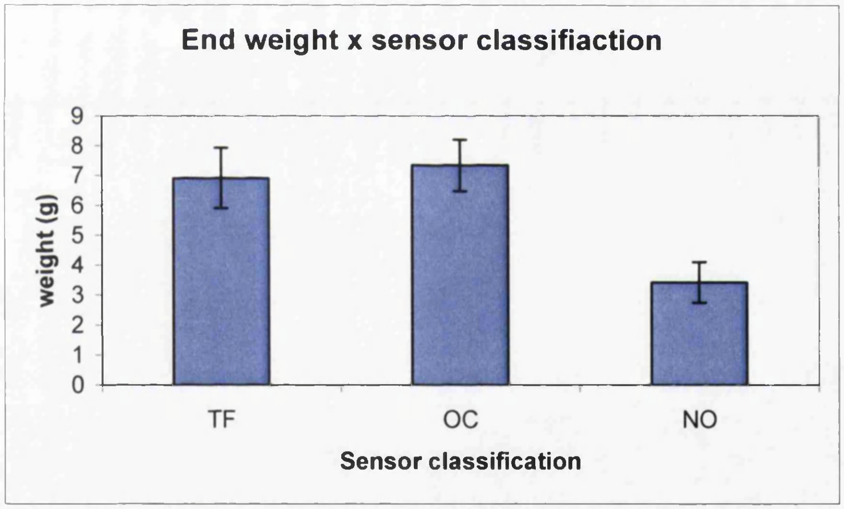


Figure 4.10b Mean (and standard error) end weight of individual fish with their sensor classification. NO = no sensor interaction OC = occasional sensor interaction and TF = the main trigger fish.

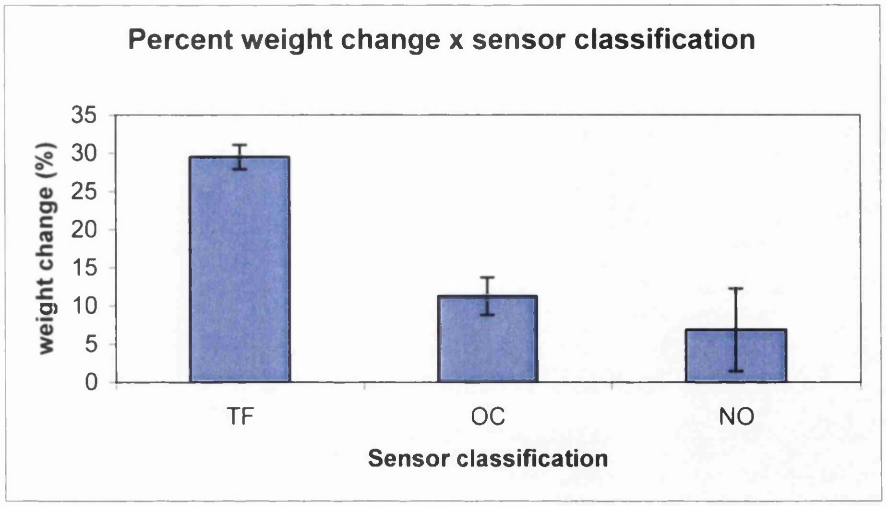


Figure 4.10c Percentage weight change of individual fish with their sensor classification. NO = no sensor interaction OC = occasional sensor interaction and TF = the main trigger fish.

Sensor use classification	n	Start weight	End weight	Percent weight change
NO	4	3.1^{a}	3.4^{a}	6.86^{a}
OC	9	6.6^{b}	7.3^{b}	11.28^{a}
TF	2	5.4^{ab}	6.9^{ab}	29.51^{b}
F		**4.27**	**4.22**	**5.53**
p		**0.040**	**0.041**	**0.020**

Table 4.3 Summery of the start, end and change in weight classified by trigger action. NO = no sensor interaction OC = occasional sensor interaction and TF = the main trigger fish, superscript letters indicate significant differences (P>0.05).

4.5.3 Response to a novel object

Groups 5, 6 and 7 were tested for their response to three different novel objects. Following observations of cod reacting to the novel object, 3 distinct responses were defined: swimming within 1 body length of the object (S), Swimming within half a body length of the object (CS) and physical interaction with the object (I). Table 4.4 shows correlations between each of the responses, S and CS are highly correlated so are combined for further analysis.

	Swim within one body length	Swim within half a body length
Swim within half a body length	0.478 0.000	
Make a physical interaction	-0.093 0.439	0.310 0.008

Table 4.4 Pearson Correlation coefficients for each of the three categories of responsiveness to the novel object with p value below

Figure 4.11 show the responses by groups 5, 6 and 7 of cod with the three novel objects, over the 40 minutes of each trial. The fish in tank one showed the most responses, with up to a total of 35 actions in one five minute period. While in tank two, there are barely 10 actions in five minutes. While the fish in tank three show an intermediate number of actions. In all tanks, there is a lower number of I interactions than S.

Tank differences were broadly consistent across the three trials, but there were no consistent trends within.

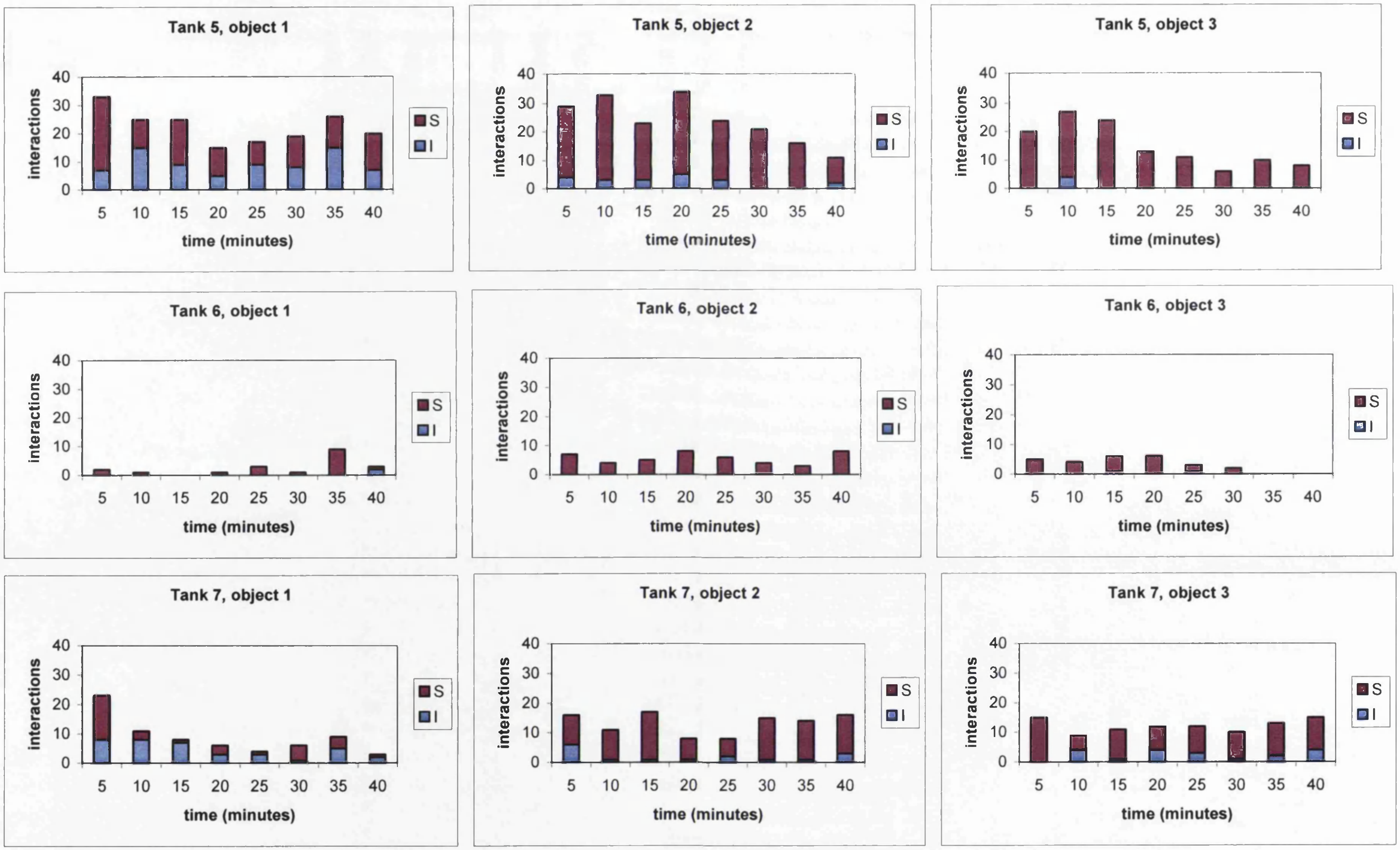


Figure 4.11 The number of interactions with novel objects in each 5 minute section over 40 minute periods. S represents swimming past within one body length (S and CS have been combined), I represents a physical interaction with the object

4.5.4 Interactions with a novel object and weight

Figure 4.12a gives the start weight of the fish and their physical interactions (I) with the novel objects. There is no significant relationship (p=0.102, R^2=19.3%, F=3.1, df=14).

Figure 4.12b gives the start weight of the fish and their swim interactions with the novel objects. There is a significant positive relationship with the equation y = 2.865x - 5.4528 (p=0.046, R^2=27.2%, F=4.85, df=14).

Figure 4.13a gives the end weight of the fish and their physical interactions with the novel objects. There is a significant positive relationship with the equation y = 1.9298x - 4.441 (p=0.034, R^2=24.8%, F=5.62, df=14).

Figure 4.13b gives the end weight of the fish and their physical interactions with the novel objects. There is a significant positive relationship with the equation y = 2.8575x - 7.4288 (p=0.020, R^2=35.2%, F=7.06, df=14).

Figure 4.14a gives the percentage weight change of the fish plotted against their swim interactions (CS and S) with the novel objects. There is no significant relationship (p=0.106, R^2=18.6%, F=3.01, df=14).

Figure 4.14b gives the percentage weight change of the fish plotted against their physical interactions with the novel objects. There is a significant positive relationship with the equation y=0.52x+1.087 (p=0.031, R^2=31.3%, F=5.87, df=14).

