UNIVERSITY OF
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# Quantifying and modelling of the nitrogenous wastes associated with the commercial culture of Atlantic cod (*Gadus morhua* L.)

Thesis submitted for the degree of
Doctor of Philosophy

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**Dedicated**

**To**

**my family**

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# Declaration

I declare that I carried out the work for and was principal contributor to the intellectual content of this thesis. No part of this work has been submitted for any other degree.

Robert Louis Andrew Oliver

# Abstract

In Scotland, environmental regulation restricts commercial cod culture to the equivalent of 66 % of that granted for commercial Atlantic salmon (*Salmo salar* L.) farms. This calculation is based on estimations of nitrogen discharge from the difference in protein content between salmon and cod diets, with the higher levels of protein in cod diets suggesting a higher nitrogen discharge compared to that observed for salmon diets. In turn, this could potentially result in increased nitrogen enrichment of a marine ecosystem.

The aims of this study (quantifying and modeling of nitrogenous wastes associated with the commercial culture of Atlantic cod (*Gadus morhua* L.) were achieved through a series of tank and cage investigations, each of which studied juvenile and adult Atlantic cod. The study provided data with respect to nitrogen excretion from juvenile and adult fish in both systems. This would allow the development of dispersion models and the calculation of nitrogen budgets for commercial cod culture, thus providing environmental regulators data independent of salmon models to create regulations that would be specifically applied to cod farming.

The tank - based studies investigated three diet formulations produced by EWOS® Innovation in Norway, as a 4 mm pellet (juvenile study) and as a 7 mm pellet (adult study). The three iso - energetic diets varied primarily in protein content (40 %, 50 % and 60 %). Two tank studies, one on juvenile and one on adult cod, investigated growth, condition and tissue composition, and the production of dissolved nitrogenous wastes over a 5 and 7 month period respectively.

At the beginning of the acclimation period prior to the adult tank study commencing, the fish had a mean weight of approximately 1275 g. The difference in the final weight promoted by each diet was not significant (with an approximate final weight of 2400 g), suggesting that a low protein diet (40 % protein) promoted similar growth to a high protein diet (60 % protein). Other growth and condition parameters were also similar for all diets with the condition improving over the course of the study. As the fish completed spawning immediately prior to the

commencement of the study, an increase in condition was not observed until approximately day 90 of the 210 - day investigation. Over the course of the 5 - month juvenile study, growth was approximately 224 g for the 40 % protein diet and approximately 275 g for the 50 % protein and 60 % protein diets. This suggests that a higher protein diet is required for optimal growth of juvenile cod and that 50 % and 60 % protein diets promote similar growth and condition, potentially reducing the protein requirement of juvenile diets. In the juvenile investigation, condition increased over the full range of the study.

In both tank studies, nitrogen digestion was directly related to protein (and associated nitrogen) content of the diets in the juvenile study expressed as a percentage of the nitrogen content of the diet as 59.19 % (40 % protein), 56.90 % (50 % protein) and 52.23 % (60 % protein) suggesting that nitrogen digestion is more efficient at lower protein content in the diet. When expressed as a percentage of the nitrogen content of the diet, nitrogen digestion observed in the adult study was 60.55 %, (40 % protein) 60.92 % (50 % protein) and 60.60 % (60 % protein) respectively, suggesting protein digestion is similar regardless of protein content in adult cod.

In the adult tank study, under a manual feeding regime, a post - prandial - peak is observed at 105 min. following the cessation of feeding. Thereafter, ammonia levels drop over the course of the sampling period. Following the afternoon meal commencing at 420 min., ammonia levels rise at least until the final samples are collected at 450 min. Under an automated regime, a lesser post - prandial - peak is observed but the ammonia concentration is lower over the sampling period compared to the respective profile under a manual regime. The ammonia profile produced throughout the juvenile tank study follows a very similar trend to that observed in the adult study under the manual feeding regime.

Two cage - based investigations took place at the No Catch® Ltd. commercial organic cod farm in Vidlin Voe on the east coast of Shetland. Both studies investigated growth, condition and tissue composition, as well as the production of dissolved nitrogenous and particulate wastes associated with the culture of juvenile and adult Atlantic cod in cage systems. Sampling for the

adult study occurred over three days during three sampling trips (September 2005, November 2005 and February 2006). Sampling for the juvenile study took place over three days on a single trip to Vidlin in late April 2006. The diets used at No Catch® Ltd. were produced by Biomar® in Grangemouth.

A relationship between feeding and ammonia concentration is less evident in the cage studies than in the tank studies, and similarly, the relationship between feeding and ammonia concentration is less evident in juvenile fish than in adult fish. As ammonia values were converted to (µg/L/tonne biomass), the ammonia concentration recorded is largely dependent upon the biomass of the sampled cages at both the nursery site and production site.

Deposition rates of organic carbon and nitrogen around the production and nursery cage sites in Vidlin Voe are related to the position of the sediment trap relative to its location and proximity of the trap to the specific cage site. Weather condition also had an impact on deposition rates with calmer weather producing lesser deposition rates.

Around the production site, deposition rates of organic carbon and nitrogen are greatest in the direction of the prevailing current. Deposition rates decrease with an increasing distance from the cages. Although sediment trap results were inconsistent, a similar trend is observed for each of the three sampling trips, although actual deposition values were different. Differences between the deposition rates at the highly dynamic production site and the low energy nursery site indicated that sedimentation of waste from cod culture is highly dependent on water currents.

Models of particulate waste deposition associated with the production cage site in Vidlin Voe were produced using the spreadsheet - based Cage Aquaculture Particulate Output and Transport (CAPOT) model, developed at the Institute of Aquaculture. The models were parameterised using the data collected and tested against an established regulatory model, DEPOMOD. The similarity in results illustrated the robustness of the highly flexible spreadsheet waste model for cod culture.

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# Glossary

| | |
|---|---|
| C | carbon |
| cm | centimetre |
| E.I.A. | Environmental Impact Assessment |
| E.Q.S. | Environmental Quality Standard |
| F.C.R. | Food Conversion Ratio |
| g | gram |
| hr | hour |
| H | hydrogen |
| H.S.I. | Hepatosomatic Index |
| kg | kilogram |
| M.E.R.L. | Machrihanish Environmental Research Laboratory |
| M.M.F. | Machrihanish Marine Farm |
| m | metre |
| mg | milligram |
| ml | millilitre |
| mm | millimetre |
| min. | minute |
| N | nitrogen |
| % L | % lipid |
| % P | % protein |
| s | second |
| S.D. | Standard Deviation |
| S.G.R. | Specific Growth Rate |
| T.A.C. | Total Allowable Catch |
| µg | microgram |

# Chapter 1

## INTRODUCTION

# INTRODUCTION

Atlantic cod (*Gadus morhua* L.) are widely distributed in arctic and temperate waters on both sides of the North Atlantic Ocean where the species live over the continental shelf and in shallow coastal waters. On the North American coast, cod can be found from North Carolina to Northern Labrador, around Greenland, around Iceland and to the coasts of Europe from the Bay of Biscay to the Barents Sea.

Cod stocks have been exploited for hundreds of years and with more recent developments in fishing technology and techniques, wild cod stocks have hit critically low levels. This has led to a sharp increase in market value and to an increased interest in commercial culture of the species. Recent research in Norway, Canada and Scotland has supported the development of commercial culture techniques for Atlantic cod.

## 1.1 Capture production

Atlantic cod has been the most commercially important fish in the north Atlantic since the discovery of the new world and has been exploited for over 1,000 years (Kurlansky, 1999). Catches of Atlantic cod rose to between 150,000 tonnes and 400,000 tonnes during the 1800's (Kurlansky, 1999). In 1885, the Canadian government claimed cod stocks would be fertile forever, but by 1902, the British government conceded that over - fishing was evident (Kurlansky, 1999).

The development of fishing technology and techniques during the 20$^{th}$ century resulted in ever increasing catches, rising to a peak of approximately 2,000,000 tonnes from the Grand Banks alone during the 1960's. A total north Atlantic catch of 4,000,000 tonnes was observed in the mid 1960's (Figure 1.1). With ever increasing catches, it was thought the stocks were

indestructible; in reality, the improved commercial fishing technologies and techniques resulted in over - fishing and by the 1970's, catches were showing rapid signs of decline (Figure 1.1).

In the second half of the 20th century, fishing nations such as the United States, Canada and Iceland gained a zone of sovereignty of 200 miles, protecting these waters from foreign vessels. The associated introduction of Total Allowable Catch (TAC) quotas for all cod stocks was hoped to develop sustainable fisheries.

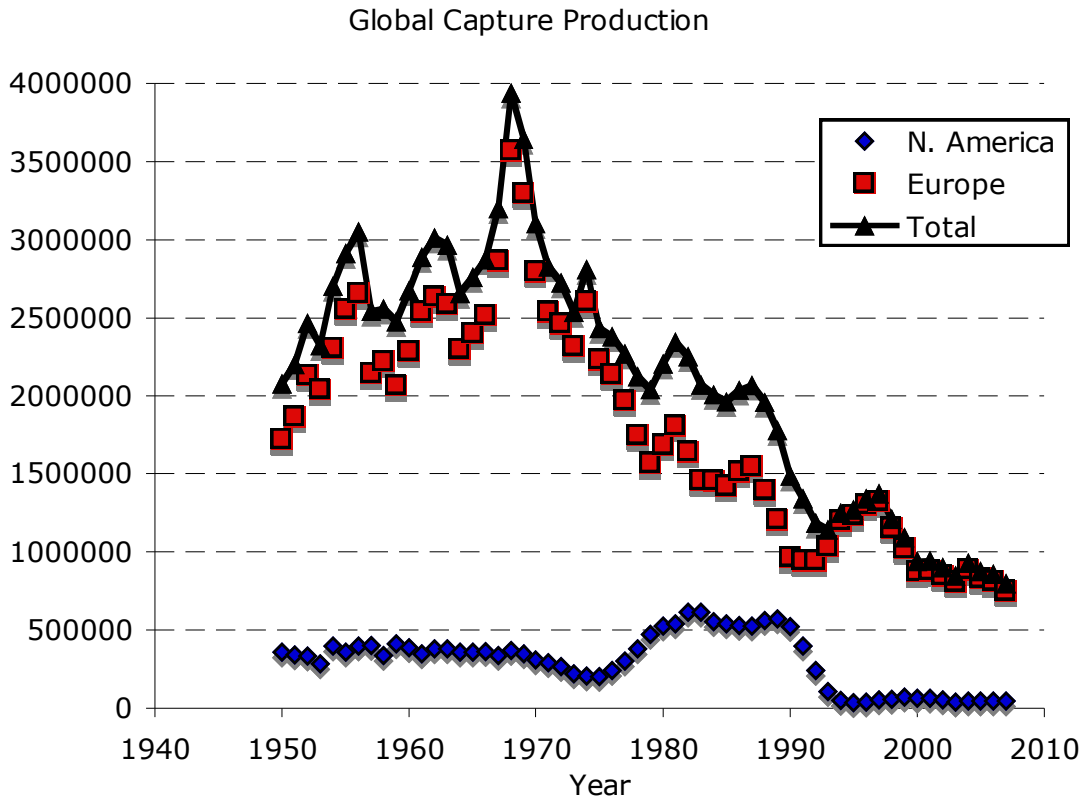



Figure 1.1 Capture production of wild Atlantic cod since 1950 (created using statistics obtained on www.fao.org).

It was thought that through introducing TAC quotas, stocks would recover, but it appears to have had little or no impact on the recovery of stocks, particularly in the Grand Banks. Credence was given to the consideration that the apparent poor recovery is due to the lack of prey in the North Atlantic. Consequently the remaining cod of the North Atlantic maybe starving as their environment cannot provide sufficient food to support or improve stocks. In a recent study, (Swain & Sinclair, 2000) the author interviewed commercial fisherman and accompanied them on fishing trips. Commercial fishers reported 'an absence of large mature fish' and have frequently reported catching 'slinky' cod. 'Slinky' is the local name (used on the Grand Banks) given to extremely thin cod that are almost emaciated in appearance. Such cod have been frequently reported, suggesting that these specimens were representative of the stocks. Wild cod should progress from demersal juveniles to pelagic adults. However, with a lack of prey, juvenile cod are forced to remain bottom feeders as they enter adulthood, developing a down-turned head and a humpback (Swain & Sinclair, 2000). A typical adult cod has a mouth suited to catching pelagic fish. However, catches of 'slinky' cod exhibit red abrasions around the mouth due to enforced bottom feeding. Such a phenomenon is having a greater effect on cod compared to other species such as haddock, a species that has large eyes and a snout, which are well suited for extensive bottom feeding. Growth rates of Canadian Atlantic cod have also fallen in recent years. In the 1970's, the cod could reach approximately 70 cm in 7 years. However, in the 2000's it is likely that cod will reach between 54 cm and 59 cm in the same period (DFO, 1998) as referenced in (Swain & Sinclair, 2000). Other factors such as over - predation were considered, although if stocks were heavily predated, the remaining cod would theoretically benefit due to an increased availability of prey. The study found remaining cod in heavily exploited cod stocks to be small, 'slinky' and in poor condition, suggesting that a lack of food is a major factor inhibiting the recovery of wild stocks. To this end, aquaculture of cod is becoming more relevant to maintaining supply.

## 1.2 Aquaculture potential of Atlantic cod

Atlantic cod has long been identified as a species with considerable potential for intensive culture in northern Europe and Canada (Eurofish, 2003; Morais *et al*, 2001; Walden, 2000). First attempts to cultivate cod started in Norway at the Flodeivigen Biological Station in 1884. The rearing of larvae in enclosed natural basins became a reality by 1886 but the first complete artificial culture of cod to maturity in laboratory conditions was only accomplished in 1977 (Hogenstad, 1984). Early cod aquaculture relied on the capture of wild adults (in poor condition) in early summer, which were then ongrown in captivity (dos Santos & Jobling, 1988).

The early studies were conducted with the only aim of producing yolk-sac larvae to enhance declining wild stocks (Walden, 2000; Torrisen *et al*, 1993; Solemdahl *et al*, 1984). The first large-scale project to stock the sea was initiated in 1950 and involved stocking the Oslofjord in Norway with larval cod. The program ran for 21 years but was terminated in 1971 because benefits of the program could not be demonstrated (Pillay & Kutty, 2005). Initial attempts to produce juvenile cod utilised semi – intensive 'mesocosms'. This involved pumping seawater into large saltwater ponds and lagoons, allowing the larvae to feed upon the plankton that were introduced with the tides. Variations in plankton quality and introduction of diseases inhibited this practice greatly (Walden, 2000). Intensive farming began in the mid 1980's (Eurofish, 2003; Walden, 2000) but production failed to achieve its potential due to factors such as availability of healthy juveniles and a low market price. This combined with high operating costs caused interest in intensive cod aquaculture to wane (Eurofish, 2003).

Recently however, unprecedented low levels of wild stocks has led to a sharp increase in market value and invigorated interest in intensive culture of the species (Eurofish, 2003; Walden, 2000). Cod is currently a very promising candidate for intensive culture (Eurofish, 2003) for many reasons; a high fecundity and the ability to spawn naturally in captivity is a distinct

advantage (Walden, 2000). Cod are a naturally hardy, shoaling and gregarious species, making them an ideal candidate for intensive culture. Growth can be high, even at low temperatures and the recent development of formulated diets has led to improved growth and Food Conversion Efficiency (Jobling *et al*, 1991; Jones, 1984; Howell & Bromley, 1983). Larval survival may be low but has been shown to at least be comparable with other marine species grown in captivity (Jobling *et al*, 1991; Jones, 1984; Howell & Bromley, 1983).

As in the past, operating costs are still a major factor. With the building of new hatcheries and production units, production needs to be high for an operation to be profitable (Eurofish, 2003), suggesting a high initial investment is required. Hatcheries are beginning to provide a larger, more reliable source of healthy juveniles and the development of live feed and formulated feeds has resulted in healthier juveniles available for on - growing. However, the requirement for live feeds and a high rate of cannibalism in larval cod contributes to increased operating costs. Similarities to salmon farming means it is possible to use a lot of experience already gained and it is hoped that new research and technology can be implemented, allowing potential growth of the industry to be rapid. This is shown in Figure 1.2 with a rapid increase from around the year 2000, particularly in Norway.

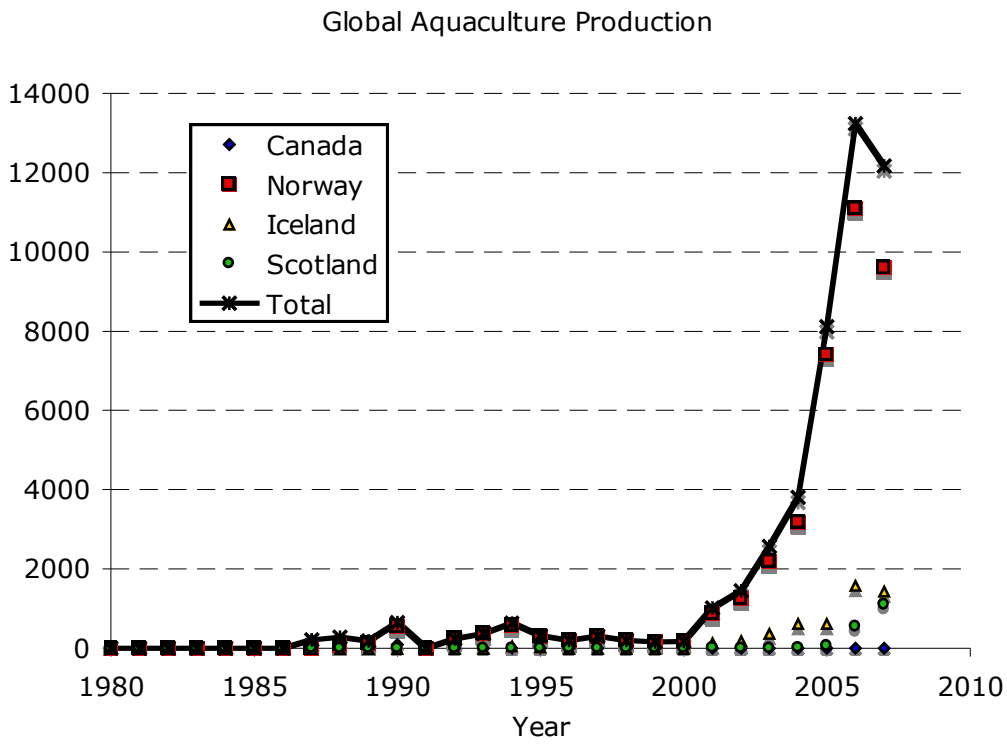



Figure 1.2 Aquaculture production of Atlantic cod since 1980 (created using statistics obtained on www.fao.org).

It is estimated that farmed cod production in Norway could reach 175, 000 tonnes by 2010 and 400,000 tonnes by 2015 (Pillay & Kutty, 2005).

### 1.2.1 Cod aquaculture techniques in Scotland

Current cod aquaculture in Scotland involves the collection of fertilised eggs from broodstock tanks. The fertilised eggs are buoyant in water and are collected through the overflow from each of the broodstock tanks prior to transfer to conical tanks until they hatch. A noticeable difference in the hatchery stage between Atlantic cod and Atlantic salmon culture is the requirement for live feed for larval cod prior to weaning onto a formulated 'dust' diet. Atlantic salmon eggs are approximately 50 times larger than cod eggs and upon hatching, the resulting

salmon larvae (alevin) utilise yolk sac reserves prior to weaning onto a formulated 'dust' diet. Following the hatching of the cod larvae, they are fed progressively on algae, rotifers and then artemia, an increasing prey size for the increasing larval size. As the larvae grow, they are weaned onto the formulated diet at approximately 30 days of age when they are between 7.5 mm and 8 mm in length.

At 5 g, all juvenile cod are injection vaccinated against vibrio and on reaching approximately 50 g, are transferred to No Catch® Ltd. at Vidlin Voe in Shetland, at present the only significant Atlantic cod production in Scotland. The 50 g cod are initially introduced to the nursery site at Vidlin and are subsequently moved to the production site in Vidlin for on - growing until harvest. Between transfer to the nursery site and harvest, the grow - out period for Atlantic cod is similar to that of Atlantic salmon (c. 24 months). Although culture methods on a commercial cage site are similar between the species, Atlantic cod require a higher protein diet than Atlantic salmon. Cod readily lay down excess lipid in the liver and a high protein diet (and associated low lipid content) inhibits excess lipid accumulation in the liver. Current on growing diets incorporate approximately 52 % protein for cod compared to approximately 40 % protein used in salmon culture. The use of the 60% protein diet provided by EWOS® in the tank studies was suggested in order to produce a range of results both in terms of protein and lipid.

### 1.2.2 Feeding in cod aquaculture

Cod are omnivorous predators and feed on a wide range of animals (www.fishbase.org; www.fao.org). Larvae and post - larvae feed on plankton before they settle on the seabed. Juveniles eat invertebrates such as copepods, amphipods, fish larvae and shellfish larvae. Larger cod are more predatory and generalistic, feeding on crustacea, molluscs, echinoderms and fish such as capelin, herring and even young cod (www.fishbase.org; www.fao.org). In times of limited food, cod become omnivorous scavengers and will eat a very large range of species

(www.fishbase.org). Stones have been found in cod intestines and as cod preferentially eat large prey swallowed whole, the stones were probably swallowed deliberately to act as gastroliths, which aid digestion (Clark *et al,* 1995; dos Santos *et al*, 1993). Such a phenomenon could be investigated in the development of feeding regimes and techniques in commercial cod aquaculture.

Intensive cod aquaculture initially utilised formulated diets adapted from the commercial culture of Atlantic salmon. However, the increasing interest in cod farming has led to the development of a variety of formulated feeds specifically for the intensive culture of Atlantic cod (Jobling *et al*, 1991; Lie *et al*, 1988).

As cod readily lay down excess lipid in the liver, they demand a low lipid diet (Pillay & Kutty, 2005; Morais *et al*, 2001). At present, formulated cod diets contain approximately 50 % - 55 % protein. As protein is the most expensive nutrient of aquafeeds, optimal regimes are essential to maintain economic sustainability of commercial culture. Current regimes such as those investigated and observed in the current study where the fish were fed at regular intervals throughout the day contradict other work (Lambert & Dutil, 2000).

### 1.2.3 Natural and artificial diets

Natural diets used in commercial cod and salmon aquaculture generally utilise fish obtained from wild capelin, herring and sand eel fisheries. The prey fish are fed to the culture species whole or minced. As wild cod will feed on prey preferably swallowed whole, supplying a natural diet will meet the nutrition requirements as closely as possible. Feeding large pieces will also reduce foraging energy expenditure. However, the use of natural prey has limited use due to costs and reliability of the supply. The quality (nutritional value) of the prey fish will also fluctuate over the year due to developmental stage, maturation and condition, reducing the reliability of a nutritionally rich diet.

Artificial diets used in aquaculture are composed of raw materials obtained from wild capelin, herring and sand eel fisheries. The main raw materials obtained from these fisheries and used in commercial Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) farming are fish meal and fish oil. The caught prey fish are ground up and the oil separated from the meal. These two raw ingredients are the supplemented with other components, nutrients and vitamins, and the mix is extruded into a stable uniform pellet. Extruded diets are generally cheaper than using wild caught fish and also provide a more reliable source of feed of a more reliable nutritional value.

The use of fish meal and oil is preferred as it promotes greatest growth due to the similarities in nutrient profiles required by carnivorous species and the nutrient profiles supplied in the fish meal and fish oil. With the decline in wild stocks of capelin, herring and sand eels, the partial substitution of fish meal and oil with plant meal and oil will have to be studied. However, such substitutions are not ideal as the plant derived meal and oil with not promote optimal growth in carnivorous species due to the differences in nutrient profiles required by the fish and supplied by the raw materials. However, such work is beyond the remit of the current study.

Much work has been carried out investigating optimal feed size, composition and feeding regimes (Lambert & Dutil, 2000; dos Santos *et al,* 1993). dos Santos *et al* (1993) compared the growth of cod fed diets from natural and artificial formulations. dos Santos *et al* (1993) found that diets formulated from natural food products (herring and herring meal) showed higher growth but also elevated lipid liver level compared to those fed on diets formulated with capelin oil. Food size was also shown to be important with smaller food sizes giving impaired growth and decreased FCR. Lambert & Dutil (2000) studied different feeding regimes on the effect of growth of adult cod, which were fed whole capelin. They conclude that in general, optimal feed frequency was three times per week to satiation, though for maturing cod there was no growth improvement at more than two meals per week. However, at high stocking densities (> 40 $kg/m^3$), adult cod fed five times a week showed greater growth than those fed three times a

week. Both studies (Lambert & Dutil, 2000; dos Santos *et al*, 1993) used wild caught cod and primarily investigated natural prey diets. However, such studies may provide an indication of an optimal formulation for aquafeeds and feeding regimes for commercial cod culture.

## 1.3 Aquaculture regulation in Scotland

The Scottish Environment Protection Agency (SEPA) regulates all freshwater and marine aquaculture in Scotland. "SEPA has a duty to control discharges to surface waters and ground - waters, including tidal waters out to the three - mile limit. SEPA does this by issuing a legally binding consent to discharge under the Control of Pollution Act 1974" (www.sepa.org.uk). Each application for consent to discharge is submitted with the findings of an Environmental Impact Assessment (EIA). An 'impact' describes changes in an environmental parameter over a specified period of time, within a defined area (Wiesner, 1995). An EIA provides a wide range of site-specific data such as, an extensive study of tidal characteristics, details of flora and fauna and chemical characteristics of the seabed (www.sepa.org.uk). From an environmental perspective the purpose of an EIA is to predict and assess the potential impacts from (in this instance) an aquaculture activity. These are then compared with the recognised Environmental Quality Standards (EQS), which then allows the regulatory authority (SEPA) with the scientific data of pollution to determine the potential impacts relative to pre - determined standards on any given site. Following consideration of the application, "SEPA will either grant or refuse consent to discharge. Where consent is granted this will include specific conditions to limit the effects that the discharge may have upon the receiving environment. Monitoring will be carried out by the discharger and SEPA, to ensure that the impacts of the discharge remain within the appropriate EQS" (www.sepa.org.uk). In an industry growing in size and diversity, SEPA must continue to develop and evolve the regulatory approach to the aquaculture industry.

At present, the majority of marine finfish aquaculture in Scotland comprises of Atlantic salmon culture. The current discharge consent granted to intensive Atlantic cod farmers has been calculated using a DEPOMOD model created for the commercial culture of Atlantic salmon. The discharge consent is based upon biomass and is calculated that 66 % of the tonnage granted for salmon will be granted for cod. At present, discharge consents are issued on the species - specific level of nitrogen discharge expressed as a 'species - factor' relative to salmon. This is shown in Table 1.1 where it can be seen that cod have a 'species – factor' of 1.5 compared to salmon, which has a 'species – factor' of 1. This is based upon the increased protein and therefore nitrogen content of commercial cod diets compared to that in commercial salmon diets.

Protein is approximately 14 % ± 1 % nitrogen, and nitrogen is the major limiting nutrient in marine ecosystems (Tucker, 1998; De Silva & Anderson, 1995; Barnabe, 1994 and Jobling, 1994). Therefore, the more protein in the diet, the more bio - available nitrogen (ammonia, nitrate, nitrite) is produced through excretory functions. This could lead to increased nutrient enrichment in a marine ecosystem. Protein sparing through increasing lipid levels in the diet permits salmon farmers to successfully use low protein diets of around 38 %. However, cod traditionally demand high protein diets (50 % - 55 %) as an increased level of lipid results in excess lipid deposition in the liver and ultimately, unhealthy fish (Morais *et al*, 2001; Jobling, 1993 and Jobling *et al*, 1991).

## 1.4 Waste and impacts

In order to quantify and model the nitrogenous waste associated with the commercial culture of Atlantic cod and ultimately create an accurate nitrogen budget, all nitrogen entering the system (nitrogen in diet), all nitrogen remaining in the system (retained in fish) and all nitrogen leaving

the system (through excretion of dissolved nitrogenous wastes via the gills and urine, and leaching from uneaten food and faecal material), has to be accounted for.

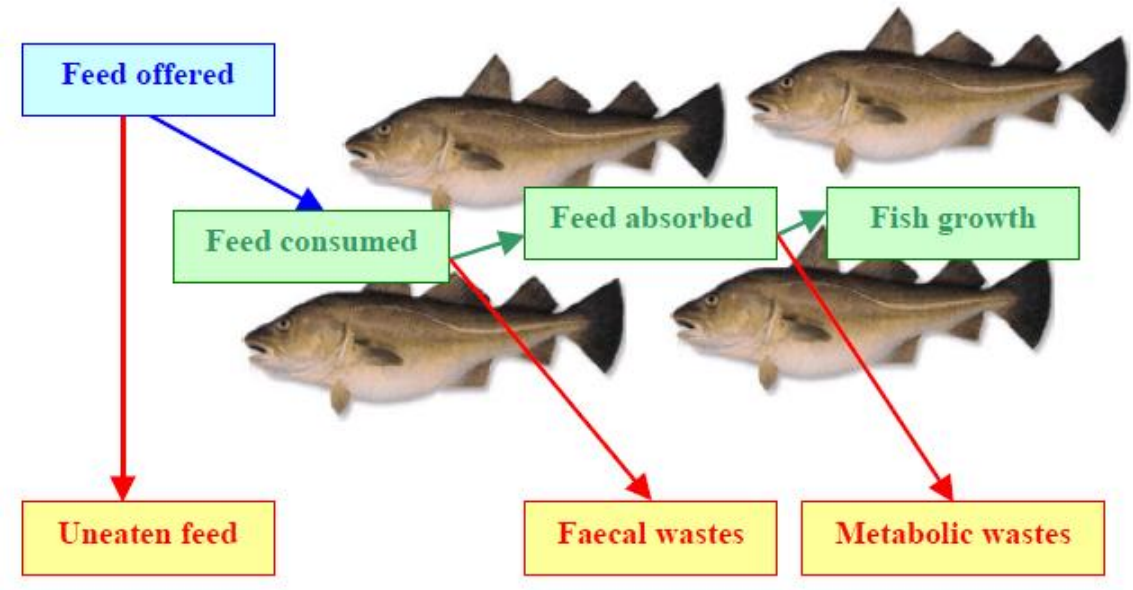



Figure 1.3 Conceptual diagram of the mass - balance model for Atlantic cod.

(Herlin 2003).

### 1.4.1 Ammonia production

Ammonia is the most significant dissolved waste product of finfish aquaculture, created through the metabolism of protein (Pillay & Kutty, 2005; Halver & Hardy, 2002; Tucker, 1998; DeSilva & Anderson, 1995 and Jobling 1994). The primary means of removal of nitrogen in fish is ammonotelism; metabolised protein is excreted from the fish as ammoniacal nitrogen, which oxidises to nitrite then nitrate, the last of which is a major source of enrichment of phytoplankton in marine systems (Pillay & Kutty, 2005; Tucker, 1998; Kardong, 1998;

Campbell, 1996; Handy & Poxton, 1993; Mosmossen & Walsh, 1992; Ramnarine *et al*, 1987 and Buttery & Lindsay, 1980).

Ammonia is toxic to the fish and must be removed quickly (Halver & Hardy, 2002) and is released across the gills and in the urine (Moskness *et al*, 2004; Tucker, 1998). The vast majority of nitrogen excretion (80 % - 90 % of ammonia and urea) occurs at the gill surface (Moskness *et al*, 2004; Dosdat *et al*, 1996). The kidneys are responsible for very little nitrogenous excretion (Moskness *et al*, 2004). Urea and creatine can be excreted in the urine, through the skin or across the gills (Moskness *et al*, 2004).

Ammonia is produced through the breakdown of amino acids by amino transferase and other enzymes such as glutamate and dehydrogenase in the mitochondria (Lovell, 1998; Jobling, 1994). Urea (another nitrogenous waste) is produced through the degradation of purines, and through arginine catabolism by arginase (Dosdat *et al*, 1996). Ammonia is produced in the liver (Pillay & Kutty, 2005; Halver & Hardy, 2002; Dodset *et al*, 1996 and Jobling, 1994) and along with urea is released into the hepatic vein and carried by the blood to be excreted at the gills (Moskness *et al*, 2004).

### 1.4.2 Excretion over gills

As well as being a means for ventilation, gills are the primary means of the removal of ammonia, urea and carbon dioxide (Kardong, 1998; Randall *et al*, 1997; Campbell, 1996). Carbon dioxide is produced through respiration and the metabolism of carbohydrates and lipids while ammonia is the main waste product resulting from protein metabolism (Pillay & Kutty, 2005; Tucker, 1998; Kardong, 1998; Campbell, 1996; Handy & Poxton, 1993; Momossen & Walsh, 1992 and Ramnarine *et al*, 1987). The gills are countercurrent: (Kardong, 1998; Campbell, 1996) meaning that blood in the gill lamellae flows in the opposite direction to water flowing through the gills. This increases the uptake of oxygen and of the waste excretion (ammonia, urea and carbon dioxide) to about 80 % efficiency as exchange occurs along the

entire length of the capillary on the secondary lamellae (Pillay & Kutty, 2005; Halver & Hardy, 2002 and Lovell, 1998).

### 1.4.3 Excretion in urine

Marine teleosts such as Atlantic cod lose water to the surrounding environment across the epithelia and gills. The tissues become hypotonic to the seawater and the fish has to ingest a lot of water to regain an osmotic balance (Kardong, 1998; Randall *et al*, 1997; Campbell, 1996). Any excess salt gained through the high ingestion of seawater is excreted across the gills and also through a small volume of concentrated urine (Kardong, 1998; Campbell, 1996; Lambert *et al*, 1994). The nephridia of the kidney are greatly reduced (Campbell, 1996) and lack glomeruli, distal tubule and Bowmans capsule (Kardong, 1998; Campbell, 1996). Aglomerular kidneys conserve water by eliminating the filtration process in renal capture (Kardong, 1998; Randall *et al*, 1997). The distal tubule absorbs salt from urine therefore, the lack of distal tubule results in no absorption of salts. As there is so little urine produced, the highest percentage of dissolved nitrogenous waste excretion occurs over the gills as discussed in Section 1.4.2.