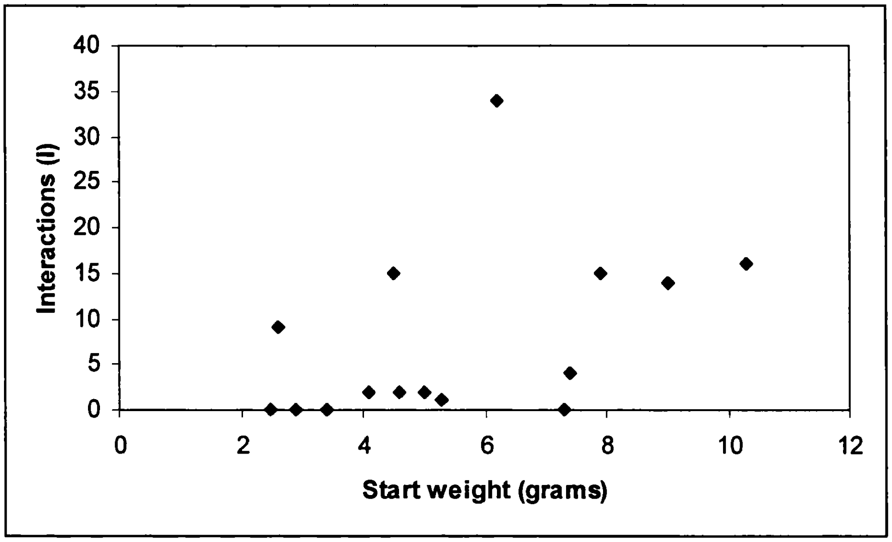


Figure 4.12a Start weight of cod plotted against the mean number of physical interactions made with a novel object

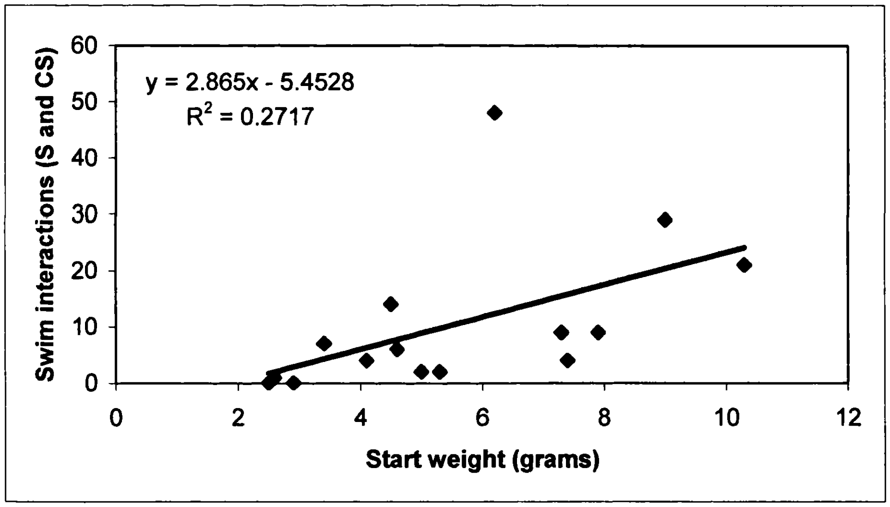


Figure 4.12b Start weight of cod plotted against the mean number of swim interactions made with a novel object

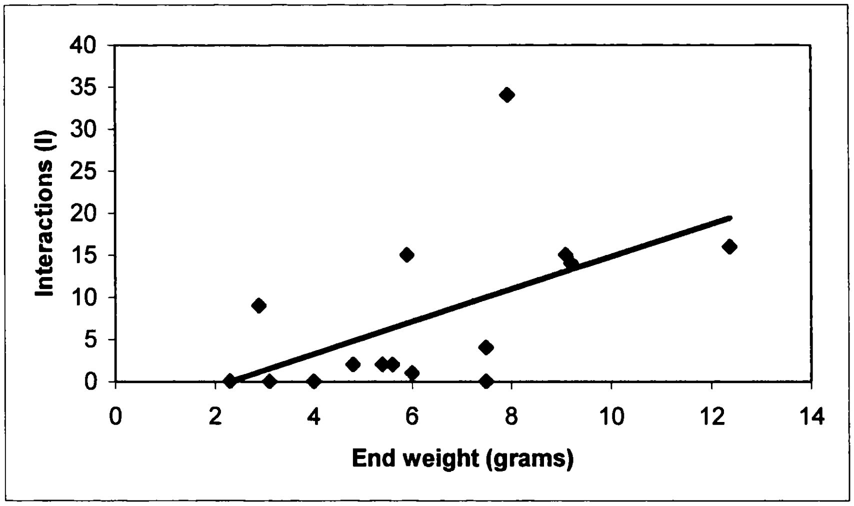


Figure 4.13a Relationship between the end weight of juvenile cod and their physical interactions with novel objects.

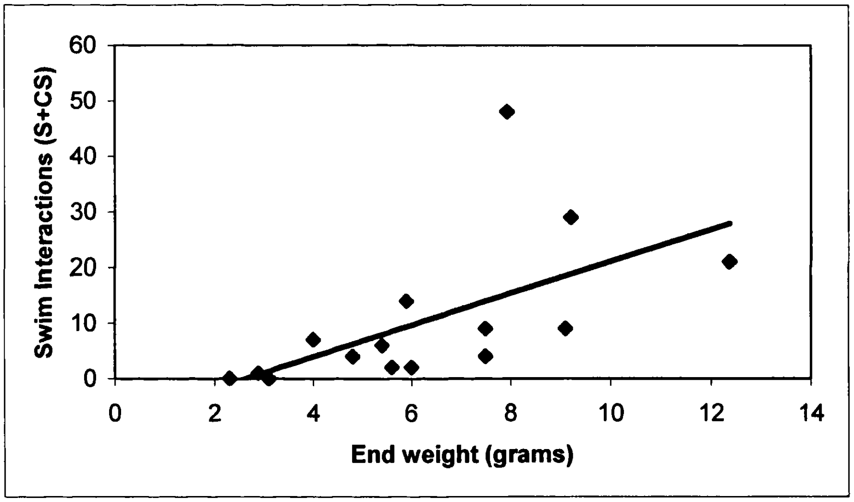


Figure 4.13b Relationship between the end weight of juvenile cod and their swim interactions with novel objects.

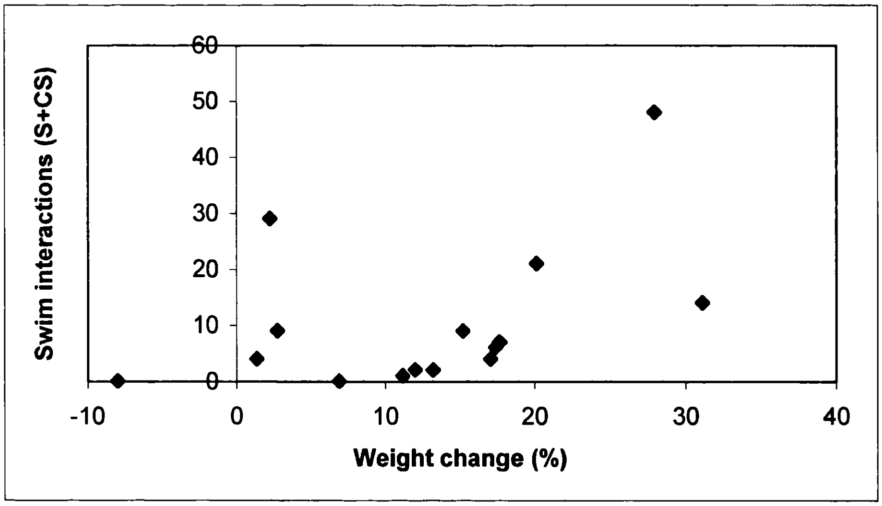


Figure 4.14a Percentage weight change across the trial plotted against the number of swim interactions (S and CS) during the novel object test.

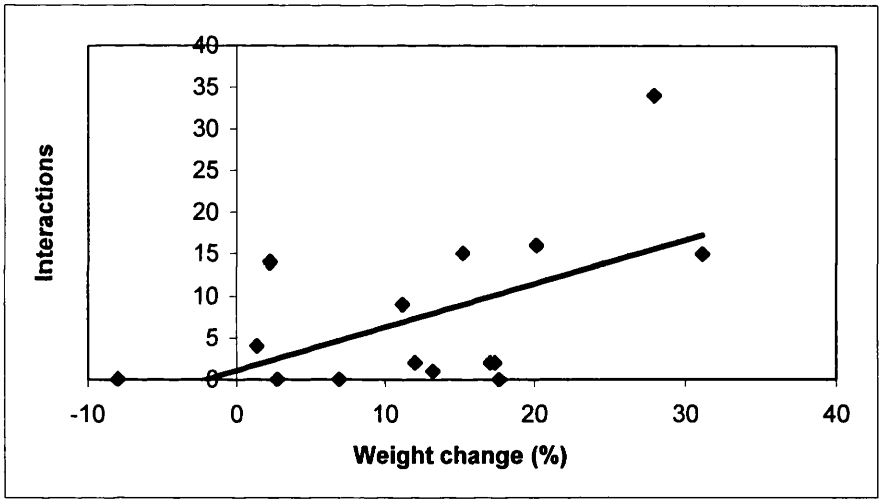


Figure 4.14b Percentage weight change across the trial plotted against the number of physical interactions (I) during the novel object test.

4.5.5 Relationship between response to novel object and trigger use

Figure 4.14 shows that when the number of novel object swimming interactions (S and CS) are compared with the feeder classification, there is a significant positive correlation (P= 0.010). Figure 4.15 shows a similar pattern is seen when comparing the number of physical interactions (I) during the novel object tests with the feeder status classification; the more interactions a fish makes, the more likely it is that the fish was responsible for using the feeder (P = 0.015).

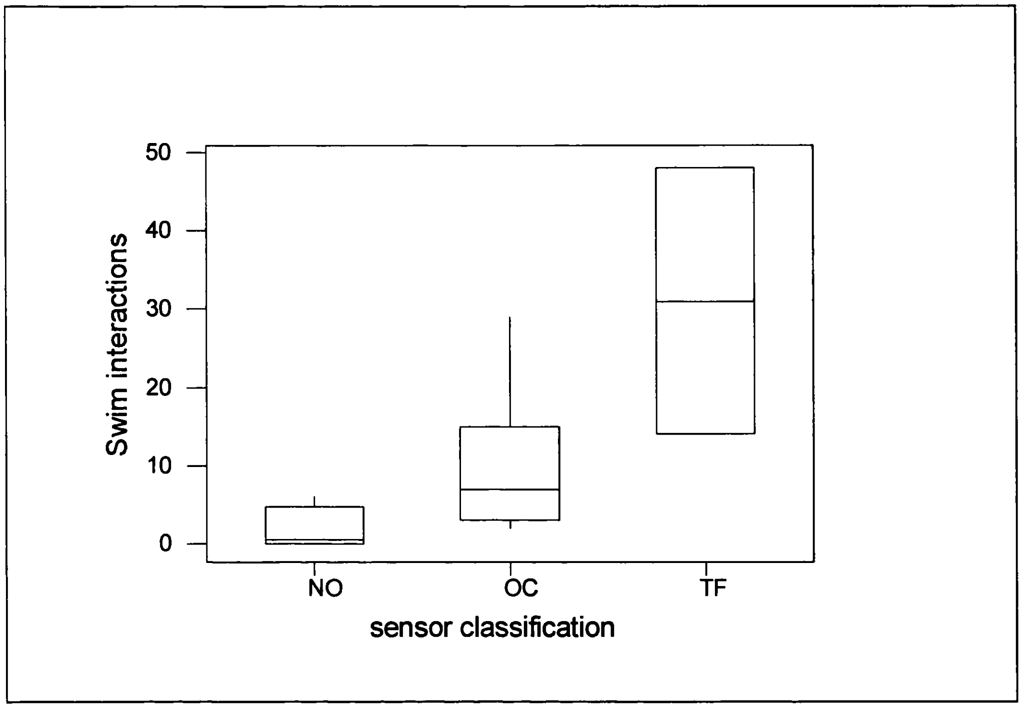


Figure 4.15 Sensor classification of individual juvenile cod against the total number of swimming interactions with a novel object, showing the median, interquartile range box and data range