### 1.4.4 Toxicity of dissolved nitrogenous wastes

Ammonia is toxic to the fish and the severity of toxicity is related to species, age, periodicity and severity of exposure. In periods of low or moderate poisoning, the fish may stay at the waters surface and occasionally gulp for air (Tucker, 1998). Acute ammonia toxicity can lead to overproduction of gill mucus which may result in hyperventilation / irregular ventilation. Other stress induced reactions such as rapid escape movement, loss of equilibrium, spiral swimming and mortalities may also result (Knoph, 1992). Chronic sub - lethal levels result in stress and reduced feeding leading to physiological imbalance and possibly blood and kidney damage.

Environmental gill disease and bacterial gill disease may also result (Moskness *et al*, 2004; Tucker, 1998).

Ammonia within water exists in equilibrium between unionised ammonia ($NH_3$) and the ammonium ion ($NH_4^+$). Ammonia is the term given to the total of $NH_3$ and $NH_4^+$. Ammonium Hydroxide dissociates in water forming the ammonium ion ($NH_4^+$) and the dissolved ammonia gas ($NH_3$) according to the following chemical equilibrium:

$$\underline{NH_3} + H_20 \Leftrightarrow \underline{NH_4\,OH} \Leftrightarrow NH_4^+ + OH^-$$

Underlined is the most toxic form. (after Jobling, 1994 & Barnabe, 1994).

Dissolved ammonia gas ($NH_3$) is the most toxic form and easily passes through the gills. In general terms, an increase in pH and / or an increase in temperature results in an increase in the proportion of $NH_3$ (Pillay & Kutty, 2005; Lovell, 1998 and Jobling 1994). The ratio of $NH_3$ to $NH_4^+$ in the environment is primarily affected by temperature and pH, and to a lesser extent, by dissolved oxygen, salinity and pressure. Consequently, there are large variations in the $NH_3$ to $NH_4^+$ ratio. Ammonia could be more toxic in saltwater as the pH is higher (Tucker, 1998). In intensive systems, the concentration of ammonia in the culture medium varies, but a common maximum tolerable level of 0.0125 mg/L has been reported. However, results between the studies (Pillay & Kutty, 2005; Lovell, 1998; Tucker, 1998 and Jobling 1994) are highly variable due to the differing species investigated and the interaction of biotic and abiotic factors affecting toxicity.

The ammonia concentration in the blood is determined by the ammonia concentration of the surrounding water. High ammonia concentration in the water reduces excretion rates and therefore increases ammonia concentration in the blood, which can be fatal to the fish. Under such conditions, ammonia is converted to urea (which is less toxic) (Halver & Hardy, 2002;

Tucker, 1998; Dosdat *et al*, 1996 and Jobling, 1994). The conversion to urea requires energy so direct excretion of ammonia is preferred. Ammonia ($NH_3$) and ammonium ions ($NH_4^+$) are the main excretory products accounting for 75 % - 90 % of dissolved nitrogenous wastes, while urea and creatine comprise a further 5 % - 15 % of these wastes (Halver & Hardy, 2002; Dosdat *et al*, 1996 and Jobling, 1994). In seawater, the availability of sodium and calcium ions involved in the active transport of ammonium ions increases gill membrane permeability and could make ammonia excretion easier than in freshwater (Soderberg & Meade, 1991).

Nitrite ($NO_2^-$) is the first product of ammonia oxidation and is approximately 10% less toxic than ionised ammonia ($NH_4^+$) and cannot be allowed to accumulate in the environment. On uptake by the fish, nitrite entering the blood binds to haemoglobin to form methaemoglobin, interfering with $O_2$ transport and can cause brown blood disease (Tucker, 1998; Jobling, 1994). Methaemoglobin is always present in the plasma in measurable quantities and a ratio of methaemoglobin 50 : 50 haemoglobin is tolerable. When methaemoglobin increases over this level, the oxygen carrying capacity of the blood is greatly reduced and can result in stress, hypoxia & hypercapnia (Jobling, 1994). Exposure at sub - lethal levels may lead to a degree of tolerance after several days (Tucker, 1998). Nitrite is less toxic in salt water due to an increased level of chloride & calcium ions providing protection by limiting uptake of nitrite and bromide, bicarbonate, sulphate, phosphate & nitrate ($NO_3^-$) (Tomasso, 1994).

Nitrate ($NO_3^-$) is relatively non-toxic compared to ammonia and nitrite but in elevated levels may lead to a weakened immune response and consequently can promote disease (Tucker, 1998).

### 1.4.5 Ammonia in the environment

In marine aquaculture systems, ammonia is produced through several different pathways; the main sources are excretion across the gills with a small volume produced in the concentrated urine. Ammonia can also be liberated from uneaten food, faecal material and mortalities (Tucker, 1998). Given the consistency of faecal material produced by Atlantic cod (c. 90 % moisture), it could be deduced that the liberation and dispersion of ammoniotelic nitrogen will be swift following excretion due to the dilute consistency of the material. As uneaten food breaks down in the environment, nitrogen is liberated as ammonia, which will break down to nitrite then nitrate which is bio-available, and could potentially lead to the enrichment of phytoplankton populations in marine ecosystems.

With the increasing interest in the commercial culture of Atlantic cod, it can no longer be acceptable that discharge consents are issued on the species - specific level of nitrogen discharge expressed as a 'species - factor' relative to salmon. This is shown in Table 1.1.

Table 1.1 Species - specific levels of nutrient nitrogen discharge, expressed as a 'species - factor' relative to salmon. (Gillibrand *et al*, 2002).

| Species | Total discharge of nutrient nitrogen (kg / tonne production) | Species Factor |
|---|---|---|
| | | |
| Salmon | 48.2 | 1.0 |
| Halibut | 67.1 | 1.4 |
| Turbot | 86.9 | 1.8 |
| Cod | 72.3 | 1.5 |
| Haddock | 72.3 | 1.5 |

However, as discussed by Herlin (2003), the credibility of such results is questionable as there are no scientific references in the publication (Gillibrand *et al*, 2002). Atlantic salmon and Atlantic cod as species are very different physiologically and have very different life histories. This strongly suggests that the current 'species - factor relative to salmon' must be superceded by species - specific scientific data providing the regulatory authority (SEPA) the necessary tools to issue discharge consents specifically for Atlantic cod.

## 1.5 Aims

Initial studies into the investigation of quantifying and modeling of the nitrogenous wastes associated with the commercial culture of Atlantic cod were previously undertaken as an MSc project at the Institute of Aquaculture (Herlin, 2003). These studies were undertaken investigating 70 g – 80 g fish in triplicate over a 42 - day period.

The current study as reported in this thesis was designed to quantify and model the production of dissolved and solid nutrients effusing into the environment from intensive cod culture. It was anticipated that the data when compiled, would be valued and potentially form the basis in the development of policy regulating cod farming. After studying and considering previous work (Herlin, 2003), the current studies were designed to fulfill the aims stated on the following page.

1. To quantify the production of dissolved nitrogenous wastes associated with the commercial culture of cod.
2. To measure and model the particulate waste outputs from farmed cod.
3. To produce a nitrogen budget specific to the commercial culture of cod.

These aims were achieved by investigating nutrient outputs from cod culture using a number of scenarios, which follows the rationale described in Section 1.6.

## 1.6 Rationale

In order to satisfy the aims and create an accurate nitrogen budget for the commercial culture of Atlantic cod, all nitrogen entering the system (nitrogen in diet), all nitrogen remaining in the system (retained in fish) and all nitrogen leaving the system (through excretion of dissolved nitrogenous wastes via the gills and urine, and leaching from uneaten food and faecal material), has to be accounted for. A series of tank - based and cage - based studies were undertaken at Machrihanish Environmental Research Laboratory (MERL) and at No Catch® commercial cod farm, respectively.

The tank-based studies undertaken at MERL incorporated a 5 - month investigation of juvenile cod and a 7 - month study of adult cod. Both studies investigated three commercially formulated diets produced by EWOS®. The three diets studied were made to the same three formulations but as a 4mm pellet for the juvenile study and as a 7 mm pellet for the adult investigation. The diets varied primarily in protein, with lipid levels adjusted accordingly to keep the diets iso-energetic. A series of measurements were taken over the course of each study to assess growth and condition of the cod, promoted by each of the formulations. For each diet in juvenile and adult studies, the flow of nitrogen through the aquaculture system was charted through a series of measurements of protein and nitrogen in the diet, tissues and faecal matter. Water samples were collected to analyse ammonia, nitrite and nitrate in the tank water arising from direct excretion from the fish as well as leaching from faecal matter and uneaten food. Water samples were collected throughout a single sampling day every fortnight throughout both studies to assess how dissolved nitrogenous waste production related to feeding regimes and protein content of the diet in both juvenile and adult fish. These elements of the work are discussed in Chapters 3 and 4.

Similar investigations were undertaken at No Catch® commercial cod farm in Shetland. The work undertaken in Shetland involved a study of adult cod in commercial sea-cages and the investigation of juvenile cod in the nursery sea - cages. Three sampling trips were made to the

cage production site over a period of 4 months, and a single sampling trip was made to the juvenile nursery site. A single diet formulation (produced by Biomar®) was used in each study in Shetland, the formulations between the studies varied with the juvenile fish being fed a slightly higher protein diet. All the measurements taken during the tank-based studies at MERL were also taken throughout both of the cage-based studies and are discussed in Chapters 5 and 6.

However, the cage - based studies were also used to investigate deposition rates and dispersal of solid waste from the commercial production site at No Catch® Ltd. The data was collected using sediment traps deployed during each trip and the results were plotted and modelled in contour charts, which are displayed and discussed in Chapter 7.

The measurements taken from both of the tank-based studies and cage-based investigations were separately compiled (for each study) to produce a nitrogen flux model for different sized cod, grown in different culture systems, fed different diet formulations under different feeding regimes. It was thought that such a comprehensive study protocol would help to answer the questions, which gave rise to the study.

# Chapter 2

## MATERIALS AND METHODS

# MATERIALS AND METHODS

## 2.1 Tank studies

Tank studies were undertaken at MERL in Argyll, on the west coast of Scotland. MERL is a land - based research facility owned and operated by the University of Stirling and is located on the South shore of Machrihanish Bay. The purpose of the work undertaken at MERL was to investigate the impact of different protein levels in food on growth, condition and tissue composition, and the related production of dissolved nitrogenous wastes associated with the culture of adult and juvenile Atlantic cod in tank systems. The three adult diets and three juvenile diets investigated at MERL were formulated to contain 40 %, 50 % and 60 % protein. The protein levels were set following discussions with EWOS® UK. It was concluded that such closed formulations would be realistic and practical diets representative of commercial diets used in the culture of Atlantic cod.

The intake pipes at MERL draw water from the shallow Machrihanish bay. Given that the bay is shallow and the tidal range large, water availability in summer months can be affected; as can the turbidity of the water supply in times of adverse weather. Water temperatures can rise to 15 ºC in September and fall to 8 ºC in February. A 7 - month adult investigation took place between June 2004 and February 2005, while a 5 - month juvenile investigation occurred between September 2004 and February 2005.

## 2.2 Cage studies

The cage - based studies were undertaken at the No Catch® Ltd. organic cod site located in Vidlin Voe on the east coast Shetland. The fieldwork investigated growth, condition and tissue composition, as well the production of dissolved nitrogenous wastes and solid wastes associated with the commercial culture of juvenile and adult Atlantic cod grown in a commercial cage site.

The study involved 4 visits to the cage sites in Vidlin Voe. The first three visits (September 2005, November 2005 and February 2006) investigated adult cod in the production site. The final visit in April 2006 investigated juvenile cod held at the nursery site.

The mouth of Vidlin Voe is to the northeast of the sea pens, exposing the site to a dynamic environment, especially during winter months. Compared to other areas in the U.K., the tidal range in Shetland in narrow; a range of 0.5 m - 1.5 m between high and low tides (www.wxtide.co.uk). Water temperatures rise to approximately 13 ºC during summer months and fall to approximately 8 ºC during the winter (data supplied by No Catch® Ltd.). Cod have been farmed on this site since 2003. Previously, Atlantic salmon (*Salmo salar* L.) were commercially produced in Vidlin Voe. The site lay fallow for 3 months following the termination of salmon farming before cod were introduced in September 2003. With a harvest size of 4 kg and an average grow - out period of 24 months, the first cod were harvested in spring 2006.

## 2.3 General sampling methods

The diets used in the studies at MERL and No Catch® Ltd. were analysed for proximate composition (Section 2.4.1), settling velocities (Section 2.4.2), leaching rates (Section 2.4.3).

At MERL, the growth rates (Section 2.5.1) were measured while the Food Conversion (FCR) (Section 2.5.2) and Specific Growth Rate (SGR) (Section 2.5.3) were calculated. The Condition Factor (K - Factor) of the fish was studied, (Section 2.5.4) and an Hepatosomatic Index (HSI) (Section 2.5.5) was produced. Ingestion rates were calculated by collecting uneaten food from the outflow of each tank (Section 3.3.1) and faecal material was stripped from sacrificed fish (Section 3.3.2) allowing digestibility rates to be measured. Such work is discussed in Chapter 3.Water samples were collected throughout a single sampling day every fourteen days from the

inflow, within the tank and from the outflow of each tank allowing a daily profile of dissolved nitrogenous waste excretion to be produced (Chapter 4).

At No Catch® Ltd., size (length and weight) and the condition (K - Factor) of the fish were studied, (Section 2.5.4) and an HSI was produced (Section 2.5.5). This is discussed in Chapter 5. Water samples were collected periodically throughout three consecutive sampling days, from beside the same cages, at a depth of 4 m allowing daily profiles of dissolved nitrogenous waste excretion to be compiled (Chapter 6). In order to assess the production and dispersion of particulate matter, a series of sediment traps were deployed along a transect on the prevailing current and a transect running perpendicular to the prevailing current (Chapter 7). The traps were deployed for approximately 72 hrs, coinciding with the duration of each sampling trip.

## 2.4 Techniques used to analyse feed

### 2.4.1 Proximate analysis

Commercial diets for intensive cod farming were initially adapted from commercial salmon diets, but with the increased interest in commercial cod culture, species - specific diets are being developed for the industry. Part of the studies at MERL investigated the impact of different diets upon the growth of cod. Three iso - energetic diets were produced by EWOS® Innovation which incorporated 40 %, 50 % and 60 % protein. The three diets investigated throughout both studies were sampled several times throughout each study (June 2004, September 2004, November 2004 and February 2005) to monitor proximate composition. All samples were collected from deep inside the respective feed bin and frozen (- 20 $^{\circ}$C ± 1 $^{\circ}$C) in labeled pots prior to analysis at the Institute of Aquaculture. The diets fed immediately before each study commenced was sampled prior to each study and anlaysed in the same manner.

Samples of the diets fed at No Catch® Ltd. were sent to the Institute of Aquaculture to be analysed. A large sample of the diet was collected by No Catch® Ltd. staff from the respective

feed barge (production or nursery site). Diet samples were collected in September 2005, November 2005, February 2006 and April 2006 and placed in a labeled pot and frozen (- 20 °C ± 1 °C) prior to analysis at the Institute of Aquaculture.

Standard proximate analysis parameters were measured using standard techniques. All diets, taken from MERL and No Catch® Ltd. were analysed for moisture (Gallenkamp Oven) [AOAC 1990-93.135], ash (Gallenkamp Muffle Furnace) [AOAC 1990-942.05], lipid (Foss Tecator Soxtec System Tecator app Note 67/83), energy (Gallenkamp autobomb), protein (Foss Tecator Kjeldahl system Tecator app. Note 30/87) and total nitrogen (Perkin-Elmer Series II CHNS/O Analyzer 2400).

### 2.4.2 Settling velocities

Settling velocities of cod feed pellets were studied to give an indication of the time taken to settle to the seabed or the time in suspension within seawater tanks. This is important for estimating the dispersion of uneaten food using models such as DEPOMOD (Cromey *et al*, 2002) and that described in Corner *et al* (2006). Pellets that remain longer within the water column are available to the fish for a longer period of time but also have a greater opportunity to leach nutrients directly into the water (Chen *et al*, 1999a).

Settling velocities for 25 pellets of each diet were timed at several water temperatures over a vertical distance of 1 m in natural seawater contained in a large perspex cylinder of 0.1 m diameter (Chen, 2000). The temperature and salinity of the water was controlled. Water temperatures varied accordingly to represent the water temperatures recorded on the date on which the diet samples were collected. The temperatures used to mimic the conditions at MERL were 13 ºC (June 2004), 15 ºC (September 2004), 10 ºC (November 2004) and 8 ºC (February 2005). The temperatures used to mimic water temperature during the cage studies were 13 ºC (September 2005), 10 ºC (November 2005), 8 ºC (February 2006) and 7.5 ºC (April 2006). Salinity was a constant 33 psu. The water was renewed after each diet was tested.

### 2.4.3 Leaching rates

Leaching rates of nitrogen from the diets were investigated under laboratory conditions by measuring the amount of dissolved inorganic nitrogen (in the form of ammonium, nitrite and nitrate).

The 6 experimental diets (40 %, 50 % and 60 % protein at 4 mm and 7 mm) were collected from the respective feed bins at MERL (see section 2.4.1) and were tested at 8 ºC and 15 ºC (temperatures representative of the range observed during the tank trials at MERL). Diets taken from the feed barges (production and nursery sites) at No Catch® Ltd. (see Section 2.4.1) were studied at 7 ºC and 11 ºC (temperatures representative of the range observed throughout the field work at No Catch® Ltd.).