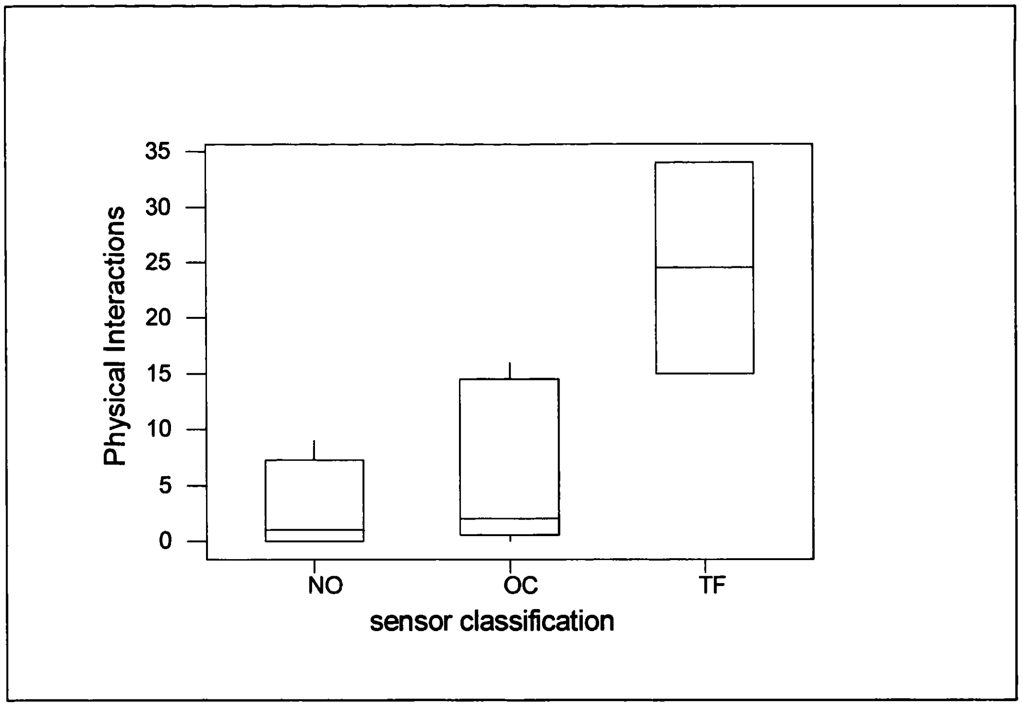


Figure 4.16 Feeder use of individual fish against the number of physical novel object interactions showing the median, interquartile range box and data range

4.5.6 Whole body immunoreactive corticosteroid

At the end of the trial, the fish in groups 5, 6 and 7 were analysed to show their whole body corticosteroid levels. Figure 4.15a gives the frequency distribution of these levels in all the fish tested while the main peak of corticosteroid levels is around the 0 – 7.5 ng.g^{-1}, with three individuals showing much higher levels. Figure 14b excludes the three outlying levels. There is a distribution of corticosteroid levels in the broken down graph of 4.15b.

Figure 4.16 gives the relationship between corticosteroid level and weight of the fish. There appears to be a negative correlation, however, there is a p value of 0.078. This makes the trend non-significant but close to the 0.05 threshold of significance. When the three high outliers are removed, there is no significant trend (p=0.897, F=0.02, df=9)

Figure 4.17 shows the level of cortisol found in whole body homogenates of each fish (ng.g^{-1}) plotted against their physical interactions (I) during the novel object test. There is a significant negative correlation with a p value of 0.014. Individuals with the highest cortisol levels made the least physical interactions with the novel objects. The fish with lower cortisol levels are spread across the range of interactions. When the three outliers are removed, there is no significant trend (p=0.306, F=1.20, df=9)

Figure 4.18 shows a plot of cortisol level against the number of swim passes individual fish did in the novel object tests. There is a significant negative correlation (p=0.026) The three fish with the highest cortisol levels did around the same number of interactions with the object as the other, lower cortisol fish. One individual with a low level of cortisol is an outlier having made over twice as many interactions as any of the other fish. When the three outliers are removed, there is no significant trend (p=0.321, F=1.12, df=9)

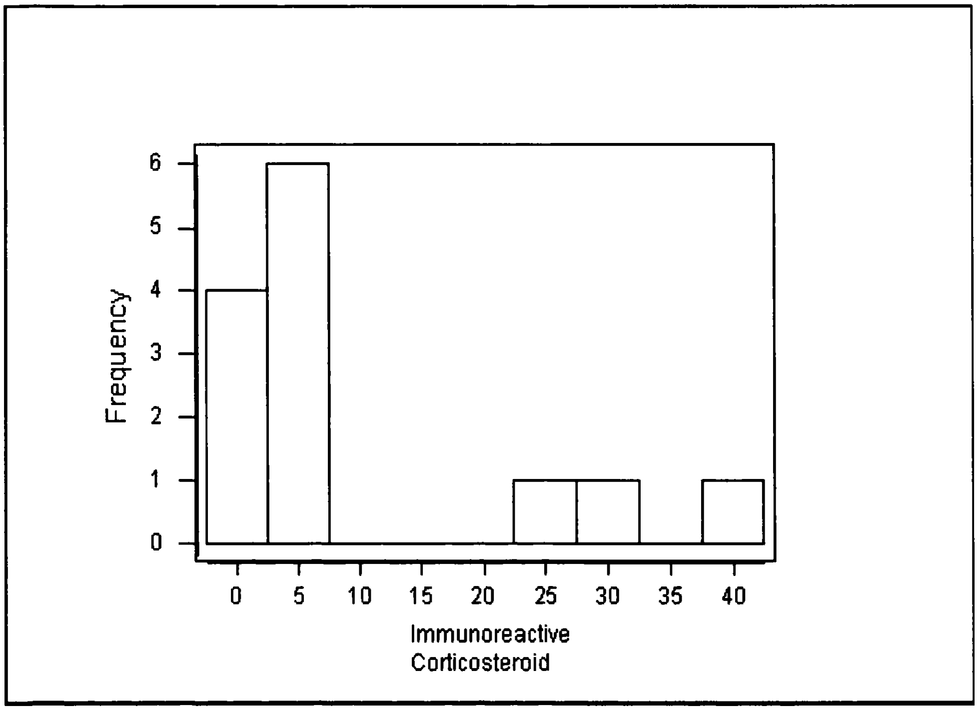


Figure 4.17a Frequency distribution of whole body immunoreactive corticosteroid levels of juvenile cod (ng.g^{-1})

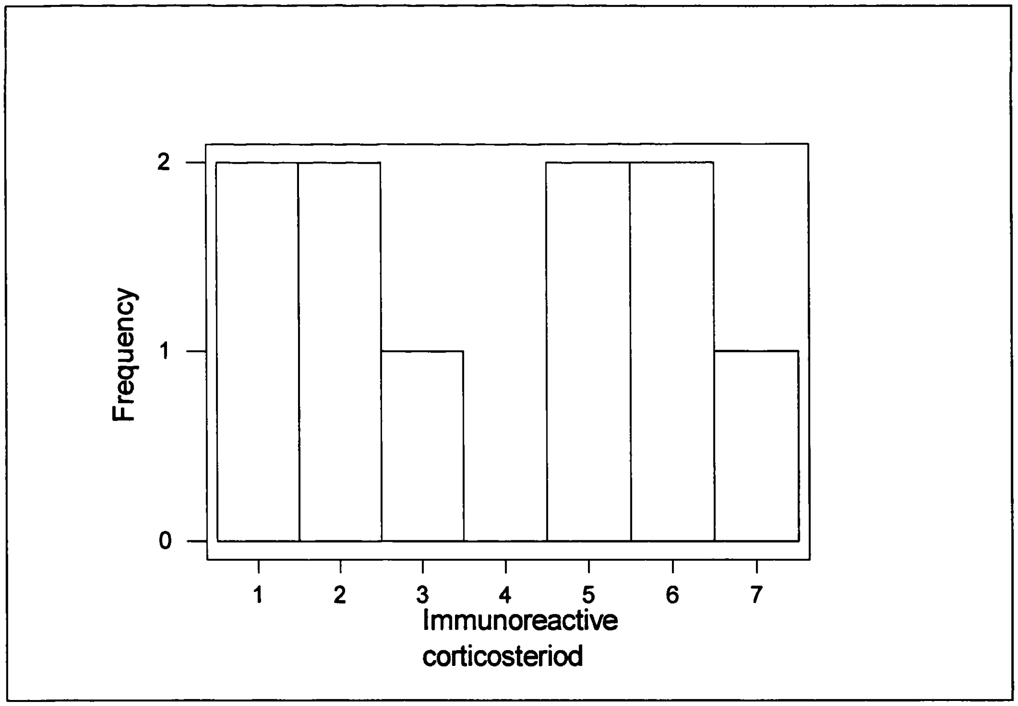


Figure 4.17b Frequency distribution of whole body immunoreactive corticosteroid levels of juvenile cod without outliers (ng.g^{-1})

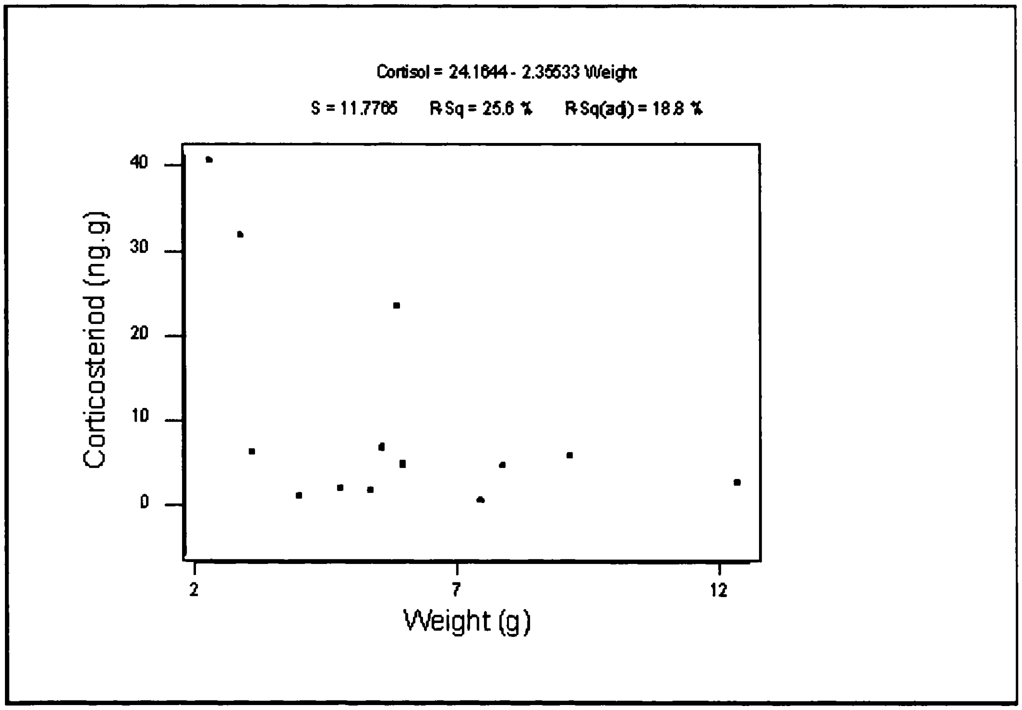


Figure 4.18 Whole body immunoreactive corticosteroid level plotted against end weight of juvenile cod