Measurements of nitrogen in the water and the diets were taken during immersion in artificial seawater in a temperature controlled incubator and automatic shaker (IKA Labortechnik HS-250 IKA Werke GmbH & Co, Stauffen, Germany). The movement of the shaker was adjusted to simulate the motion of a pellet settling slowly in the water column at each specific temperature. Artificial seawater (33 psu) was made using Tropic Marine (GmBH, AquarienTechnik, Wartenberg, Germany) premix diluted to the recommended level in distilled water.

Samples of diet collected at each of the sampling dates were tested in duplicate for leaching against duplicate controls (artificial seawater as described above). Approximately 1 g of each diet was placed into each of the duplicate beakers, containing 500 ml of the artificial seawater. The diets were immersed for a total of 30 min and water samples of 25 ml were collected from each beaker after 0 min., 2.5 min., 5 min., 10 min., 15 min. and 30 min. Water samples were collected from the control at the same intervals in order to compare measured leaching to ambient leaching. All water samples were collected in labelled Universal tubes and frozen (- 20 $^{\circ}$C ± 1 $^{\circ}$C). The samples were later thawed and analysed for ammonia, nitrite and nitrate using a Digital Calorimeter (Bran & Luebbe, auto Analyser 3).

Retention of nitrogen within all pellets examined was studied and recorded. It was however concluded that this data supplied little additional information to that given by the leaching rates. However, as the work has been undertaken, the results are not discussed in the thesis, but are shown in Appendix 3 (MERL) and Appendix 21 (No Catch®).

## 2.5 Measurement of growth, condition and tissue analysis

### 2.5.1 Growth measurement

During the tank trials at MERL, individual lengths and weights were taken for all fish prior to the acclimation period; fish were subsequently moved to ensure the starting biomass in each tank was as similar as possible. Every twenty - eight days during the trial, 30 fish were randomly sampled from each tank and the lengths and weights were measured and recorded. FCR (Section 2.5.2) and SGR (Section 2.5.3) were also calculated. From the lengths and weights, the condition (K - Factor) was calculated (Section 2.5.4) and an HSI was created (Section 2.5.5). All fish were anaesthetised in a Benzocaine bath (30 ppm, but adjusted accordingly) before lengths and weights were taken.

At No Catch® Ltd., twelve fish from the sampling cages were randomly caught and sacrificed by No Catch® Ltd. staff following every trip and sent to the Institute for analysis. Individual lengths and weights were taken, the condition (K - Factor) of the fish was calculated (Section 2.5.4) and an HSI was created (Section 2.5.5). Data on FCR and SGR was provided by No Catch ® Ltd.

### 2.5.2 Food Conversion Ratio (FCR)

The Food Conversion Ratio was calculated for both studies using the weights taken from the thirty randomly sampled fish from each tank every twenty - eight days. The calculation is shown in Equation 1 on the following page:

$$FCR = (W_t - W_o)/F_t \qquad \text{(Equation 1)}$$

$W_t$ = wet weight after time $_t$ (g)

$W_o$ = wet weight at time $_o$ (g)

F = feed fed over time $_t$ (g)

### 2.5.3 Specific Growth Rate (SGR)

The Specific Growth Rate was calculated for both studies using weights taken from the thirty randomly sampled fish from each tank every twenty - eight days. The calculation is shown in Equation 2 below:

$$SGR = (\ln W_t - \ln W_o) * 100/t \qquad \text{(Equation 2)}$$

$W_t$ = wet weight after time t (g)

$W_o$ = wet weight at time 0 (g)

t = time of study (days)

ln = natural log

### 2.5.4 K - Factor

The lengths and weights recorded from the thirty randomly caught fish from each tank every twenty - eight days were used to calculate the condition (K-Factor). The condition factor is a

volumetric calculation and was calculated using Equation 3 on the following page. Initially, in an attempt to monitor changes in the condition of individual fish in each tank, five fish from each tank were randomly caught, anaesthsetised in Benzocaine (30 ppm), PIT tagged and given a blue panjet mark on the belly between the pectoral fins. The blue mark made the tagged fish easily recognisable so that they would not be sacrificed during the studies. However, the tagged fish were seldom caught throughout the studies and so the condition factor was calculated using the thirty fish sampled every twenty - eight days.

$$K = W / L^3 \times 100 \qquad \text{(Equation 3)}$$

W = weight (g)

L = length (cm)

As the K-Factor is a volumetric calculation, it can only give an indication of the condition of the fish, which can then be related to the size and life stage of the fish.

### 2.5.5 Hepatosomatic Index (HSI)

The HSI is the ratio of the weight of the liver as a percentage of total body weight and gives an indication of the condition of the fish. The HSI was measured every fourteen days during the tank trials at MERL and following each field trip to No Catch® Ltd. Once the fish were sacrificed, (Section 2.6) the whole fish was weighed (wet weight) then the liver was removed and weighed separately (wet weight).

## 2.6 Tissue analysis

During both studies at MERL, three fish were randomly caught and sacrificed from each tank every fourteen days. The fish were sacrificed in accordance with Home Office regulations

(Animals (Scientific Proceedures) Act 1986); anaethsetised in Benzocaine (>30 ppm) then sacrificed with a sharp blow to the top of the head. Once the HSI had been calculated for the sacrificed fish, tissue samples were collected from the same fish for analysis of proximate composition. For convenience, a 'steak' was taken from each adult fish for analysis; cut between the anterior and the posterior of the second dorsal fin. Each 'steak' was approximately 50 mm – 75 mm thick. The juvenile fish were small enough to be analysed as whole, cleaned fish. All tissue samples were placed in labelled bags and frozen (- 20 $^{\circ}$C ± 1 $^{\circ}$C) awaiting analysis.

While working at No Catch® Ltd, twelve fish from the sampling cages were randomly caught and sacrificed on each of the four sampling trips. The whole fish were sent on ice, to Stirling for analysis. A 'steak' was cut from the adult fish (cut between the anterior and the posterior of the second dorsal fin). Each 'steak' was approximately 50 mm – 75 mm thick. Again, the juvenile fish were small enough to be analysed as whole, cleaned fish.

Both flesh and liver samples were analysed for proximate composition using standard techniques. Tissue samples were thawed, sliced into small pieces, weighed and dried (analysed for moisture (Gallenkamp Oven) [AOAC 1990-93.135]). Following such analysis, the dried tissue was homogenized then analysed for ash (Gallenkamp Muffle Furnace) [AOAC 1990-942.05], lipid (Foss Tecator Soxtec System Tecator app notev67/83), energy (Gallenkamp autobomb), protein (Foss Tecator Kjeldahl system Tecator app. Note 30/87) and total nitrogen (Perkin-Elmer Series II CHNS/O Analyser 2400). Moisture, ash protein and lipid and nitrogen were measured as % wet-weight. Energy was measured as Kilo joules (KJ) per gram (dry weight).

# Chapter 3

## GROWTH AND TISSUE STUDIES IN TANK SYSTEMS

# GROWTH AND TISSUE STUDIES IN TANK SYSTEMS

## 3.1 Introduction

The aim of the tank trials at MERL was to investigate the impact of three different protein levels in the diet upon growth, condition, digestibility and tissue composition associated with these diets in the culture of adult and juvenile Atlantic cod in flow -through tank systems. The tank studies permitted the investigation of the aforementioned parameters (as well as those discussed in Chapter 4), in a highly controlled environment. This would offer a comparison with the cage studies which were undertaken in a highly dynamic environment. The studies at MERL involved the growth of adult and juvenile cod using experimental iso - energetic diets formulated to contain 40 %, 50 % and 60 % protein. Feeding, ingestion, growth, condition, digestibility and tissue composition were measured for the different diets over the 5 - month (juvenile cod) and 7 - month (adult cod) study periods.

## 3.2 Adult Atlantic cod in tank systems

### 3.2.1 Experimental design

Six flow-through experimental tanks each measuring 3 m diameter (as shown in Figure 3.1) and containing 105 adult cod, were set up as two replicates for each of the three experimental diet formulations. Seawater was filtered through a 60 μm drum filter, with water flow in each tank set to 90 L/min. The experimental tanks were set - up on the 17$^{th}$ and 18$^{th}$ May 2004. The fish acclimation period commenced on 2$^{nd}$ July 2004 (day -8) while the study began on 8$^{th}$ July 2004 (day 0) and terminated 7 months later on the 2$^{nd}$ February 2005 (day 210). The photoperiod was achieved using artificial light and maintained at an ambient regime which mirrored changes in the natural day length as the year progressed. For fourteen days prior to the study commencing and throughout the study, several water quality parameters; temperature, dissolved oxygen and

oxygen saturation (%) were monitored using an Oxyguard Handy MkII meter (Oxyguard International, AS, Denmark) and recorded to ensure that water quality remained at optimal levels. Fish behaviour was also monitored to assess for indications of stress resulting from poor water quality. A high water was maintained throughout the study but protocols were in place in the event of any unforeseen changes in water quality.

Figure 3.1 Tanks used in the adult study at MERL.

### 3.2.2 Adult diets investigated

Prior to the study, the adult cod were fed a Dana® commercial cod diet as 7 mm pellets over two meals per day. Fish were fed the trial diets during a 7 - day pre - experiment acclimation period. The three diets investigated during the trial were produced by EWOS® Innovation Norway, as a 7 mm pellet. All diets were iso - energetic and varied in a protein inclusion at 40 %, 50 % and 60 %. In order to keep the diets iso - energetic, lipid levels were varied at 14 %, 12 % and 10 % respectively. Yttrium Oxide was added as an inert marker to each of the three diet formulations at the rate of 0.5 g/kg to allow apparent digestibility to be measured. Each diet was randomly allocated to two tanks allowing each diet to be investigated in duplicate. The fish were fed approximately 0.5 % b/w per day; the ration was adjusted according to appetite. Feeding was by hand between July 2004 and November 2004 (two 15 - min. meals at 9 am and 4 pm) and using an automated feeder from November 2004 to February 2005 (which altered the feeding regime to two smaller 15 - min. meals beginning at 9 am and 4 pm with the remainder of the ration being fed consistently throughout the day).

### 3.2.3 Adult fish investigated

Port Erin Marine Laboratory supplied the fish as juveniles in July 2002. These fish first matured in early spring 2004 and upon completion of spawning, in early May 2004, were used in the current study. On the 17$^{th}$ and 18$^{th}$ May 2004, individual lengths and weights of all 630 cod from the stock population were measured and recorded. The cod were then randomly introduced into the 6 trial tanks ensuring that the initial total biomass in each tank would be as similar as possible. A total of 105 cod with a mean weight of approximately 1140 g were introduced to each of the tanks giving an approximate initial biomass of 120 kg - 130 kg per tank.

### 3.2.4 Adult sampling regime

The diets were sampled periodically (see Table 3.3) throughout the adult study and analysed for proximate composition (Section 2.4.1), ingestion rates (Section 3.4.2), digestion (Section 3.4.3) and growth (Section 2.5.1) were measured every 28 days, as were the FCR (Section 2.5.2), SGR (Section 2.5.3), and K - Factor (Section 2.5.4). HSI (Section 2.5.5) was measured at 14 day intervals for each tank, as was the proximate composition of adult tissue (Section 2.6) and ingestion rates (Section 3.4.1). Sampling and analysis techniques specific to the growth and tissue studies of adult cod are discussed in section 3.4.

Table 3.1 below shows the dates of the 14 day sampling intervals throughout the 7-month adult tank investigation at MERL.

Table 3.1 Sampling dates throughout the adult study (see Section 3.2.4).

| Date | Week |
|---|---|
| 30/6/04 | -1 |
| 7/7/04 - 8/7/04 | 0 |
| 21/7/04 - 22/7/04 | 2 |
| 4/8/04 - 5/8/04 | 4 |
| 18/8/04 - 19/8/04 | 6 |
| 1/9/04 - 2/9/04 | 8 |
| 15/9/04 - 16/9/04 | 10 |
| 29/9/04 - 30/9/04 | 12 |
| 13/10/04 - 14/10/04 | 14 |
| 27/10/04 - 28/10/04 | 16 |
| 10/11/04 - 11/11/04 | 18 |
| 24/11/04 - 25/11/04 | 20 |
| 9/12/04 - 10/12/04 | 22 |
| 23/12/04 - 24/12/04 | 24 |
| 3/1/05 - 4/1/05 | 26 |
| 17/1/05 - 18/1/05 | 28 |
| 2/2/05 - 3/2/05 | 30 |

## 3.3 Juvenile Atlantic cod in tank systems

### 3.3.1 Experimental design

This study used 9 x 1 m diameter tanks, each holding approximately 400 l of seawater (as shown in Figure 3.2). The system was initially set - up on the 28th June 2004 after which there was a long enforced holding period until the beginning of the fish acclimation period on the 2nd September 2004 (day -12). The enforced holding period arose as the allocated juvenile cod were being utilised in another study. The study commenced on the 14th September 2004 (day 0) and lasted for a 5 - month period terminating on 16th February 2005 (day 154). Due to growth of the fish during the holding period, large juveniles (> 150 g) and small juveniles (< 95 g) were graded out on 31st August 2004, and the remaining 630 fish (mean weight ~ 100 g) randomly allocated between the 9 tanks. The flow rate was adjusted to approximately 12 L/min. The photoperiod was achieved using artificial light and maintained at an ambient regime which mirrored changes in the natural day length as the year progressed. For 14 days prior to the study and throughout the study, several water quality parameters such as temperature, dissolved oxygen and oxygen saturation (%) were monitored using an Oxyguard Handy MkII meter (Oxyguard International, AS, Denmark) and recorded to ensure that water quality remained at optimal levels. Fish behaviour was also monitored to assess for indications of stress resulting from poor water quality. A high water was maintained throughout the study but protocols were in place in the event of any unforeseen changes in water quality.

Figure 3.2 A 400 L tank used in the juvenile study at MERL.

### 3.3.2 Juvenile diets investigated

During the holding period, the trial fish were fed several diets, each increasing in size to allow continual optimal foraging as the juveniles grew. The cod were initially fed a 2 mm diet produced by EWOS® (58 % protein : 12 % lipid), followed by a 3 mm diet produced by Skretting® (55 % protein : 15 % lipid), then a 4 mm diet produced by EWOS® (60 % protein : 10 % lipid). During the holding period, each of the aforementioned diets was fed for approximately 3 weeks. Although the fish were fed three diets which varied in composition prior to the trial commencing, it was thought that as all fish were fed the same diets, such a regime would have little or no impact on the outcome of the study.

In order to acclimatise the fish to the experimental diets, an acclimation period of 12 days commenced on the 2nd of September 2004. The three diets used in the investigation were produced by EWOS® Innovation Norway as a 4 mm pellet. All diets were iso - energetic and varied in a protein inclusion of 40 %, 50 % and 60 % protein. In order to keep the diets iso - energetic, lipid levels were varied to 14 %, 12 % and 10 % respectively. Yttrium Oxide was added as an inert marker to each of the three diet formulations at the rate of 0.5 g/kg to allow apparent digestibility to be measured. Each diet was randomly allocated to three tanks allowing each diet to be investigated in triplicate. The three diets were made to the same formulations as those diets investigated in the adult study (Section 3.1.2). The fish were fed by hand throughout the investigation at 1.5 % bw/day, which was offered at two distinct 15 - min. meals during the day at 9 am and 4 pm.

### 3.3.3 Juvenile fish investigated

The juvenile fish originated from Machrihanish Marine Farm (MMF) and were hatched in October 2003. The fry were transferred to MERL in February 2004 (when approximately 5 g in weight), where they were injection vaccinated against Vibrio.

On the 28th of June 2004, 754 cod with a mean weight of 35.2 g were randomly allocated into the 9 tanks. However, these fish had been graded out as large and small fish from another study, so growth (weight gain) of fish in each tank were monitored over the following weeks. Ten fish were randomly caught from each tank and weighed on the 22nd of July and again on the 5th of August and although growth in each tank was similar over the following weeks, depensation was evident. Due to the growth of fish during the holding period, large juveniles (> 150 g) and small juveniles (< 95 g) were graded out on 31st August 2004, and the remaining 630 fish with a mean weight of approximately 100 g were randomly allocated between the 9 tanks giving each tank an initial biomass of between 8 kg and 9 kg.

### 3.3.4 Juvenile sampling regime

The diets were sampled periodically (see Table 3.4) throughout the juvenile study and analysed for proximate composition (Section 2.4.1), ingestion rates (Section 3.4.2), digestion (Section 3.4.3) and growth (Section 2.5.1) were measured every 28 days, as were the FCR (Section 2.5.2), SGR (Section 2.5.3), and K - Factor (Section 2.5.4). HSI (Section 2.5.5) was measured at fourteen - day intervals for each tank, as was the proximate composition of juvenile tissue (Section 2.6) and ingestion rates (Section 3.4.1). Sampling and analysis techniques specific to the growth and tissue studies of juvenile cod are discussed in section 3.4.

Table 3.2 below shows the dates of the fourteen - day sampling intervals throughout the 5 - month juvenile tank investigation at MERL.