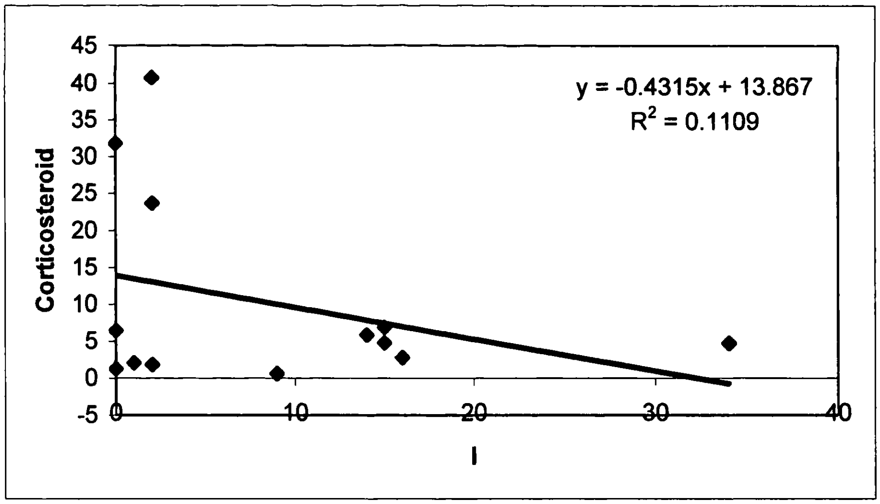


Figure 4.19 Regression plots of the physical interactions of individual fish with their immunoreactive corticosteroid level

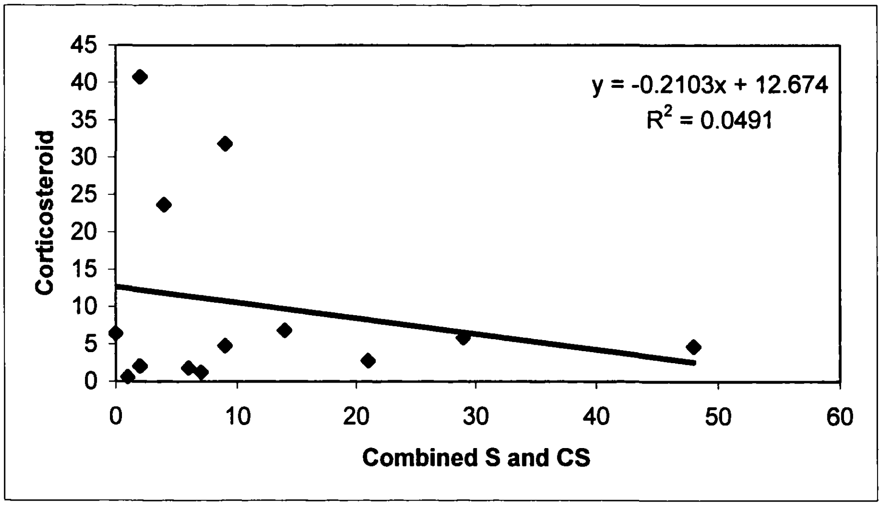


Figure 4.20 Regression plot of the combined swim interactions of individual fish with their immunoreactive corticosteroid level

4.6 Discussion

The main aim of this trial was to determine whether juvenile cod could be trained to use a demand feeding system and to investigate the process of leaning to use it, to relate individual's response to the demand feeder to the individual's response to the novel object, size and stress levels at the end of the experiment (indicated by whole body corticosteroid level).

The feeding system was designed and initial tests showed that, with some improvements, it would work well enough to be used to show feeding patterns of small fish. Initial tests showed that a plain sensor did not attract the attention of the fish and, as they spent most of their time at the bottom of the tanks and the sensor could not be held deep enough to cross their swimming routes, chance encounters between a fish and the sensor were unlikely. Sensors were therefore modified to make them attractive to the cod. This meant eventually the sensor tip was covered with green glitter mixed with clear aquarium silicon which did attract the fish's attention so the training could take place more quickly. The fish used in this trial were held at very low stocking densities, if the density was increased to commercial levels, there would be more chance of a fish encountering the sensor and learning may be more rapid, although if the stocking density is increased beyond an optimum level, the number of demands will decrease as the fish become more stressed and the water quality declines (Alanärä, 1996).

4.6.1 Learning to use the feeder

The fish in each tank successfully learnt to use the trigger to introduce feed, in between 3 and 26 days. During the learning period, the number of actuations per day increased over the first 20 days then fell back a little up to day 30. In common with other studies, for example, charr and rainbow trout (Brännäs and Alanärä, 1994, Landless, 1976), it was found that only one fish in each group was responsible for triggering the feeder. In one group, the fish responsible for triggering died after jumping out of the tank; the remaining fish took another 5 days to learn to use the sensor. In the literature, other species of fish learnt to use a demand feeder in a variety of times; rainbow trout took up to 25 days to reach a stable level of self feeding (Alanärä, 1996). Where as in Coves et al (1998), European sea bass (*D. labrax*) took around ten days to learn.

During training, only one fish in each group routinely touched the sensor, this fish was referred to as the trigger fish. A number of other fish occasionally activated the sensor; these fish were responsible for less than 10% of the total activations, while the trigger fish were responsible for the remaining 90%. The start weight of the first that took no part in triggering (NO) was significantly lower than the fish that did (OC and TF). There was no significant difference between the start and end weights of the main triggering fish and those made occasional contact. However, the percentage weight increase was highest in the main triggering fish, with no significant difference between those rated NO or OC for feeder activity. If it is assumed that the larger fish have a higher status in the hierarchy, then these results are similar to those found in rainbow trout by Landless 1976, Alanärä and Brännäs 1993 and Brännäs and Alanärä, 1994. The results found in Brown et al. (2007), a study of the poeciliid fish *Brachyrhaphis episcopi* similarly found that larger fish were always bolder than their smaller counterparts.

4.6.2 Novel object test

In the novel object test, three variables were measured; a physical interaction (I) where the fish made contact with the object, Close swimming (CS), where the fish approached the object and came within half a body length of it, and swim (S) past where a fish swims within one body length of the object. In nearly all instances, the most frequent action of the fish to the objects was CS. It was found that S and CS were significantly correlated so the two were combined. It can be seen from figure 4.11 that the fish in the three tanks reacted differently to the novel object. Those in group 5 were the most curious and made many more interactions than those in groups 6 and 7. In general, like the feeder training, one main fish from each tank that carried out most of the investigation. The response to each object was similar in each tank, the fish in tank one reacted in a similar way to object 1, 2 and 3. There were fewer physical interactions than swim interactions, although in group 5 object 1, close to half the interactions were physical. In group 6 there were only four physical interactions across all the objects. For the first object in tank three, in a number of the five minute sections, there were more physical than swim interactions.

4.6.3 Immuinoreactive corticosteroid

The majority of the fish had corticosteroid levels between 0.5 and 7ng.g^{-1} wet weight apart from three fish with levels of 24, 31 and 42 ng.g^{-1}. This is comparable with the levels found by King and Berlinsky, (2006), who found mean resting levels of below 10 ng.g^{-1}, the level raised to a mean of approximately 60 ng.g^{-1} after being subjected to a stressor. The fish with high levels tended to be the smallest, although if the three outliers are removed, there is not a significant trend of size among the remaining fish. Apart from the three outliers, the level of corticosteroid measured was consistent which indicates that the sampling was successful; the fish were quickly killed with the minimum stress. If the fish had been exposed to a stressful situation prior to the sampling, the level would be much higher. The three high readings could have been caused by sampling stress, but they were not the final fish to be sampled from each tank, so it may be assumed that those fish were chronically stressed. The two main 'personality types' of animals are the aggressive bold type which have low plasma levels of cortisol and work on the 'flight or flight' basis, the other type is are timid and tend to freeze, but have more flexible behaviour (Korte et al., 2005). Although this is a small study, this trend appears to be upheld, with fish higher corticosteroid levels having fewer interactions with the novel object, although if the three outlying fish with the highest corticosteroid levels were removed, there was no significant trend. No aggression was observed during the trial and there was no evidence for it, such as fin damage. But the stress found in the outlying individuals could have been from avoiding contact with the more dominant fish, as there were no hiding places provided in the tanks.

4.6.4 Linking feeder behaviour with the results of the novel object test and corticosteroid level

When comparing the results of the feeder learning and the novel object tests, there is a strong link between the fish that learnt to activate the feeder and those that approached the novel object most often and are therefore referred to as the most 'bold'. The boldest fish were not necessarily the largest at the start of the trial, but they did grow most over the course of the experiment. Mean percentage growth was 29.51% for the main triggering fish compared with only 6.86% for the most timid fish, which did not come into contact with the sensor throughout the trial. An interesting experiment that could be set up in the future would be to look at the smallest fish and investigate if their boldness increases as they consume less. Borcherding and Magnhagen, (2008) found that underfed perch became bolder than those that were well fed. However, Gregory and Wood (1999) found that chronic plasma cortisol elevation had the effect of reducing feed intake and therefore growth of juvenile rainbow trout (*O. mykiss*) even when held alone, although in that paper, the cortisol level was sustained artificially by an implant so there was no chance for the level to drop and for the fish to 'recover' and start to consume larger amounts. There is a trend for the fish that made the most interactions had the lower corticosteroid level, this is caused by the three outliers which all had very low interaction rates. This is true for both physical and swim interactions.

4.6.5 Problems

The main problem encountered was the maturing of the filter and maintaining acceptable water conditions. As it was a marine cold water environment, the filter was slow to mature, and the very process of maturation meant that the fish had to be exposed to ammonia in order to build up the bacterial load. It too around 3 months to build enough nitrosomonas to start the oxidising of ammonia to nitrites, and another month to start production of nitrate from the nitrite by nitrobacter and nitrospira. This meant that the water conditions were not optimal for growth and had to be kept under control with large water changes. The water quality did not have any acute effects on the fish; however, the effects of chronic background level ammonia can have negative effects on fish, although Francois et al. (2008) concluded that juvenile cod were resistant to chronic low level ammonia exposure, so there may not have been any effects on the results.