Table 3.2 Sampling dates throughout juvenile investigation (see Section 3.34 above).

| Date | Week |
|---|---|
| 2/9/04 | -1 |
| 13/9/04 - 14/9/04 | 0 |
| 27/9/04 - 28/9/04 | 2 |
| 11/10/04 - 12/10/04 | 4 |
| 25/10/04 - 26/10/04 | 6 |
| 8/11/04 - 9/11/04 | 8 |
| 22/11/04 - 23/11//04 | 10 |
| 6/12//04 - 7/12/04 | 12 |
| 20/12/04 - 21/12/04 | 14 |
| 3/1/05 - 4/1/05 | 16 |
| 17/1/05 - 18/1/05 | 18 |
| 31/1/05 - 1/2/05 | 20 |
| 16/2/05 - 17/2/05 | 22 |

## 3.4 General methods

### 3.4.1 Ingestion rates

Uneaten food was collected and measured throughout a sampling day every fourteen to give an indication of feeding success and allow accurate rations to be calculated. The calculated ingestion rates were used in conjunction with feeding quantities, FCR's and digestibility (all discussed later in this Chapter), as a method of calculating nitrogen loading (Beveridge, 2004). An investigation to assess the moisture uptake of the pellets was undertaken to account for water uptake so that an accurate dry weight of uneaten food could be calculated. This was undertaken by submersing a weighed sample of each diet in a beaker of natural seawater at ambient temperature for 120 mins. During this period, the pellets were removed at measured time intervals (Table 3.3) and weighed. The details were recorded and the pellets were returned to the seawater until the next time interval. The recorded weights following immersion are shown in Table 3.3 below.

Table 3.3 The impact of immersion on the weight of a sample of the investigated diets.

| Time (mins) | Weight (g) |
|---|---|
| | |
| 0 | 43 |
| 5 | 52 |
| 10 | 52 |
| 15 | 53 |
| 30 | 55 |
| 45 | 57 |
| 60 | 59 |
| 90 | 63 |
| 120 | 66 |

As it can be seen in Table 3.3, there is progressive weight gain of the sample of pellets as a result of water uptake. It is important that these values are considered when estimating ingestion rates based solely upon the weight of retrieved immersed pellets.

Several methods of collection of uneaten food were considered and attempted:

A shallow mesh sock placed in the outflow standpipe of each tank.

A deep mesh sock placed in the outflow standpipe of each tank.

Syphoning the outflow mesh in base of each tank.

However, it was thought that the larger tanks used in the adult study were too big for the such collection methods to be successful as most of the uneaten food remained in the outflow sump below the tank. It was therefore concluded that flushing each tank following feeding would be the most successful method of collection. Each tank was flushed at 10 - min. intervals over a period of 30 min. following each meal. A hand net (mesh size of 2mm) was held across the outflow pipe as the tanks were flushed. From the immersion studies (see previous page) the integrity of the extruded pellets remained constant over the 30 min. period and consequently, no pellets would be lost using the 2mm mesh hand net. The collected feed was then weighed (wet weight) on a Mettler PM 6000 scale balance and the values recorded.

The night prior to sampling the juvenile tanks for ingestion rates, a 2 mm metal mesh disc was placed around the outflow standpipe in each tank. The following day, the outflow mesh was syphoned every 10 - min. over a 30 min. period following each meal. The collected pellets were then weighed and recorded as above.

### 3.4.2 Collection of faecal material

Due to the consistency of faecal material excreted by cod, it proved impossible to collect samples from within the tank or from the outflow. The same methods attempted when collecting uneaten food (Section 3.3.1) were considered but had no success. Flushing the tanks to collect the material also proved unsuccessful. Consequently, faecal material was stripped from three sacrificed fish every 28 days and pooled for each treatment to provide sufficient quantities for analysis. The collected faeces were stored in labeled pots and frozen (- 20 $^{\circ}$C ± 1 $^{\circ}$C) prior to analysis. The samples were later thawed then weighed and transferred to pre - weighed labelled foil trays and placed in a Gallenkamp hotbox oven at 60 ºC for 48 hours.

### 3.4.3 Apparent digestibility

Yttrium Oxide is an inert marker incorporated into diets to permit the calculation of apparent digestibility. In this study, Yttrium Oxide was incorporated into the juvenile and adult diets at the rate of 1 g / kg. This is an accepted inclusion rate, which has been use in other digestibility studies. Samples of dry weight diet and dry weight faecal material (0.06 g – 0.12 g) were weighed into acid washed beakers and 4 ml of nitric acid was added. Each beaker was then covered with an acid washed watch glass and left to stand at room temperature for two hours. The beakers were then placed on a hot plate and gently boiled (so as not to boil dry) for 1 hour. After cooling, the contents of each were separately emptied into an acid washed volumetric flask and diluted to 25 ml using 2 % nitric acid. The samples were then analysed in a Thermo-Electron X-Series 2 IC-PMS, which had previously been calibrated against standards prepared in 2 % nitric acid. In order to reduce the acid concentration of the samples and protect the Thermo-Electron X-Series 2 IC-PMS, 1 ml of each 25 ml sample was diluted in 9 ml double distilled water prior to analysis. Yttrium Oxide analysis results are shown in Table 3.6 and Table 3.7 (diet samples) and Table 3.8 (faecal samples).

### 3.4.4 Calculation of nitrogen digestibility

Samples of the investigated diets were collected periodically throughout both studies and dry weight samples were analysed for total nitrogen (Perkin - Elmer Series II CHNS/O Analyser 2400). The collected faecal samples were pooled for each treatment, dried and analysed for total nitrogen (Perkin - Elmer Series II CHNS/O Analyser 2400). The difference in total nitrogen content between the diet and respective collected faecal sample provided values for nitrogen digested through the fish gut. The nitrogen digestibility was measured as a % difference between nitrogen in the diet and nitrogen in the faecal material (see Figures 3.7 and 3.8).

### 3.4.5 Statistical analysis

All data sets were tested for normality and homogeneity of variance. Statistical significance of differences between treatments (diets) with respect to growth were analysed using a non-parametric 2-sample t-test (Mann-Whitney), followed by a one - way analysis of variance (ANOVA). The Mann Whitney test was used to analyse statistical differences in each diet over the study periods while the one – way ANOVA testes differences between each treatment. Statistical analysis of differences within and between treatments (diets) on all measured parameters were analysed using a two-way ANOVA implementing Minitab™ version statistical software (Ryan & Joiner, 1994). In all cases, where $P < 0.05$, the statistical difference was considered significant.

## 3.5 Results - diets

### 3.5.1 Proximate composition

Proximate composition of the investigated diets are shown in Table 3.4 for the adult study and in Table 3.5 for the juvenile study. The raw data from which the Tables were compiled are shown in Appendices 1 and 2 respectively.

Table 3.4 Proximate composition of the diets investigated in the adult fish study and compared to EWOS analysis. (% dry weight)

| Diet | Composition | EWOS | 30/6/04 | 2/9/04 | 11/11/04 | 9/12/04 | 2/2/05 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| A | Moisture (%) | 11.50 | 12.00 | 12.11 | 12.58 | 12.64 | 12.60 |
| | Ash (%) | 7.70 | 9.54 ± 0.01 | 8.07 ± 0.02 | 8.23 ± 0.09 | 8.32 ± 0.02 | 8.29 ± 0.04 |
| | Lipid (%) | 16.70 | 15.32 ± 0.02 | 15.42 ± 0.07 | 15.34 ± 0.30 | 15.41 ± 0.05 | 15.35 ± 0.15 |
| | Protein (%) | 41.40 | 46.23 ± 0.21 | 46.83 ± 0.22 | 46.24 ± 0.49 | 46.67 ± 0.21 | 46.28 ± 0.18 |
| | Energy (kJ) | | 21.58 ± 0.06 | 21.49 ± 0.13 | 21.22 ± 0.06 | 21.10 ± 0.09 | 21.23 ± 0.04 |
| | Nitrogen (%) | | 7.52 | 7.62 | 7.38 | 7.66 | 7.70 |
| | | | | | | | |
| B | Moisture (%) | 11.40 | 11.22 | 12.08 | 12.18 | 12.26 | 12.83 |
| | Ash (%) | 9.40 | 9.02 ± 0.03 | 9.02 ± 0.14 | 10.47 ± 0.08 | 10.53 ± 0.07 | 10.16 ± 0.07 |
| | Lipid (%) | 14.10 | 12.59 ± 0.05 | 12.36 ± 0.11 | 12.57 ± 0.07 | 12.05 ± 0.05 | 12.99 ± 0.01 |
| | Protein (%) | 50.20 | 55.58 ± 0.73 | 56.33 ± 0.42 | 56.53 ± 0.05 | 56.74 ± 0.63 | 55.29 ± 0.11 |
| | Energy (kJ) | | 21.58 ± 0.12 | 21.47 ± 0.01 | 21.12 ± 0.01 | 21.01 ± 0.01 | 21.22 ± 0.03 |
| | Nitrogen (%) | | 9.07 | 9.03 | 9.01 | 9.18 | 9.26 ± |
| | | | | | | | |
| C | Moisture (%) | 11.40 | 12.14 | 11.83 | 12.25 | 12.34 | 12.66 |
| | Ash (%) | 10.90 | 8.83 ± 0.00 | 12.25 ± 0.05 | 12.48 ± 0.05 | 12.32 ± 0.01 | 12.01 ± 0.04 |
| | Lipid (%) | 11.90 | 11.39 ± 0.02 | 11.24 ± 0.06 | 11.26 ± 0.15 | 11.38 ± 0.29 | 11.49 ± 0.18 |
| | Protein (%) | 58.60 | 65.39 ± 0.19 | 65.37 ± 0.32 | 66.36 ± 0.36 | 66.20 ± 0.85 | 65.22 ± 1.11 |
| | Energy (kJ) | | 21.17 ± 0.04 | 21.13 ± 0.04 | 20.97 ± 0.09 | 21.14 ± 0.07 | 21.243 ± 0.07 |
| | Nitrogen (%) | | 10.55 | 10.55 | 10.55 | 10.64 | 10.64 |

Table 3.5 Proximate composition of the diets investigated in the juvenile fish study and compared to EWOS analysis. (% dry weight)

| Diet | Composition (%) | EWOS | 2/9/04 | 11/11/04 | 9/12/04 | 2/2/04 |
|---|---|---|---|---|---|---|
| D | Moisture (%) | 10.70 | 12.02 | 11.74 | 11.32 | 11.61 |
| | Ash (%) | 7.60 | 8.18 ± 0.05 | 8.44 ± 0.11 | 8.43 ± 0.05 | 8.33 ± 0.01 |
| | Lipid (%) | 16.20 | 15.27 ± 0.13 | 15.74 ± 0.21 | 14.40 ± 0.19 | 15.19 ± 0.28 |
| | Protein (%) | 41.60 | 46.97 ± 0.27 | 46.96 ± 0.66 | 47.05 ± 0.50 | 46.77 ± 0.47 |
| | Energy (kJ) | | 21.58 ± 0.06 | 21.49 ± 0.13 | 21.22 ± 0.06 | 21.10 ± 0.09 |
| | Nitrogen (%) | | 7.52 | 7.62 | 7.38 | 7.66 |
| | | | | | | |
| E | Moisture (%) | 11.10 | 11.47 | 11.37 | 11.35 | 11.09 |
| | Ash (%) | 9.40 | 10.17 ± 0.03 | 10.30 ± 0.01 | 10.26 ± 0.12 | 10.44 ± 0.01 |
| | Lipid (%) | 13.90 | 12.90 ± 0.03 | 13.29 ± 0.22 | 12.54 ± 0.38 | 12.98 ± 0.10 |
| | Protein (%) | 50.30 | 56.13 ± 0.32 | 56.54 ± 0.76 | 55.51 ± 0.08 | 56.39 ± 0.44 |
| | Energy (kJ) | | 21.27 ± 0.02 | 21.13 ±0.06 | 21.01 ± 0.01 | 21.17 ± 0.07 |
| | Nitrogen (%) | | 8.97 | 9.09 | 9.35 | 9.31 |
| | | | | | | |
| F | Moisture (%) | 10.60 | 10.67 | 11.67 | 11.14 | 11.86 |
| | Ash (%) | 11.10 | 12.47 ± 0.12 | 12.69 ± 0.06 | 12.29 ± 0.06 | 12.53 ± 0.07 |
| | Lipid (%) | 11.30 | 10.66 ± 0.09 | 10.74 ± 0.03 | 10.46 ± 0.11 | 10.73 ± 0.07 |
| | Protein (%) | 59.30 | 65.48 ± 0.12 | 65.90 ± 0.25 | 64.64 ± 0.21 | 65.96 ± 0.28 |
| | Energy (kJ) | | 21.08 ± 0.12 | 21.23 ± 0.08 | 21.13 ± 0.05 | 21.27 ± 0.03 |
| | Nitrogen (%) | | 10.35 | 10.57 | 10.66 | 10.58 |

It can be seen from the Tables 3.4 and 3.5 that proximate composition varies little for the three formulations throughout both investigations. Standard deviation is also small suggesting that there is little variation between the replicates. This shows accuracy as the single samples were sub - divided and analysed. It should be noted that the difference in the protein and lipid values stated by EWOS® to those found throughout the studies are due to differences in analytical methods. The present study analysed dried diet samples compared to the hydrated values stated by EWOS®. The EWOS® analysis methods on wet weight samples provide accurate values, however, all analytical protocol at the Institute of Aquaculture is based on using dried material, also providing accurate results.

### 3.5.2 Ingestion rates

Ingestion rates were measured and recorded in order to calculate accurate rations but also to calculate FCR (Section 2.5.2). The uptake of water as discussed in Section 3.4.1 was considered when calculating accurate ingestion rates for both the adult and juvenile tank – based studies. Ingestion rates are shown on the following page for the adult tank study (Figure 3.3) and the juvenile tank study (Figure 3.4). All raw data is shown in Appendix 4.

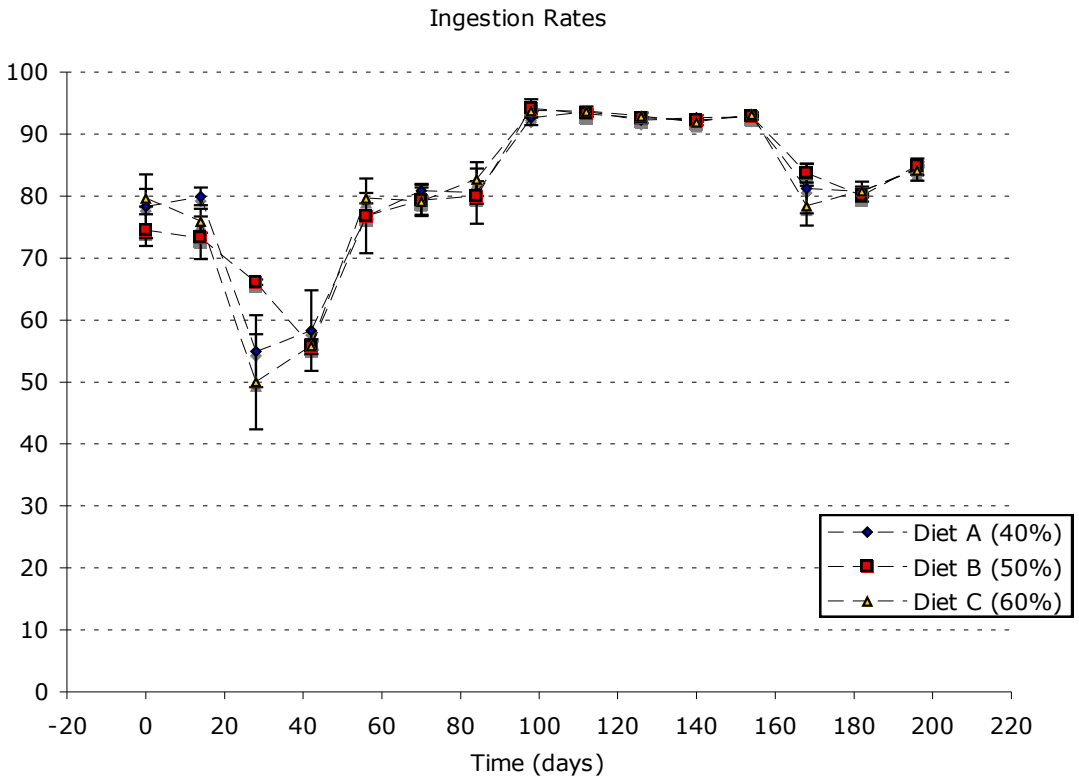



Figure 3.3 Ingestion rates measured throughout adult tank study (± 1 SD).