If the experiment were to be repeated, the main improvement to the system would be redesigning the layout so that the tanks could be accessed more easily. As it was, the tanks were placed on top of the feed collectors which were approximately one meter off the ground to allow the waste water to drain back to the sump tank. This meant that the top of the tanks were only a short distance from the ceiling and so observing the fish was difficult. In addition to this, the video cameras could not be hung far enough away from the surface to allow a full picture of the tank and the lighting was inconsistent between the tanks as lights were directly above some tanks and not others. This did not affect the results, but would have made observations and tank maintenance simpler.

4.6.6 Future work

Future work is needed to investigate if the removal of the sensor from the tank when a defined level of feeding has occurred is a viable method of demand feeding juvenile cod. This is a technology that is not seen in commercially available systems, which generally do not have any system to limit over feeding. One problem that may affect the success of the feeding system is the opportunity for accidental actuations by the fish swimming too closely to the sensor. In the current set-up, this was not an issue as the tanks were not heavily stocked and the fish tended to spend the majority of time close to the bottom of the tank. If it was used with a higher stocking density or fish that more readily swim to the surface, measures may have to be taken to prevent false actuations. In other systems, this problem has been prevented by having a different sensor type; for example, Rubio et al. 2004, used a string sensor with sea bass that the fish have to bite and pull in order for an actuation to be registered. This type of sensor was not used in the current trial, because the experimental fish had a small start weight (as small as 5 grams) and it was not thought that they could generate enough power for a signal to be registered.

Another method first used by Coves at al. (1998) involves surrounding the sensor with a screen, so fish have to enter the area to trigger the feeder. This method could be incorporated in future work with this feeding system.

Another possible improvement would be to use more fish. The small number of participant fish means that the data analysis is not strong and only the trends can be taken into account. However, if more fish were to be used, it would be harder to identify individual fish if they were marked by the same method (injected elastomers).

In conclusion, this trial has shown that small groups of juvenile cod can be trained to use a demand feeing system. In a small group as described here, the sensor is activated by one fish in the group, this fish tended to be one of the boldest (as determined by a novel object test). The boldest fish grew the most during the trial, and had the lowest whole body corticosteroid levels.

Chapter 5 General discussion

5.1 Overview of results

5.1.1 Seasonal feeding patterns defined by the AQ1 system (Chapter 2)

The AQ1 system was successfully configured to adapt to tank held juvenile cod feeding patterns, and the fish grew satisfactorily throughout the trial, though there were problems associated with the small pellet size that will be discussed later. This gave an overview of the seasonal feeding patterns. It appears that the daily feed intake is negatively affected by longer day length; this is not the expected result as longer day length is associated with higher temperature which leads to an increased need for energy (Jobling 1988). In three of the tanks, the higher temperature led to decreased feed intake, in one tank there was no effect and in the final tank there was a positive correlation between water temperature and feed intake.

Although it was not one of the main aims, the production data and FCR were recorded. It was found that the temperature was positively correlated with the FCR in three of the tanks and with no significant effects in the remaining ones. There was also no effect of photoperiod on FCR.

The daily feed input as a percentage of total feed input was found to have significant autocorrelation with itself giving repeating feed intake patterns of up to 8 days.

5.1.2 Daily feeding patterns as defined by the AQ1 system (Chapter 3)

The main feeding pattern found in part 1 was a feeding peak at dawn, followed by continual grazing across the rest of the day. In part 2, there was a less defined pattern, at times there was a higher level of feeding at night than during the day, but all the feeding tended to be continuous with fewer large peaks.

The peak feeding hour in part one occurred around dawn from March until July, and then becomes more erratic with no obvious pattern. In part 2 the peak feeding hour showed no significant pattern and had high variation across tanks. As day length increased, the peak feeding hour became earlier, but there was no significant impact of temperature in part 1. In part 2, as the temperature decreases, the peak feeding hour also decreases.

The proportion of feed consumed at night was much higher in part 2 than part 1. As the day length increased, the percentage of feed taken nocturnally decreased in part 1 and increased in part 2. In part 1, as the temperature increased, the percentage nocturnal feed intake increased, but there was no significant impact of temperature in part 2.

5.1.3 Response of cod fed by a demand feeder (Chapter 4)

An on-demand feeding system for juvenile cod was designed and produced by the bioelectronics department of the University of Glasgow. Seven groups of five fish were successfully trained to use the system. In each group one fish was mainly responsible for activating the feeder, named the trigger fish. When the final three groups to be trained were given novel object tests, there was only one fish in each tank that activated the feeder. The majority of the other fish only occasionally approached the novel object and never approached the trigger. There was no difference in start or end weight between the trigger fish and the fish that only occasionally approached the object. There was a third group that made no approaches to the sensor or the novel object and were significantly smaller at the start and end of that trial than all the other fish. The trigger fish showed significantly higher growth across the trial than the other fish. The stress levels of the final three groups was also established by whole body corticosteroid assays. The results show that all except three outlying fish had no significant difference. These three fish were all small and had no interaction with the novel object or trigger had corticosteroid levels up to eight times higher than the mean level of the remaining fish.

5.2 Problems in experimental technique

5.2.1 The AQ1 trial (Chapters 2 and 3)

The main problem using the AQ1 system was the accuracy of the sensors at the outlet of each tank, along with the need to use very small feed, which was at the edge of the system's sensing ability. Because all the water leaving the tanks had to flow past the small sensor, vortex effect was created, with the exiting water spiralling through quickly, making it even more difficult for the sensor to recognise. The sensors were originally designed to be used at the bottom of net cages, where the waste feed pellets would sink through at a slow rate. If the trial were to be repeated, a possible modification to the methods could be to use a slower flow rate, although a lower cod biomass may also be necessary.

It was also found that water quality played a role in the accuracy of the sensors. At times when there were high suspended solids, it was often mistaken for waste feed pellets. This could cause counts of over one hundred phantom pellets in just one minute. Leading to chronic underfeeding if the system went unchecked. The data set was cleaned according to a set of rules drawn up to remove days where there were obvious sensor faults. There is a section explaining the removal of days with errors in the methods section of chapter 2. The days following removed days were left in the data set because, although under or overfeeding on the previous day would have an influence on the food intake on the following day, removal of these days would seriously deplete the size of the data set. If it is assumed that the AQ1 system delivers the maximum amount of feed that the fish can consume, there should be very little difference in feed consumption between an over feeding day and a normal day. However, on the days following an under feeding day, it is likely that feed intake will have increased to compensate, so this may have slightly affected that results in Chapter 2.

5.2.2 Training problems with the demand feeder setup (Chapter 4)

In this trial, the main two problems were controlling the water quality and accessing and observing the tanks. The water quality was difficult to control because the trial took place in a system with an immature biofilter, which, being marine and cold water, were very slow to mature (High salinity causes slower rate of maturation of biofilter bacteria, for example Grommen *et al.* 2005). This meant that the fish had to be exposed to chronic low levels of ammonia and later, nitrite. Foss *et al.*, (2004) recommends that the maximum concentration of unionised ammonia that cod can be exposed to (in the long term) without adverse effects is $0.06 mg.l^{-1}$, in this trial the level did occasionally exceed this level before water changes by approximately $0.03 mg.l^{-1}$. The quality was controlled by large water changes, but the ammonia concentration had to be maintained otherwise the filter would not colonise with the correct bacteria. Ammonia is known to cause gill damage and osmoregulation problems (Svbodová *et al.*, 2007, Le François, *et al.*, 2008, Foss *et al.*, 2005)

The other main problem was accessing and observing the tanks as their tops were close to the ceiling. This did not have any impact on the results, but did make aspects of the experiment harder. For example, it was difficult to observe fish at the bottom of the tanks and a full view of the surface of the tanks was not possible through the camera systems. The other main impact of the low ceiling, combined with placement of room lights, was that there was a differing light intensity at the surface of each tank. This may have changed the fishes' likelihood of approaching the surface, where the sensor and one of the novel objects were placed.

5.3 Significance and implications for aquaculture

5.3.1 Seasonal patterns

Most of the results found in the part of the trial are the opposite of what was expected. For example, the feed intake in four out of the five tanks was negatively affected by longer day lengths. Only one of the tanks had an increase in feed intake with higher temperature, in the remaining tanks, there was either no significant correlation or a negative association. Most papers measuring this relationship have found higher growth rates and therefore greater food intake associated with higher temperature, up to an optimum, for example, Solberg *et al.*, (2006), Chambers and Howell, (2006), Jobling (1988) It would be useful to repeat the trials perhaps using larger industrial sized tanks so the same fish could be used throughout a full year. It is possible that the light intensity was too strong for the fish, especially as the tanks were quite shallow, this may have negatively affected the fish and influenced the results. Repeating using larger, deeper tanks may change the results, especially as even at this small size, cod are naturally benthic feeders (Brawn, 1969) and being unable to retreat to darker, deep water may have been stressful for them.

One of the most striking results from this part of the study was the strong rhythmic patterns of feed intake, as indicated by the autocorrelation. The patterns found were tank wide, suggesting that the whole tank of fish had the same fluctuations in feed intake, this could be because the whole tank was subjected to the same variations in water quality and stressful events such as disturbance and noise from human activity in the area. A possible reason for this is that cod store a lot of energy in the liver (Dos Santos *et al.*, 1993, Lie *et al.*, 1986). On day one, it may take in a large meal, storing excess energy, then in the following days, it eats less, spending more time avoiding' predators. Another reason for the periodicy suggested in Bailey and Alanärä, (2006) is that the rhythms are the time taken for the stomach to empty and for the appetite to return.

5.3.2 Cod daily feeding rhythms

The seasonal feeding patterns that were found and the effects of temperature and photoperiod were, in most cases, the opposite of what was expected. For example, shorter day lengths appeared to induce a higher feed intake and higher temperature led to lower feed intake. In addition to this, the two parts of the trial gave different results, indicating that the cod in the two parts of the trial did not have feeding patterns that are simply affected by time of year, it is impossible to separate group and seasonal factors in this case. The two different groups of fish may have been influenced by the presence of more or less feed as the program changes as outlined in the discussion of chapter 3.

It seems to be common practice for cod farms to give a main feed in the morning and evening, and sometimes to use an automatic feeder delivering supplementary feed during the daylight hours. In the results of this trial, the fed intake in the remaining daylight part of the day accounted for between 60% in March, where there was no nocturnal feeding because of the restricted program and 35% in November, where there was continuous browsing throughout the 24 hour period. Taking the present results into consideration, it would seem that this is a good basis for planning a feeding regime for cod. However, there are several points to bear in mind. As different daily patterns were found for days with similar conditions, their feeding patterns can not be simply based on temperature and day length. In some instances, it seems that the presence of food is the reason for a feeding peak, which is then continued despite the excess feed being removed. For instance, where the trial first began in March 2004, there was a morning increase in feed availability as the program shifted from night to day. The dawn feeding peak continued for many months, even when the program for day and night became the same. When the trial was restarted with a different group of fish and no sudden change in amount of feed provided, the morning peak does not reappear. A further trial would be needed to further test this theory.