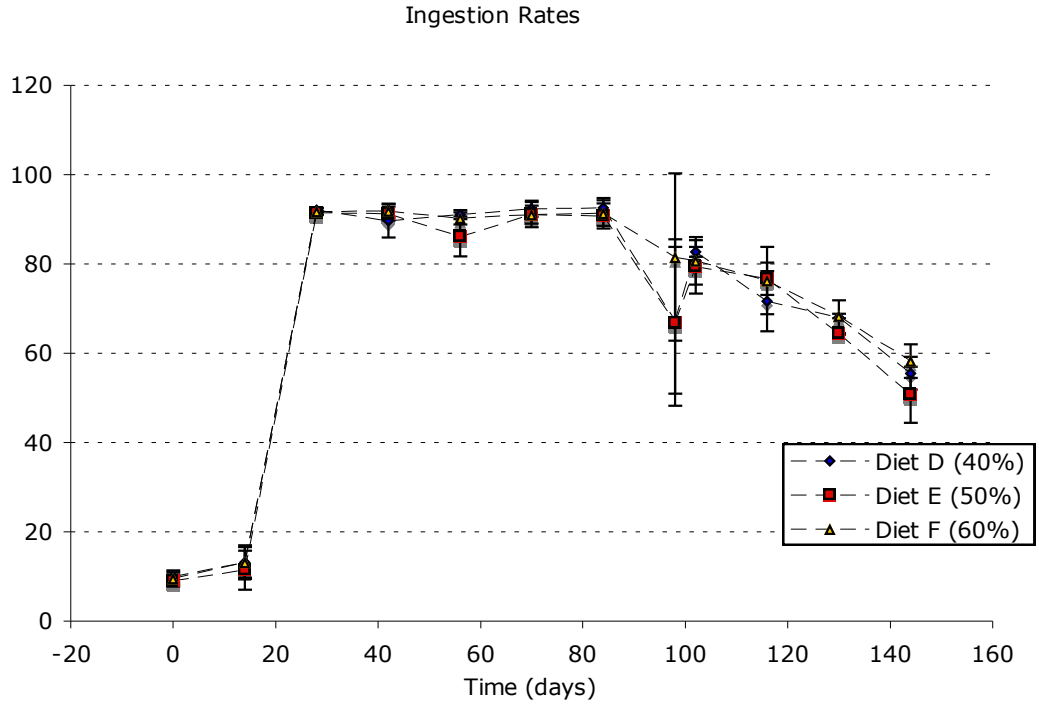



Figure 3.4 Ingestion rates measured throughout juvenile tank study (± 1 SD).

Ingestion rates during the adult study were constant around 93 %. However, a significant dip at the beginning of the study can be explained by an error made when calculating rations. The drop in ingestion rates toward the end of the study can be explained by a decrease in appetite as the fish begin to mature (see Section 3.9.1).

Initial ingestion rates measured during the juvenile study were exceptionally low and could be explained by a sampling error. By placing the fine collecting mesh (2 mm mesh) around the outflow on the sampling day, the disturbed fish ingested little. Once the error was rectified, ingestion rates rose sharply to approximately 90 %. As the fish matured, ingestion rates began to drop. Such a phenomenon was observed in other studies (see Section 3.9.1).

### 3.5.3 Apparent digestibility

In order to measure apparent digestibility, dry weight diet and faecal samples collected throughout both tank studies were analysed for Yttirum (Section 3.4.3). However, even after pooling the replicate faecal samples collected during the juvenile tank study, there was insufficient material to conduct Yttirum analysis.

Yttirum concentration results obtained for the diets investigated in the juvenile and adult studies are shown in Table 3.6 and Table 3.7 respectively and the concentration of Yttirum in the dry weight adult faecal material collected is shown in Table 3.8. The associated raw data is shown in Appendix 5.

Table 3.6 Yttirum content of the diets investigated in the juvenile tank study (± 1 S.D.)

| Juvenile | 2/9/04 | 11/11/04 | 9/12/04 | 2/2/05 |
|---|---|---|---|---|
| Diet | µg/mg | µg/mg | µg/mg | µg/mg |
| | | | | |
| 4757 (40%) | 0.0024 ± 0.0020 | 0.0005 ± 0.0000 | 0.0017 ± 0.0025 | 0.0018 ± 0.0023 |
| 4758 (50%) | 0.0041 ± 0.0033 | 0.0015 ± 0.0018 | 0.0005 ± 0.0019 | 0.0006 ± 0.0004 |
| 4759 (60%) | 0.0016 ± 0.0011 | 0.0015 ± 0.0022 | 0.0014 ± 0.0011 | 0.0011 ± 0.0013 |

Table 3.7 Yttirum content of the diets investigated in the adult tank study (± 1 S.D.)

| Adult | 2/9/04 | 11/11/04 | 9/12/04 | 2/2/05 |
|---|---|---|---|---|
| Diet | µg/mg | µg/mg | µg/mg | µg/mg |
| | | | | |
| 4760 (40%) | 0.0037 ± 0.0015 | 0.0009 ± 0.0008 | 0.0031 ± 0.0037 | 0.2106 ± 0.1359 |
| 4761 (50%) | 0.0016 ± 0.0008 | 0.0006 ± 0.0003 | 0.0002 ± 0.0009 | 0.0521 ± 0.0299 |
| 4762 (60%) | 0.0054 ± 0.0018 | 0.0046 ± 0.0031 | 0.0022 ± 0.0013 | 0.1862 ± 0.0000 |

Table 3.8 Yttirum content of the faecal samples collected throughout the adult tank study (± 1 S.D.)

| Faecal | | | |
|---|---|---|---|
| Diet | 4760 (40%) | 4761 (50%) | 4762 (60%) |
| | | | |
| August | 0.0012 ± 0.0002 | 0.0026 ± 0.0021 | 0.0083 ± 0.0008 |
| Septmber | 0.0006 ± 0.0008 | 0.0010 ± 0.0001 | 0.0238 ± 0.0010 |
| October | 0.0027 ± 0.0002 | 0.0011 ± 0.0002 | 0.0305 ± 0.0018 |
| November | 0.0025 ± 0.0029 | 0.0024 ± 0.0017 | 0.0179 ± 0.0019 |
| December | 0.0008 ± 0.0001 | 0.0013 ± 0.0001 | 0.0428 ± 0.0041 |
| January | 0.0011 ± 0.0001 | 0.0020 ± 0.0005 | 0.0173 ± 0.0149 |
| February | 0.0010 ± 0.0002 | 0.0211 ± 0.0171 | 0.0221 ± 0.0252 |

From Tables 3.5, 3.6 and 3.7, and the charts in Appendix 5, the results show great inconsistency and variation with high standard deviations highlighting the wide range of results found. This is consistent in both adult and juvenile diet samples questioning the accuracy of the analysis and the value it brings to the current study. The Yttrium Oxide results for both analysed diets were far lower than the inclusion rate stated by EWOS® (1 g / kg).

### 3.5.4 Nitrogen digestibility

Diet and faecal samples collected throughout both tank studies were analysed for nitrogen (Section 3.3.3). Nitrogen digestion was calculated as the difference between nitrogen in the diet samples (dry weight) and nitrogen in the stripped faecal material (dry weight). Results obtained for the adult tank study are shown in Figure 3.5, while the results obtained for the juvenile study are shown in and Figure 3.6. The associated raw data is shown in Appendix 5.

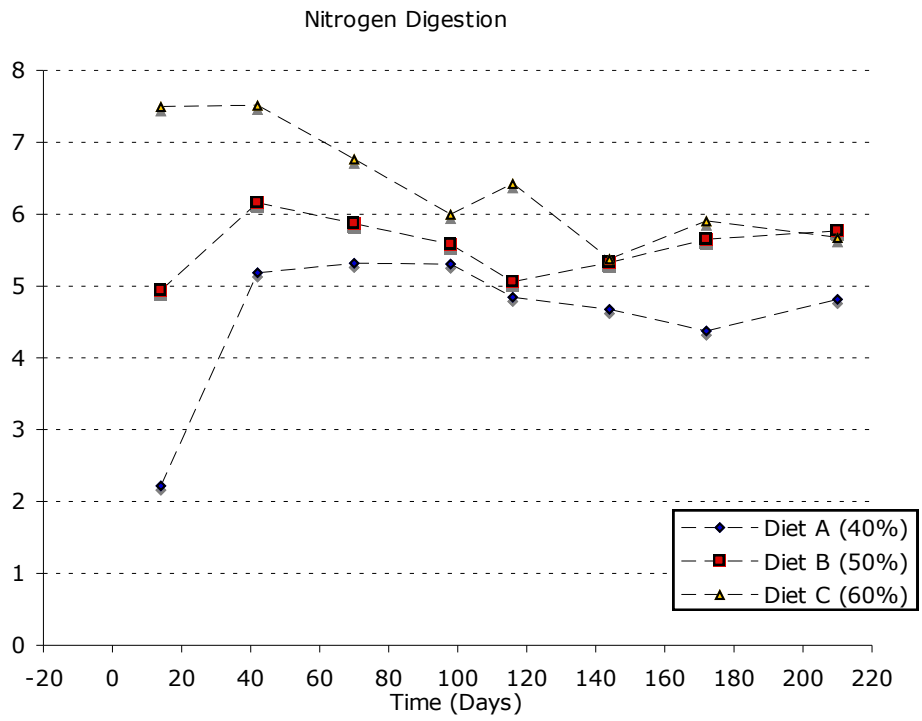



Figure 3.5 Nitrogen digestion observed throughout the adult tank study (7mm pellet).

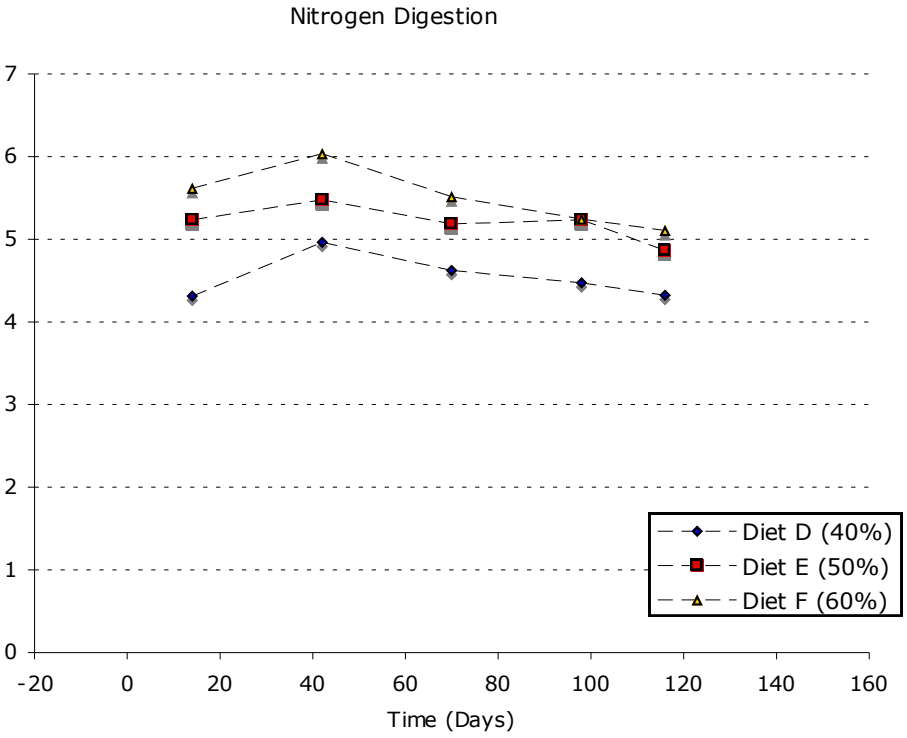



Figure 3.6 Nitrogen digestion observed throughout juvenile study (4mm pellet).

Nitrogen digestibility could only be measured in the tank studies and was initially calculated as a difference in the percentage of the nitrogen content between the dried diet sample and the corresponding dried stripped faecal sample. In both tank studies, nitrogen digestion was directly related to protein (and associated nitrogen) content of the diets and in the juvenile study (Table 3.6) the mean value was measured as follows: 4.54 % (40 % protein), 5.20 % (50 % protein) and 5.50 % (60 % protein), and a expressed as a percentage of the nitrogen content of the diet as 59.19 %, 56.90 % and 52.23 % respectively. After an initial increase under each treatment (day 40 of the study), nitrogen digestion fell in all cases toward the end of the study.

In the case of the adult study (with the exception of sampling on day 14) nitrogen digestion (calculated as a difference in the percentage of the nitrogen content between the diet sample (dry weight) and the corresponding stripped faecal sample (dry weight)) remained constant over the duration of the study. A mean value of 4.59 % (40 % protein), 5.50 % (50 % protein) and 6.40 % (60 % protein), and expressed as a percentage of the nitrogen content of the diet as 60.55 %, 60.92 % and 60.60 % respectively.

## 3.6 Results - growth

An increase in weight (g) observed throughout the adult study is shown in Figure 3.7 and throughout the juvenile study is shown in Figure 3.8

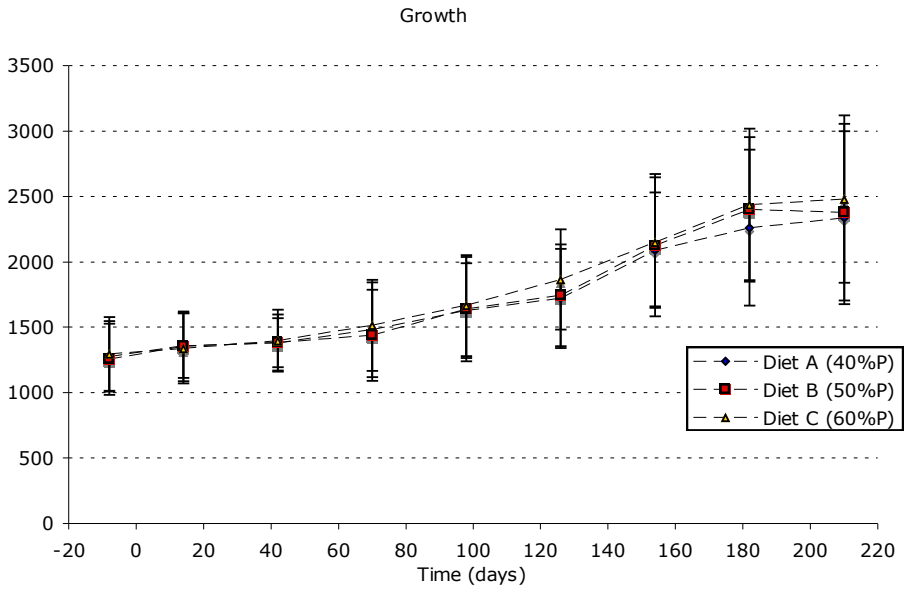



Figure 3.7 Mean weight of adult fish in each treatment (error bars are ± 1 SD).

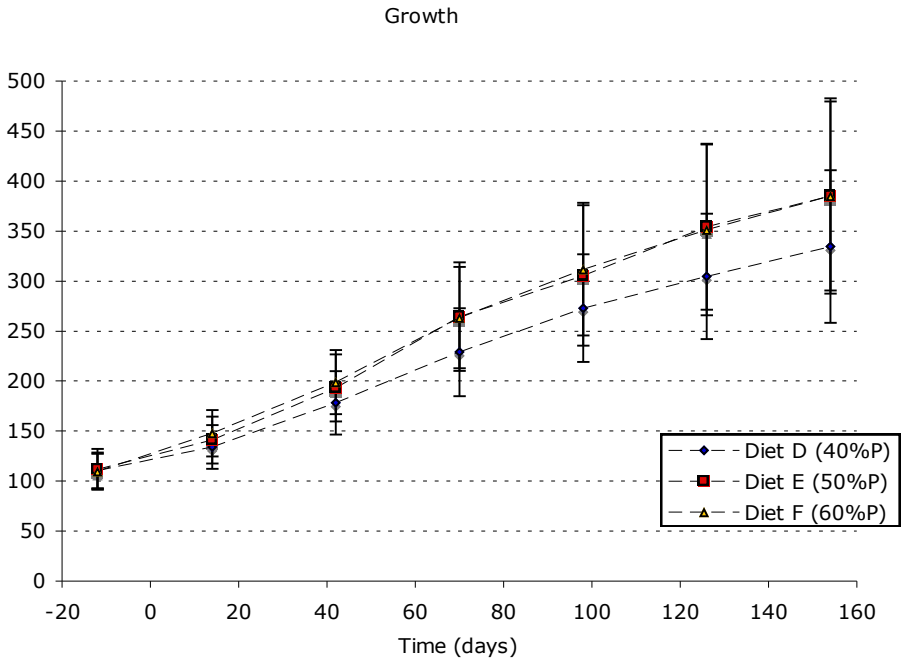



Figure 3.8 Mean weight of juvenile cod in each treatment (error bars are ± 1 SD).

Growth in terms of weight gain is shown in Figure 3.7 for the adult study and 3.8 for the juvenile study. Lengths were also taken from the thirty cod randomly sampled per tank every four weeks and all the associated raw data is shown in Appendix 6 (by taking lengths, the fluctuations in K-Factor of the cod could be measured). Although relative weight gain between treatments within each study, the individual growth trends are mirrored under each treatment in both studies; in the adult study, there is no significant difference in growth (weight) promoted between Diet A, diet B and Diet C. However, in the juvenile trial, Diets E and F promote greater growth (weight) than that promoted by Diet D.

It can be seen that at the beginning of the acclimation period (30$^{th}$ June, 2004) prior to the adult study commencing (8$^{th}$ July, 2004), the cod had an initial mean weight of approximately 1250 g. The final weights show no significant difference in growth promoted by the 40 %, 50 % or 60 % protein diets suggesting that adult cod can be successfully fed on a 40 %P diet, reducing feed costs and reducing nitrogen impact upon the environment. All juvenile fish were approximately 110 g at the beginning of the acclimation period (2$^{nd}$ September, 2004) prior to the study commencing (14$^{th}$ September, 2004). Figure 3.8 shows that there is no significant difference in growth promoted by Diet E (50 % protein) and Diet F (60 % protein), with a mean final weight of approximately 380 g ± 95 g ($n = 18$, $F = 2.08$, $P > 0.05$). Growth promoted by Diet D (40 % protein) is significantly lower with a mean final weight of 340 g ± 76 g, ($n = 27$, $F = 9.42$, $P < 0.05$) suggesting that a higher than 40 % protein level may be required to promote optimal growth in juvenile cod. However, although higher protein levels are required for juvenile cod, the use of a 50 % protein diet as opposed to a 60% protein diet would reduce feed costs and nitrogen impact upon the environment in the juvenile stages of cod culture.

### 3.6.1 FCR

The FCR (see Equation 1) measured throughout the adult study is shown in Figure 3.9 and for the juvenile study (Figure 3.10). All raw data for both studies is shown in Appendix 7.

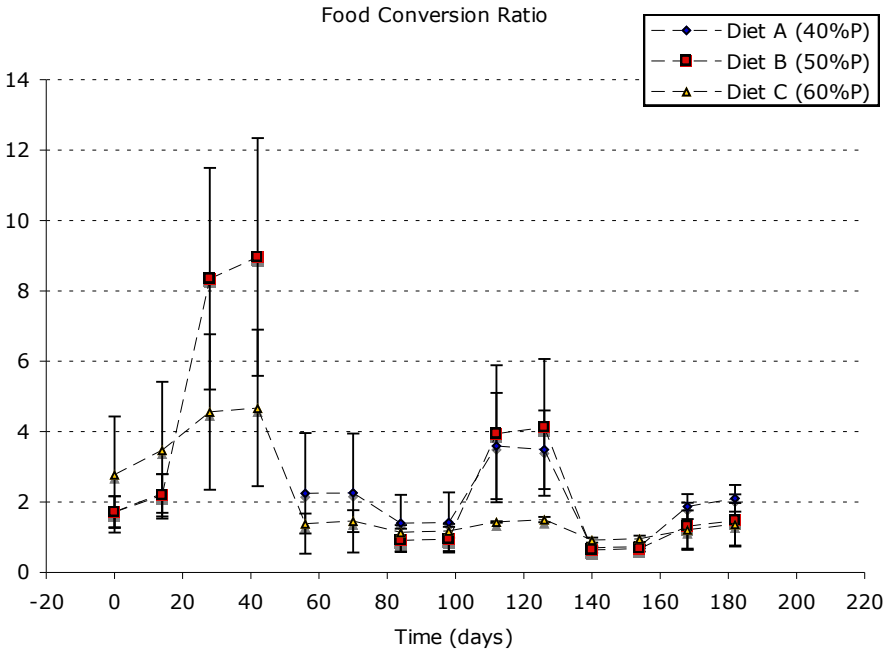



Figure 3.9 FCR observed throughout the adult study (error bars are ± 1 SD).

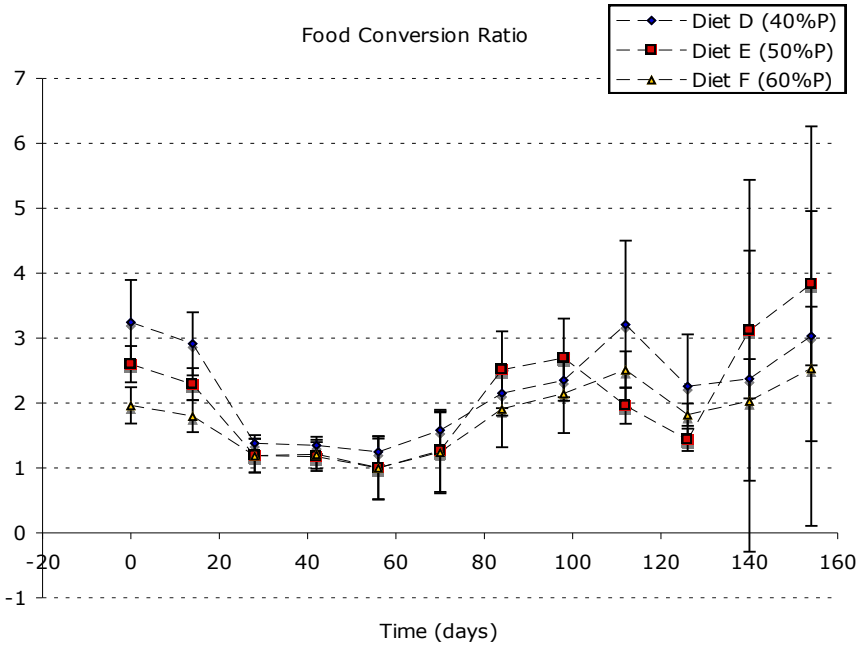



Figure 3.10 FCR observed throughout the juvenile study (error bars are ± 1 SD).

It was seen that the FCR calculated throughout that adult investigation (Figure 3.9) fluctuates greatly over the course of the study although the majority of FCR's observed were between 1 and 2. The fluctuations may have occurred due to errors made through managerial concerns when calculating rations in the early stages of the study and also due to adverse weather causing rapid fluctuations in water temperature and visibility. This may have inflicted stress upon the fish, reducing appetite, as poor visibility would inhibit foraging success. Factors such as poor condition during the early part of the trial and sexual maturation in the latter stages can also account for an elevated FCR. Statistical analysis shows that differences in FCR within each treatment over the course of the study are not significant ($n = 16$, $F = 0.50$, $P = 0.075$).

The FCR calculated for the juvenile study (Figure 3.10) were generally lower than those observed throughout the adult study, but also fluctuated throughout the juvenile study. Initial FCR was high but this could be linked to sampling error when installing the mesh grids to catch uneaten food to calculate ingestion rates. This would have disturbed the fish during the early stages of the investigation reducing appetite. Once this error was amended, FCR drops to between 1 and 2. Statistical analysis showed that the difference in FCR recorded within each treatment over the course of the study period was significant ($n = 12$, $F = 9.37$, $P < 0.05$).

### 3.6.2 SGR

SGR is a measure of growth as a percentage of total fish weight. SGR is higher in juvenile fish than adult fish. Such a phenomenon would be expected as younger fish have higher growth rates than older fish. SGR were calculated (see Equation 2) throughout both the adult study (Figure 3.11) and the juvenile study (Figure 3.12). The associated raw data is shown in Appendix 8.

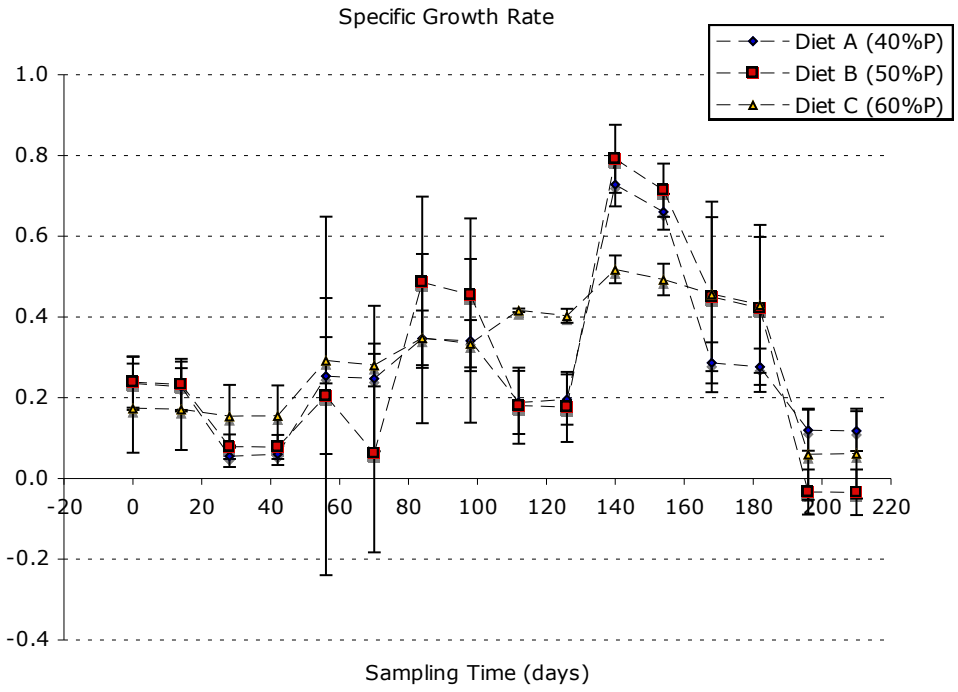



Figure 3.11 SGR recorded throughout the adult study (error bars are ± 1 SD).

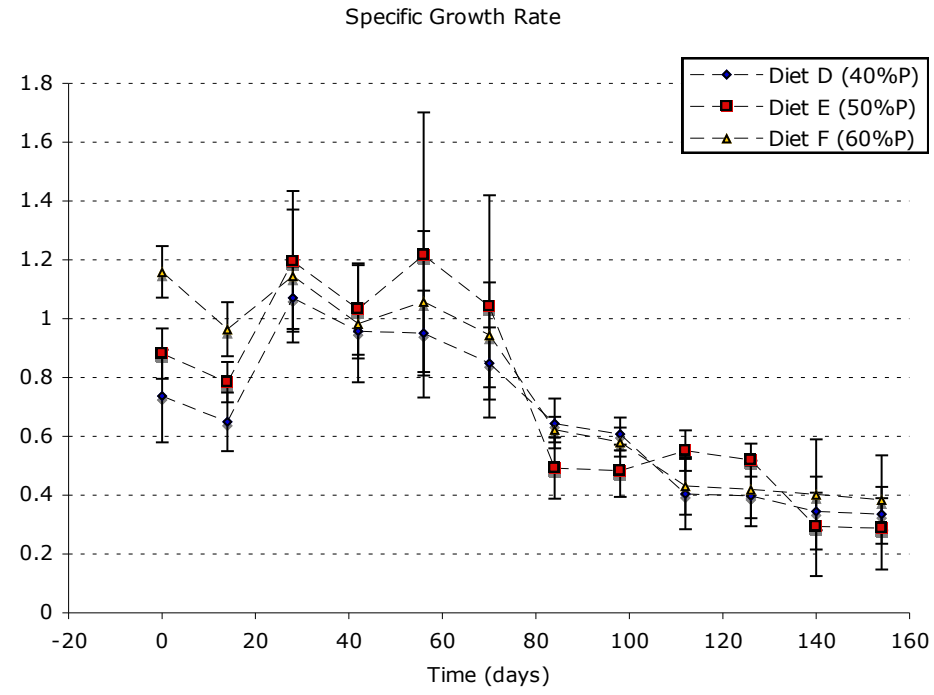



Figure 3.12 SGR measured throughout the juvenile study (error bars are ± 1 SD).

At the beginning of the investigation, SGR observed in the adult investigation (Figure 3.11) were low (around 0.2 % bw/day) as energy is mainly directed toward reconditioning following spawning. As the fish regained condition around day 100 (mid October 2004), SGR increased for each treatment to a peak (between 0.5 % bw/day and 0.8 % bw/day) around day 150 (early December 2004). However, as the fish began to mature, energy was directed from growth to the production of gametes and the SGR fell toward the end of the study.

It can be seen in Figure 3.12 that the initial SGR for juvenile cod was high around 1 % bw/day. By day 80 (early December), SGR dropped to approximately 0.4 % bw/day as the cod begin to mature and more energy is directed toward the production of gametes – precocious maturation was observed in both male and female juvenile fish that were sacrificed toward the end of the study. Statistical analysis showed that for the adult study, differences in SGR were significant for each diet over the course of the study ($n = 16$, $F = 12.03$, $P < 0.05$) and also between treatments ($n = 3$, $F = 4.44$, $P < 0.05$). Similar statistical results are shown for the juvenile study ($n = 12$, $F = 28.11$, $P < 0.05$) for each diet over the study, and ($n = 3$, $F = 38.29$, $P < 0.05$) between diets.

## 3.7 Results - condition

### 3.7.1 K - Factor

The K - Factor was calculated using an equation commonly used for Atlantic salmon and represents the relationship between length and weight of the fish. The K - Factor was calculated throughout both the adult study (Figure 3.13) and the juvenile study (Figure 3.14). All raw data is shown in Appendix 9. At the beginning of the investigations, five random fish from each tank were caught, panjet marked and PIT tagged in order to monitor changes in condition of these individual fish throughout both studies. However, as the tagged fish were rarely caught when sampling, the K-Factor was calculated using the lengths and weights recorded from the thirty random fish sampled from each tank every twenty - eight days.

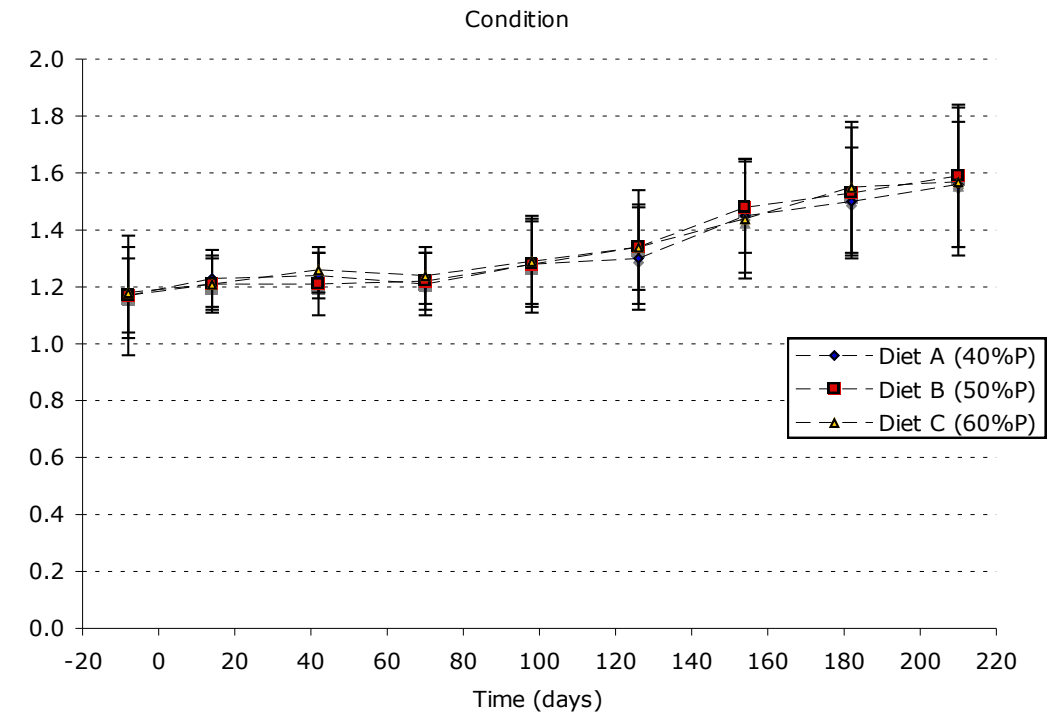



Figure 3.13 K-Factor observed in adult study (error bars are ± 1 SD).

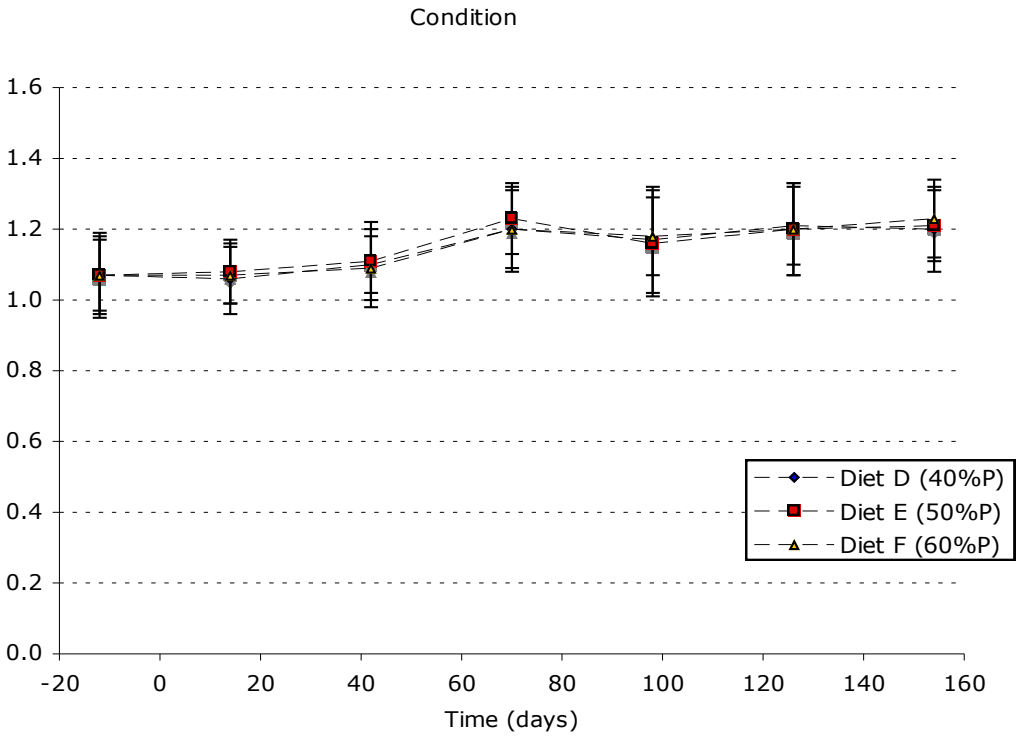



Figure 3.14 K-Factor observed in juvenile study (error bars are ± 1 SD).