This means that cod have flexible feeding patterns and may settle into a feeding rhythm prescribed by the farmer. The literature on feeding in wild cod states that gadoids undertake a nocturnal vertical migration away from the sea bed to feed during the night (Bozzano *et al.*, 2005, Lough *et al.*, 1989), therefore, more investigations of nocturnal feeding would be interesting

5.3.3 Training juvenile cod to use a demand feeder

This section investigated the process of cod learning to use a demand feeder, focusing on which fish was responsible for triggering the feeder and how that fish compares with the others in the group. In other papers, it was found that only one or two fish in a group is the trigger fish (Brännäs and Alanärä, 1993, Alanärä, 1996, that was the situation found here as well. The trigger fish were not necessarily the largest fish, but they did show the highest percentage growth across the 30 day trial period when compared with the other fish.

As well as the trigger fish, there was a group of fish that did not thrive, they either put on very little, or lost weight, they did not interact either with the sensor or any of the novel objects. It has been found in other studies that smaller fish tend to be less bold, for example, Brown *et al*, 2007. However, although small fish are generally less bold, Laakkonen and Hirvonen, 2007 found that slow growing Arctic charr (Salvelinus alpinus) were no less bold than a faster growing population. These cod either did not grow, or lost weight during the experiment and may be equivalent to the runt fish found in cod culture at the transplant of juvenile fish to sea cages from hatchery tanks. At this stage a significant percentage of cod stop feeding and waste away, this is a bottle-neck in cod production that is being investigated. Discussions with one of Scotland's main juvenile cod producers (Treasurer, Viking Fish Farms) has brought this problem to light. Some producers simply grade the runts out, but as the production costs of raising them to this stage are high, methods to induce feeding such as moist diets, different feeding regimes and feed stimulants in them will be tried.

Stress (as measured by whole body corticosteroid level) was, in some of these fish, very high in comparison with the others. The reason for this is not clear, it may be that as slightly weaker fish, the low levels of ammonia in the water stressed them more than the other fish, or perhaps there was aggressive behaviour towards them from the other fish that was unobserved. It is possible that the high corticosteroid level was caused by stress during sampling, but the fish with the highest concentrations were not the last to be sampled so a stressor did not affect all fish. In general, there was no difference in stress level as indicated by cortisol, but the three fish with raised corticosteroid levels were all amongst the smallest. As the whole fish were homogenised, it is now impossible to look more closely at their health. As the ammonia concentration in the tank may have been an issue, it would have been interesting to study the gills of these and some of the other 'healthy' fish to look for inflammation or clubbing of the lamellae.

The final three groups of fish trained to use the feeder were those that took part in the novel object tests. The fish supplied for these tests were from the final grade of that year of fish and therefore were made up from the smallest grade. This may have meant that all the fish in this part were likely to be the weakest of the year class and that was perhaps why there was a proportion of fish that were failing to thrive. Unfortunately, the novel object test was not carried out with the previous groups of fish, so no comparison is available.

5.4 Future work

These trials gave some interesting results and have raised a number of questions that can be answered by continuing research in this area. A natural progression of the daily and seasonal feeding patters trials is to set up commercial systems with the AQ1 or similar system. In deeper tanks or cages, different results may be gained, for example, as discussed earlier, longer day length caused lower feed intake, when the opposite should have been true, and a possible reason for this could have been that the shallow tanks were too bright for the cod. Repeating the trial using deeper tanks may bring about different results.

Further investigations of the juvenile cods feeding flexibility would be interesting. The results from this trial showed that the fish appeared to adapt to daily changes in the program by feeding harder when feed has been absent or low for some time, producing feeding peaks before and after food absences.

Additional work could be done on the demand feeding system that was designed as part of this project. Time limits restricted the amount of calibrating work that could be carried out on the system, initial tests showed that all parts of the system worked, when operated manually, but there was no opportunity to test it with fish. Further development of the software would make it more adaptable for use in aquaculture and research. For example, separating the tanks so that each one can operate at different times and with different levels and having control of the amount of time the feeder would run for at each actuation rather than it being controlled deeper in the programming.

More work is needed on the demand feeding behaviour of cod. This could be in larger groups such as in an aquaculture setting and comparative studies of the effects of demand feeding verses automatic or hand feeding. This would give information on the best way to feed cod for growth in aquaculture and keeping track of what is eaten will give an idea of the level of waste food which is also an important factor both environmentally and economically.

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Appendix 1

Block 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6																								
7	x	x	x	x	x	x																		
8	x	x	x	x	x	x																		
9							x	x																
10	x	x	x	x	x	x	x	x																
11	x	x	x	x	x	x	x																	
12							x	x																
13	x	x	x	x	x	x	x	x																
14	x	x	x	x	x	x	x	x																
15							x	x																
16							x	x																
17							x	x																
18							x	x																
19							x	x			x													
20							x	x		x	x		x	x										
21							x	x		x	x		x	x										
22							x	x		x	x		x	x										
23							x	x		x	x		x	x										
24							x	x		x	x		x	x										

Significant differences between mean percentage feed consumed per hour, identified by Tukey test ($p \leq 0.05$). Block 1.

Block 2

	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12a	13a	14a	15a	16a	17a	18a	19a	20a	21a	22a	23a	24a
1																								
2																								
3																								
4																								
5																								
6																								
7	x	x	x	x	x	x																		
8							x																	
9							x																	
10							x																	
11							x																	
12							x																	
13							x																	
14							x																	
15							x																	
16							x																	
17							x																	
18							x																	
19							x																	
20							x																	
21							x																	
22							x																	
23							x																	
24							x																	

Significant differences between mean percentage feed consumed per hour, identified by Tukey test ($p \leq 0.05$). Block 2.

Block 3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6																								
7	x	x	x	x	x	x																		
8	x	x	x	x																				
9	x	x	x	x	x	x																		
10	x	x	x	x																				
11	x	x	x	x	x																			
12	x	x	x	x																				
13	x	x	x	x																				
14	x	x	x	x	x																			
15	x	x	x	x																				
16	x	x																						
17																								
18							x																	
19							x		x															
20							x		x															
21							x		x		x		x	x										
22							x	x	x	x	x	x	x	x	x	x								
23							x	x	x	x	x	x	x	x	x	x								
24							x	x	x	x	x	x	x	x	x	x								

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test (p≤0.05). Block 3.

Block 4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6																								
7	x	x	x	x	x	x																		
8	x	x	x	x	x	x																		
9	x	x	x	x	x	x																		
10	x	x	x	x	x	x		x																
11	x	x	x	x	x	x																		
12	x	x	x	x	x	x				x														
13	x	x	x	x	x	x						x												
14	x	x	x	x	x	x																		
15	x	x	x	x	x	x																		
16	x	x	x	x	x	x																		
17	x	x	x	x	x	x						x												
18	x	x	x	x	x			x				x												
19	x	x	x	x	x			x				x		x										
20							x	x			x	x		x		x								
21							x	x	x		x	x		x	x	x								
22							x	x	x	x	x	x	x	x	x	x	x	x						
23							x	x	x	x	x	x	x	x	x	x	x	x	x					
24							x	x	x	x	x	x	x	x	x	x	x	x	x					

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test (p≤0.05). Block 4.

Block 5

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6																								
7	x	x	x	x	x	x																		
8	x	x	x	x	x		x																	
9	x	x	x	x	x		x																	
10	x	x	x	x	x		x																	
11	x	x	x	x	x	x																		
12	x	x	x	x	x		x																	
13	x	x	x	x	x		x																	
14	x	x	x	x	x		x																	
15	x	x	x	x	x		x																	
16	x	x	x	x	x		x																	
17	x	x	x	x	x		x																	
18	x	x	x	x	x		x																	
19	x	x	x	x	x		x																	
20	x	x	x	x	x		x																	
21							x				x													
22							x				x		x											
23							x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
24							x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test (p≤0.05). Block 5.

Block 6

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6	x	x	x	x	x																			
7	x	x	x	x	x																			
8	x	x	x	x	x	x	x																	
9	x	x	x	x	x	x																		
10	x	x	x	x	x	x																		
11	x	x	x	x	x																			
12	x	x	x	x	x																			
13	x	x	x	x	x																			
14	x	x	x	x	x	x																		
15	x	x	x	x	x																			
16	x	x	x	x	x	x																		
17	x	x	x	x	x	x																		
18	x	x	x	x	x	x	x																	
19	x	x	x	x	x	x																		
20	x	x	x	x	x	x	x																	
21	x	x	x	x		x	x																	
22						x	x		x	x	x	x	x	x	x	x								
23						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
24						x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 6.

Block 7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6	x	x	x	x	x																			
7						x																		
8						x																		
9	x	x	x	x	x	x																		
10	x	x	x	x	x	x	x	x																
11	x	x	x	x	x	x	x	x																
12	x	x	x	x	x	x	x	x	x															
13	x	x	x	x	x	x	x	x																
14	x	x	x	x	x	x	x	x	x															
15	x	x	x	x	x	x	x	x																
16	x	x	x	x	x	x	x	x																
17	x	x	x	x	x	x																		
18	x	x	x	x	x	x	x	x																
19	x	x	x	x	x	x																		
20						x				x	x	x	x	x	x	x		x						
21						x				x	x	x	x	x	x	x		x						
22						x				x	x	x	x	x	x	x	x	x						
23						x			x	x	x	x	x	x	x	x	x	x						
24						x			x	x	x	x	x	x	x	x	x	x						

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 7.

Block 8

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6	x	x	x	x	x																			
7						x																		
8						x																		
9	x	x	x	x		x																		
10	x	x	x	x		x																		
11	x	x	x	x	x	x	x																	
12	x	x	x	x	x	x	x	x																
13	x	x	x	x	x	x	x	x																
14	x	x	x	x	x	x	x	x																
15	x	x	x	x	x	x	x	x																
16	x	x	x	x	x	x																		
17	x	x	x	x		x																		
18	x	x	x	x		x																		
19	x	x	x	x		x																		
20						x					x	x	x	x	x									
21						x					x	x	x	x	x									
22						x						x	x	x										
23						x			x		x	x	x	x	x	x	x	x	x	x				
24						x			x	x	x	x	x	x	x	x	x	x	x	x				

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 8.

Block 9

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5	x	x	x	x																				
6	x	x	x	x	x																			
7					x	x																		
8						x																		
9	x	x	x			x																		
10	x	x	x			x	x																	
11	x	x	x	x		x	x																	
12	x	x	x	x		x	x																	
13	x	x	x	x		x	x																	
14	x	x	x	x		x	x																	
15	x	x	x	x		x	x																	
16	x	x	x			x																		
17						x																		
18	x	x	x			x	x																	
19		x				x																		
20					x	x					x	x	x	x										
21					x	x					x	x	x	x	x									
22						x						x												
23					x	x						x	x	x										
24					x	x			x	x	x	x	x	x	x			x						

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 9.

Block 10

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5	x	x	x	x																				
6					x																			
7					x																			
8	x	x			x																			
9					x																			
10					x																			
11	x	x	x		x																			
12	x	x	x		x																			
13	x	x	x	x	x		x																	
14	x	x	x	x	x	x	x		x															
15	x	x	x		x																			
16	x	x	x	x	x		x																	
17					x								x	x		x								
18	x	x	x		x																			
19					x								x	x		x								
20					x									x										
21					x							x	x	x	x	x								
22					x							x	x	x		x								
23					x							x	x	x	x	x								
24					x			x				x	x	x	x	x	x		x					

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 10

Block 11

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5	x	x	x	x																				
6					x																			
7					x																			
8					x																			
9					x																			
10					x																			
11					x																			
12					x																			
13					x																			
14	x	x	x		x																			
15					x																			
16					x																			
17					x																			
18	x	x	x	x	x																			
19					x																			
20					x																			
21					x																			
22					x																			
23					x																			
24					x									x					x					

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 11.

Block 12

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5	x	x	x	x																				
6					x																			
7					x																			
8					x																			
9					x																			
10					x																			
11			x		x																			
12	x	x	x	x	x		x																	
13			x		x																			
14					x																			
15					x																			
16			x		x																			
17					x																			
18					x																			
19	x	x	x		x	x																		
20					x																			
21					x																			
22					x																			
23					x							x							x					
24					x							x							x					

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 12.

Block 14

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6	x	x	x	x	x																			
7						x																		
8						x																		
9						x																		
10						x																		
11						x																		
12	x	x	x	x				x																
13	x	x	x	x																				
14	x	x	x	x																				
15	x	x	x	x																				
16						x																		
17			x			x																		
18						x																		
19						x																		
20						x																		
21						x																		
22						x																		
23						x																		
24						x						x	x	x										

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 14.

Block 15

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6	x	x	x	x																				
7																								
8																								
9																								
10																								
11			x	x																				
12																								
13																								
14																								
15			x	x																				
16																								
17		x	x	x																				
18																								
19	x	x	x	x																				
20																								
21																								
22																								
23																								
24						x					x				x		x		x					

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 15.

Block 16

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6		x	x	x	x																			
7						x																		
8						x																		
9																								
10						x																		
11																								
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14																								
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16																								
17						x																		
18																								
19																								
20						x																		
21						x																		
22						x																		
23																								
24						x																		

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 16.

Block 27

6																								
7																								
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10																								
11																								
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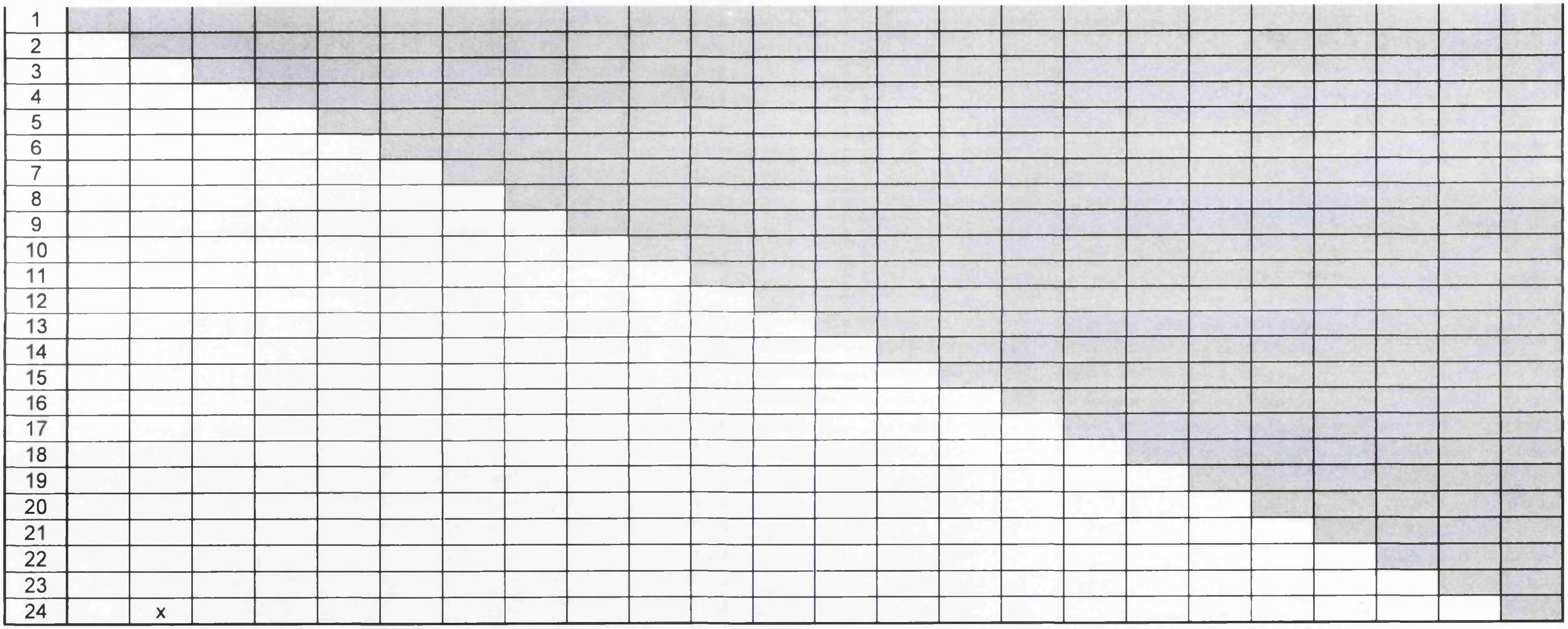
18	x					x	x	x		x														
19																								
20																								
21																								
22																								
23																		x						
24																		x						

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test (p≤0.05). Block 27.

Block 28



1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
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4				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
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6						▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
7							▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
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11											▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
12												▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
13													▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
14														▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
15															▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
16																▨	▨	▨	▨	▨	▨	▨	▨	▨
17																	▨	▨	▨	▨	▨	▨	▨	▨
18																		▨	▨	▨	▨	▨	▨	▨
19																			▨	▨	▨	▨	▨	▨
20																				▨	▨	▨	▨	▨
21																					▨	▨	▨	▨
22																						▨	▨	▨
23																							▨	▨
24		x																						▨

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test (p≤0.05). Block 28.

Block 30

6				x		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
7				x			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
8				x				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
9				x					▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
10				x						▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
11				x							▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
12				x								▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
13				x									▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
14				x										▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
15				x											▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
16				x												▨	▨	▨	▨	▨	▨	▨	▨	▨
17				x													▨	▨	▨	▨	▨	▨	▨	▨
18				x														▨	▨	▨	▨	▨	▨	▨
19				x															▨	▨	▨	▨	▨	▨
20				x																▨	▨	▨	▨	▨
21				x																	▨	▨	▨	▨
22				x																		▨	▨	▨
23				x																			▨	▨
24																								▨

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 30.

Block 32

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
2		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
3			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
4				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
5					▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
6		x				▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
7		x					▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
8		x						▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
9		x							▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
10										▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
11		x									▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
12		x										▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
13		x											▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
14		x												▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
15		x													▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
16		x														▨	▨	▨	▨	▨	▨	▨	▨	▨
17																	▨	▨	▨	▨	▨	▨	▨	▨
18																		▨	▨	▨	▨	▨	▨	▨
19																			▨	▨	▨	▨	▨	▨
20																				▨	▨	▨	▨	▨
21																					▨	▨	▨	▨
22																						▨	▨	▨
23																							▨	▨
24																								▨

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 32.

Block 34

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2																								
3																								
4																								
5																								
6																								
7																								
8																								
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15			x	x	x	x	x	x																
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23																								
24																								

Significant differences between mean percentage feed consumed per hour, identifyed by Tukey test ($p \leq 0.05$). Block 34.

Appendix 2 Components of the demand feeding system

The sensor

The sensor is a bimorphic pizo electric switch, encased in a stainless steel tube. The switch was covered in a silicone strip, which in turn was enclosed in a transparent silicone tube. The silicone strip was given a green colour by mixing silicone with green enamel paint. Green was chosen as it is as cod's retina has the highest absorption around the green / blue wavelength.

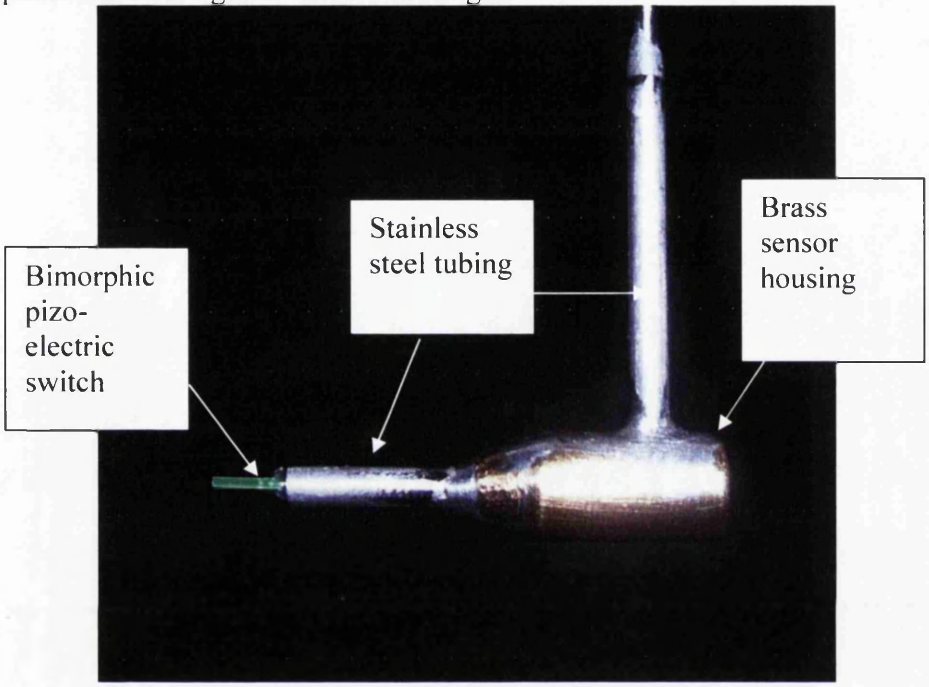

Figure 1 the sensor mechanism

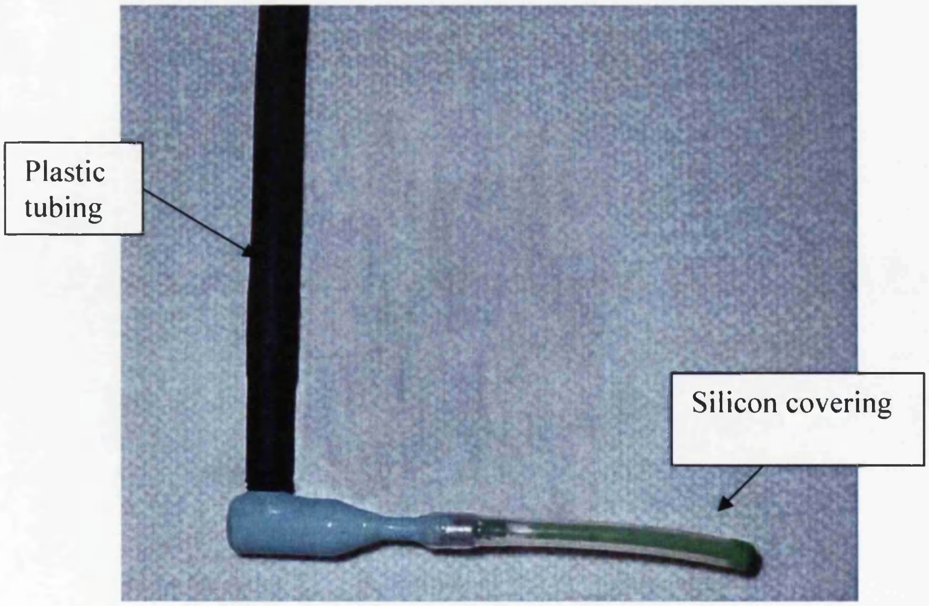

Figure 2 the waterproofed sensor housing

The sensor motor and counter balance

As the fish were being held in a recirculation system, a major consideration for the feeding system was the ability to prevent overfeeding. Therefore the sensor was attached to a stepper motor, which, when activated, turned the sensor through 90º so that the trigger section was lifted out of the water.
Once the sensor is out of the water, the motor stops exerting pressure on the it and it was found that the weight of the trigger end pulled itself back down. So a brass counterweight was added to the each sensor shaft.

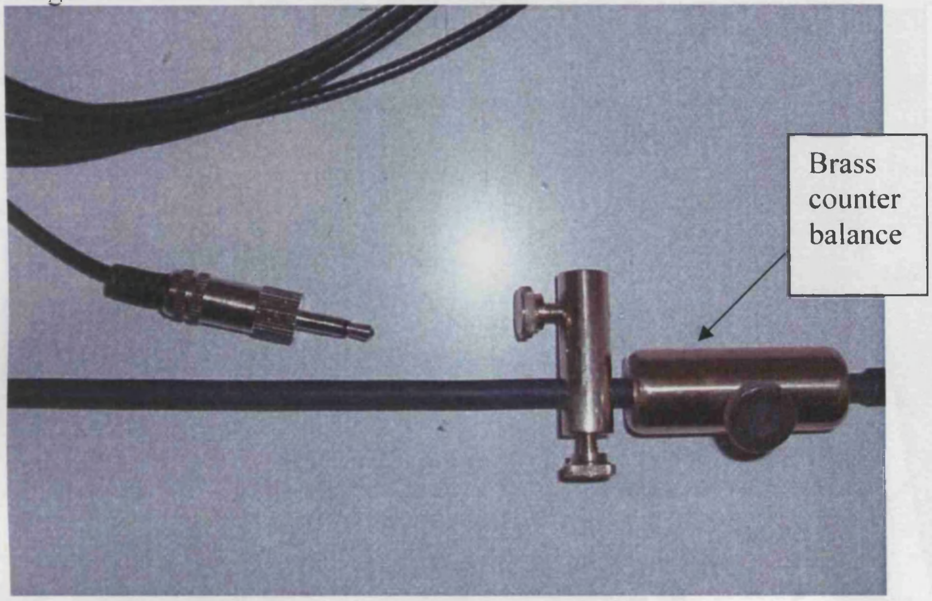

Figure 3 the brass counter balance

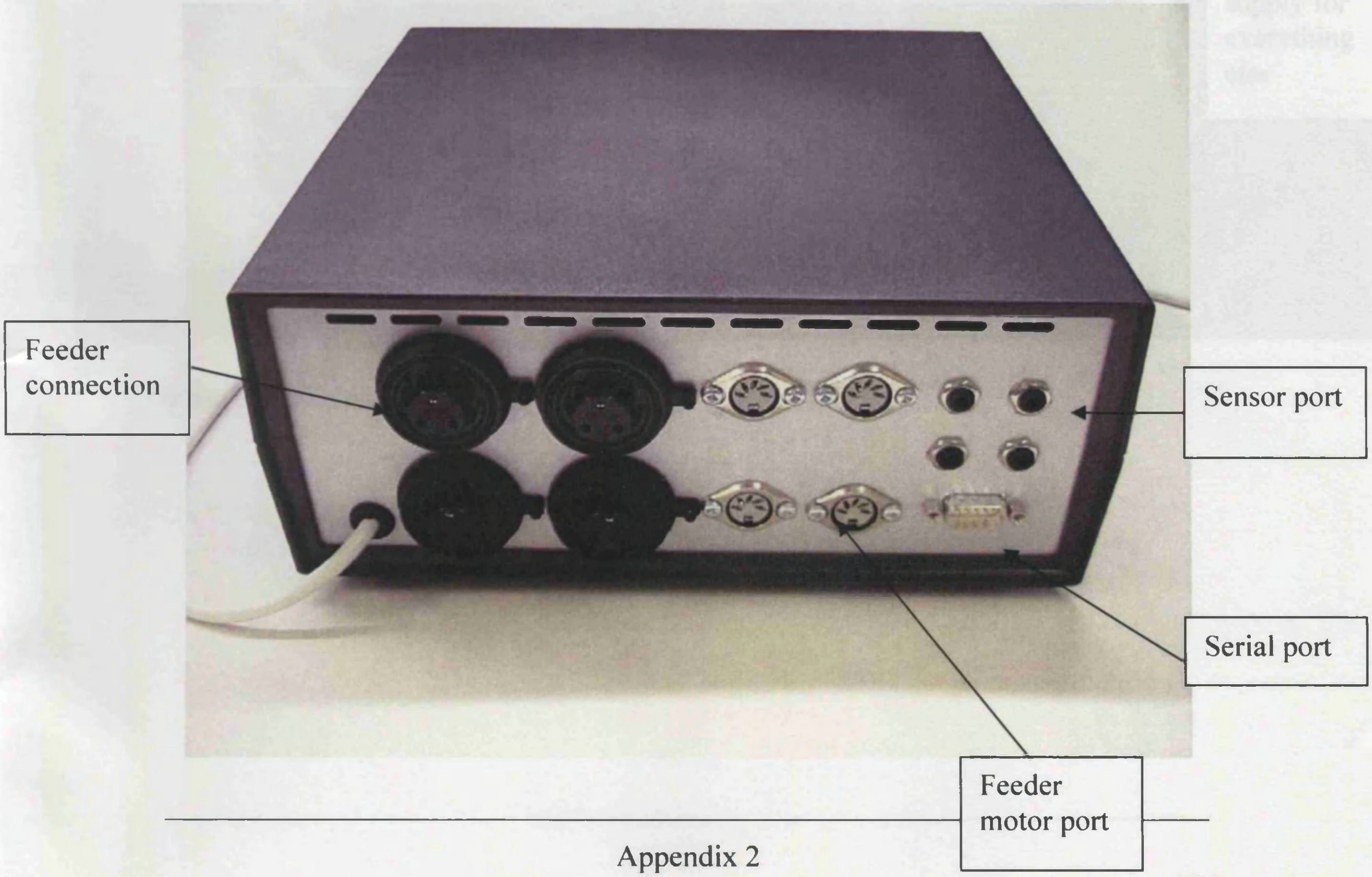

Figure 4 The control box ports

The control box

The feeder, sensor, sensor motor and computer are linked to a control box. There are LEDs set in the front to indicate the status of each device.
Internally there are 4 potentiometers, one for each sensor. These could be adjusted to set the gain (sensitivity level) of each sensor.

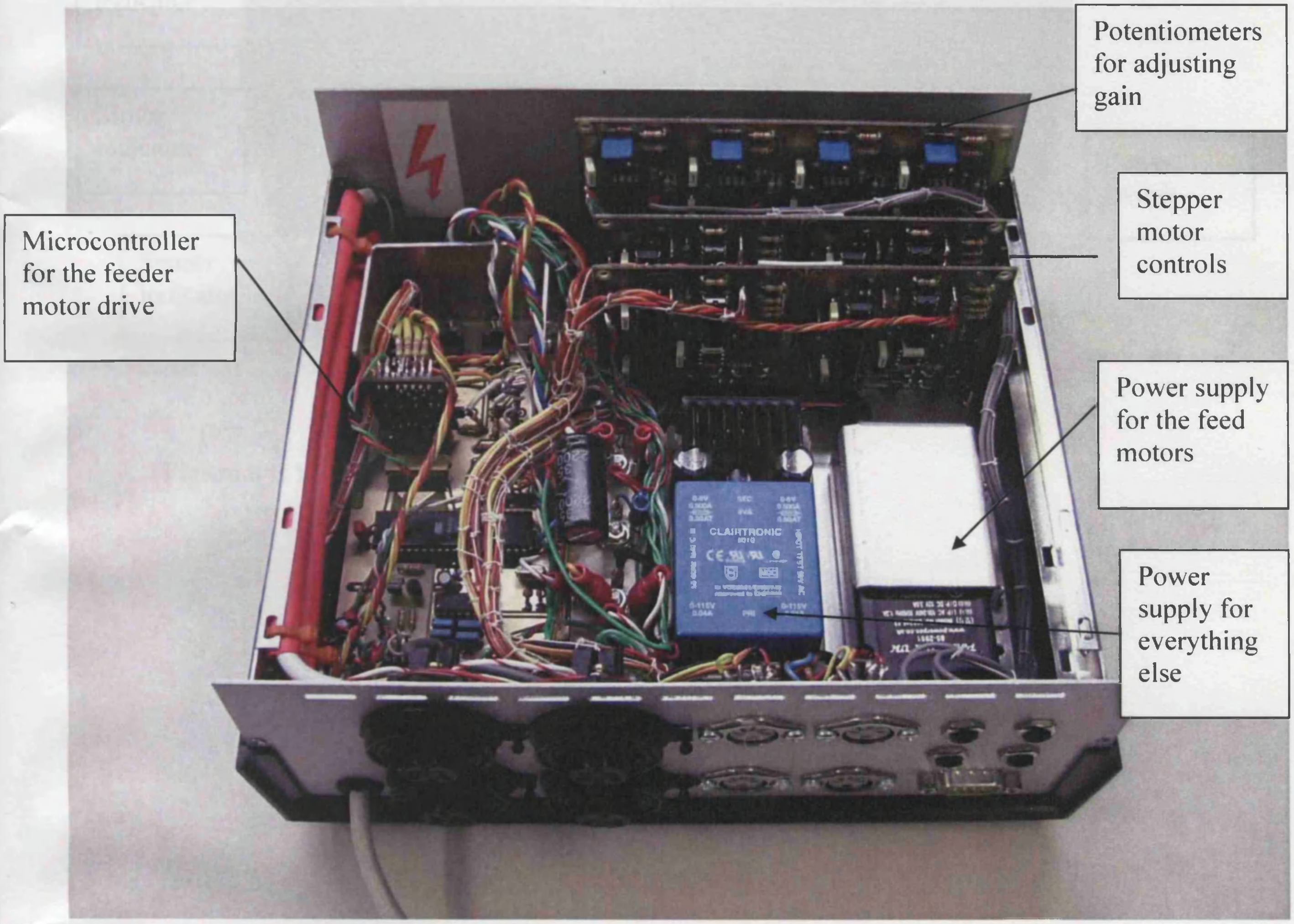

Figure 5 The control box

Figure 6 The control box casing