From Figure 3.13, it is evident that the condition of the adult fish improved over the course of the study. However, as the fish completed spawning immediately prior to the commencement of the study, an increase in condition was not observed until approximately day 90 of the 210 - day investigation. Over the study period the K - Factor increased from approximately 1.17 ± 0.17 for all diets investigated, to give final values of 1.56 ± 0.22 (40 % protein), 1.59 ± 0.25 (50 % protein) and 1.57 ± 0.26 (60 % protein). There was no significant difference in the K - Factor promoted by each of the three adult diet formulations ($n = 12$, $F = 3.41$, $P > 0.05$). It was seen that similar growth and K - Factor are promoted by the 40 %, 50 % and 60 % protein diets which has important implications on the formulation of commercial adult cod diets. The K - Factor measured throughout the juvenile study (Figure 3.14) increased over the full range of the 154 day study from a mean value of approximately 1.07 ± 0.11 for all diets investigated, to give final values of 1.20 ± 0.12 (40 % protein), 1.21 ± 0.10 (50 % protein) and 1.23 ± 0.11 (60 % protein). There is no significant difference in the condition promoted by each of the three juvenile diet formulations ($n = 18$, $F = 2.96$, $P > 0.05$).

### 3.7.2 HSI

The HSI is a ratio of the wet weight of the liver as a percentage of the total wet weight of the fish and gives an indication of the condition of the fish. The HSI was monitored throughout both the adult study (Figure 3.15) and the juvenile study (Figure 3.16). The raw data for both studies is shown in Appendix 10.

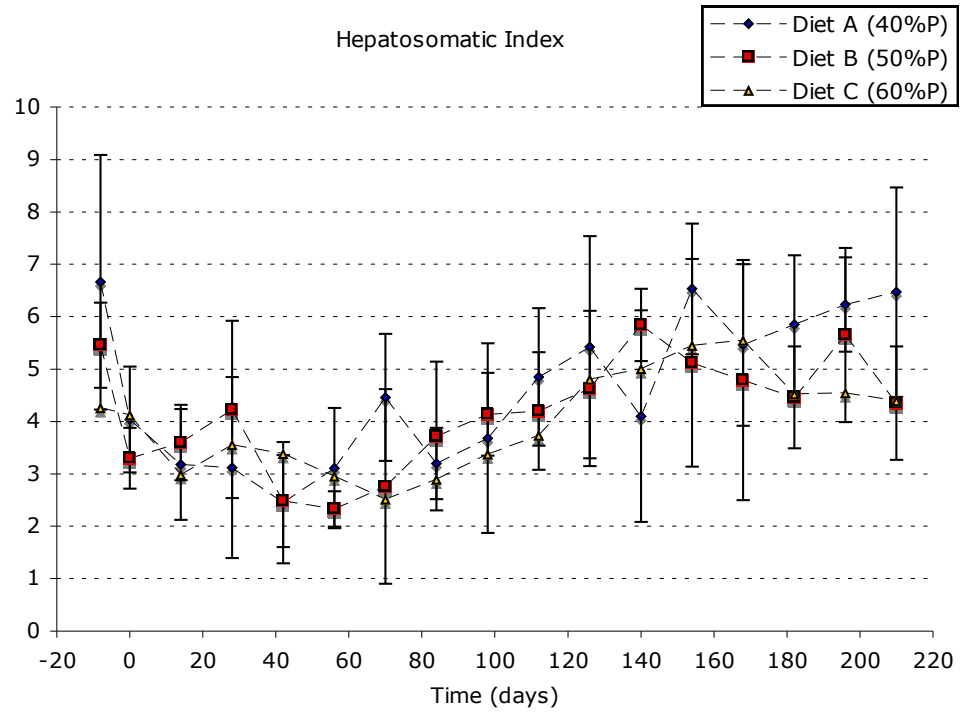



Figure 3.15 HSI observed throughout the adult investigation (error bars are ± 1 SD).

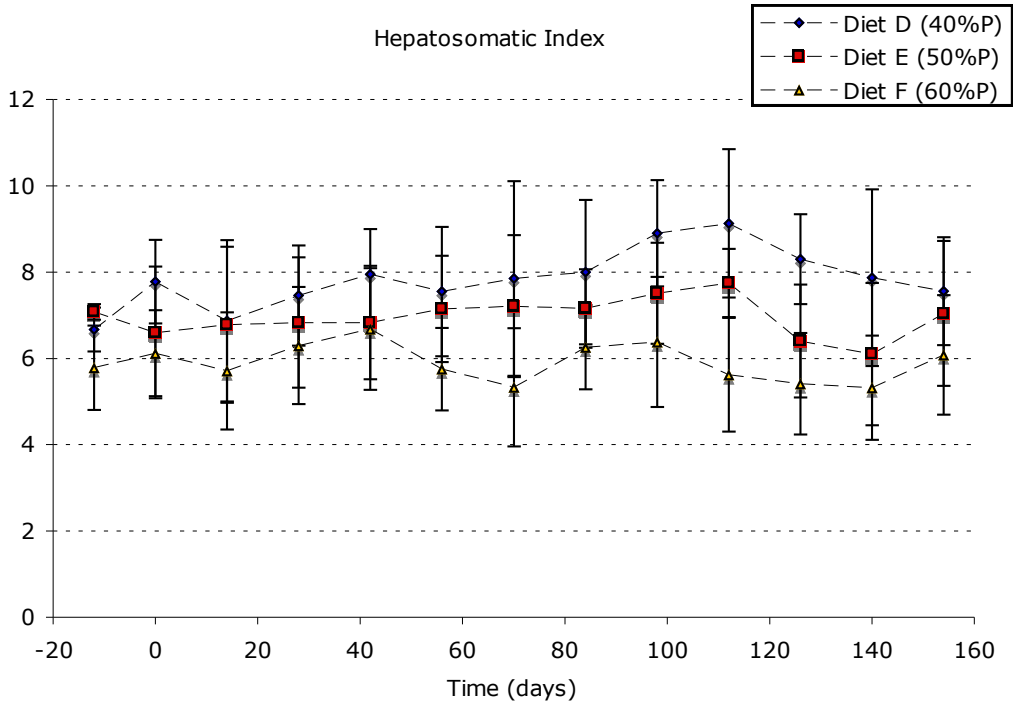



Figure 3.16 HSI observed during the juvenile investigation (error bars are ± 1 SD).

With the adult cod in poor condition following spawning immediately prior to the investigation, initial HSI was low (Figure 3.15). As the fish regained condition, the HSI began to increase as excess lipid was deposited in the liver. When using similar sized fish of a similar condition, the higher the lipid content of the diet, the greater the degree of lipid deposition in the liver, resulting in a greater HSI. Such a trend could be observed in Figure 3.115 where the final HSI promoted by Diet A (14 % lipid) was greater than those promoted by Diet B (12 % lipid) and Diet C (10 % lipid). Statistical analysis showed that differences within each diet throughout the study and between diets throughout the study were significant ($n = 90$, $F = 6.08$, $P < 0.05$) for the whole study and between treatments ($n = 90$, $F = 14.13$, $P < 0.05$).

The relationship between lipid in the diet and HSI was more apparent in the juvenile study and a more distinct difference between the diets was observed. The defining trend observed in Figure 3.16 showed that Diet D (14 % lipid) promoted the greatest HSI (mean of 7.8 %) compared to 7 % promoted by Diet E (12 % lipid) and 6 % by Diet F (10 % lipid) over the 5-month period. It was seen that the HSI promoted by each diet remained fairly constant throughout the investigation. This is confirmed by the statistical analysis that over the whole study, differences within the diets were not significant ($n = 90$, $F = 1.25$, $P = 0.262$). However, between treatments, statistical analysis shows that there is a significant difference ($n = 90$, $F = 3.06$, $P < 0.05$).

## 3.8 Results - proximate composition of tissue

Proximate composition of tissue (muscle and liver) was analysed throughout the adult study while whole cleaned carcass and liver was analysed throughout the juvenile study (Section 2.6). Given the title of the thesis, proximate composition for protein and nitrogen are discussed and all adult muscle and juvenile carcass, proximate composition data (moisture, ash, lipid, protein and nitrogen) are shown in Appendices 11 (adult cod) and 12 (juvenile cod).

### 3.8.1 Protein in muscle

Protein content of dried adult muscle and whole cleaned juvenile carcass collected from the sacrificed fish was measured to monitor the degree of dietary protein retained in the tissues. Protein is composed of approximately 14 % ± 1 % nitrogen so the proportion of protein will be indicative of the levels of nitrogen retained in the adult muscle and juvenile carcass samples. Protein content of adult muscle is shown in Figure 3.17 and of whole cleaned juvenile carcass is shown in Figure 3.18.

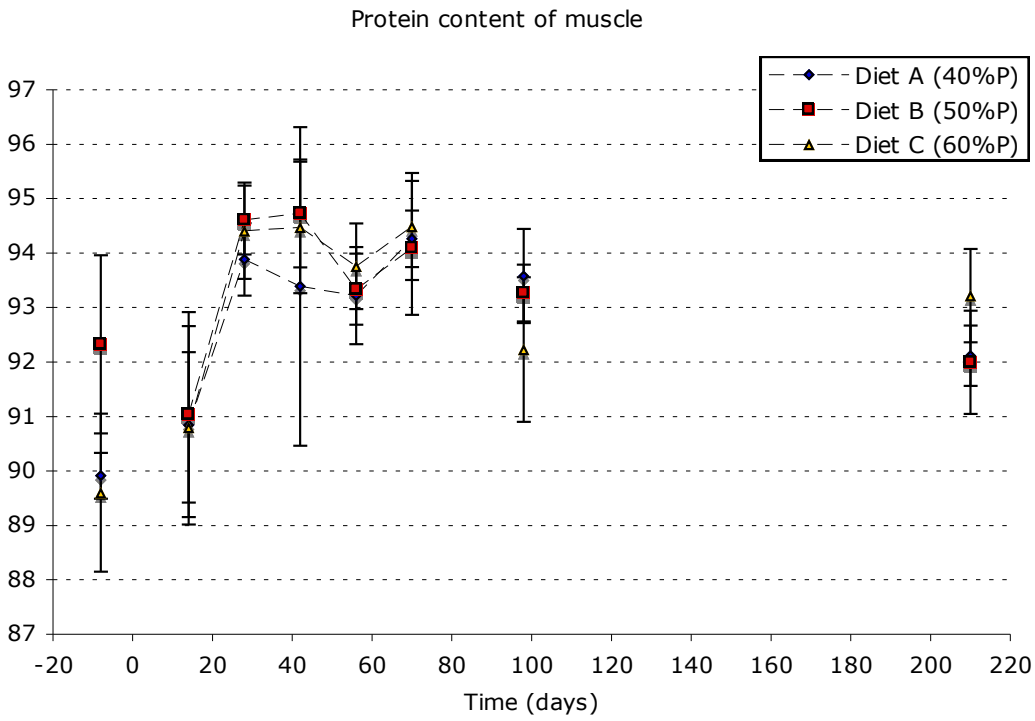



Figure 3.17 Protein content of adult muscle (dry weight) (error bars are ± 1 SD).

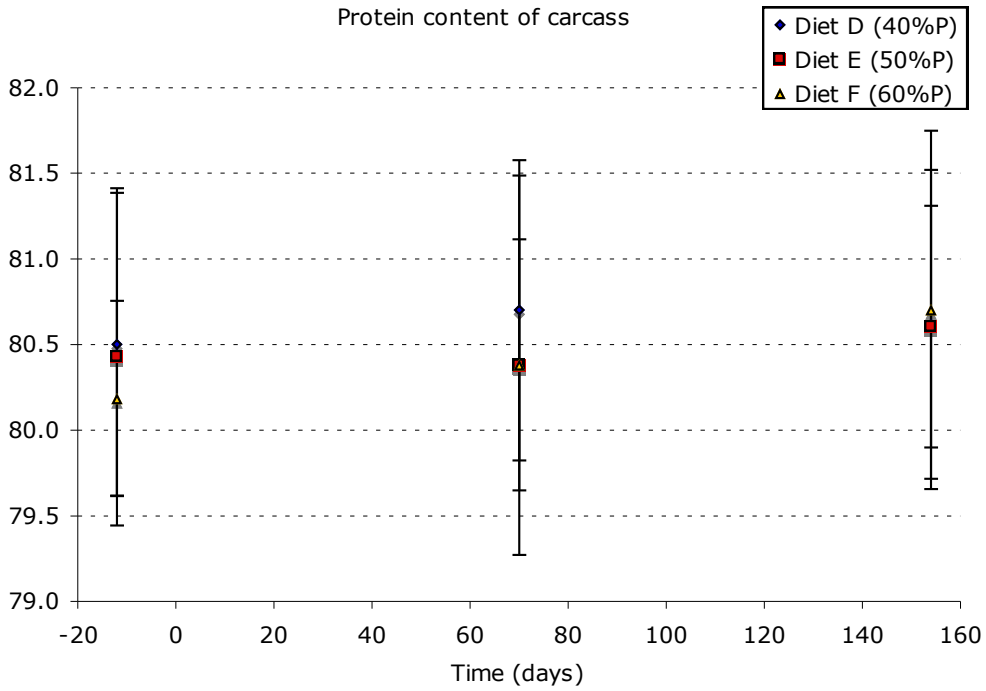



Figure 3.18 Protein content of juvenile carcass (dry weight) (error bars are ± 1 SD).

Figure 3.17 shows that protein levels observed in the adult study fluctuate under each treatment during the study with the majority of results falling between 92 % and 95 % dry weight. That said, statistical analysis showed that variation within each diet over the course of the study is significant ($n = 7$, $F = 4.25$, $P < 0.05$) and also between treatments was significant ($n = 3$, $F = 20.09$, $P < 0.05$).

Protein content of the juvenile carcass remained constant throughout the investigation (Figure 3.18). The protein levels were lower (between 80 % and 80.7% dry weight) than those observed in Figure 3.19. This was most probably due to the greater proportion of ash in the juvenile carcass (approximately 15 % dry weight) compared to approximately 7 % dry weight in the adult muscle. The elevated ash levels observed in the juvenile carcass was probably due to the whole skeleton being included in the analysed sample. Protein content of the juvenile carcass

showed no significant difference either within each diet over the whole study (n = 9, F = 0.19, P = 0.825), or between treatments (n = 3, F = 0.47, P = 0.598).

### 3.8.2 Nitrogen in muscle

Nitrogen content of muscle (dry weight) collected from the sacrificed fish was measured to monitor the degree of dietary nitrogen retained in the fish. Nitrogen content of adult muscle is shown in Figure 3.19 and for the whole cleaned juvenile fish refer to Figure 3.20. All associated raw data is shown in Appendix 11 and Appendix 12 respectively.

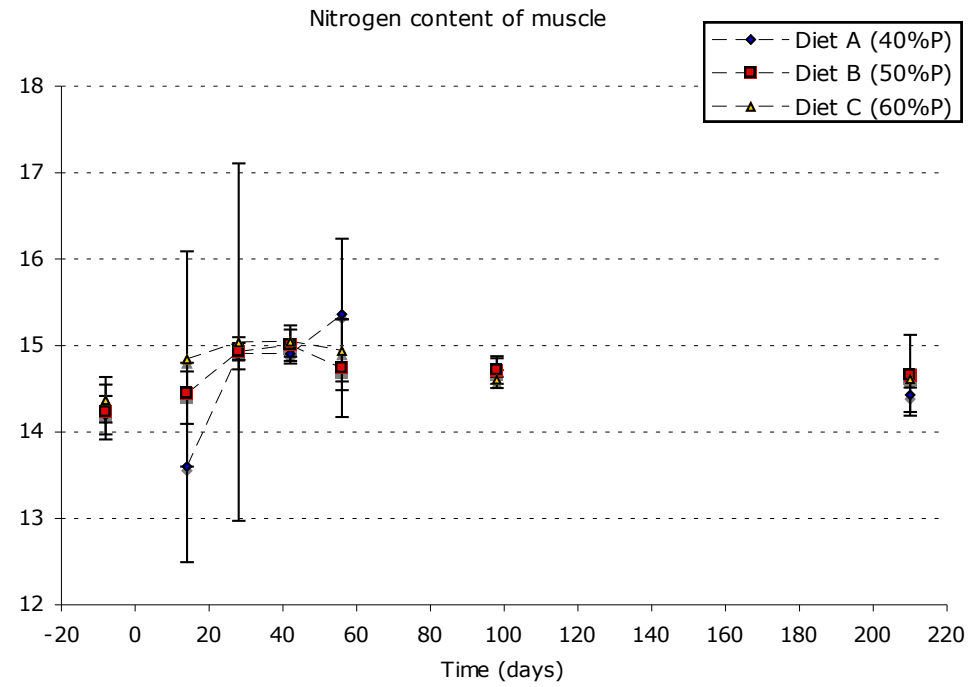



Figure 3.19 Nitrogen content of adult muscle (dry weight) (error bars are ± 1 SD).

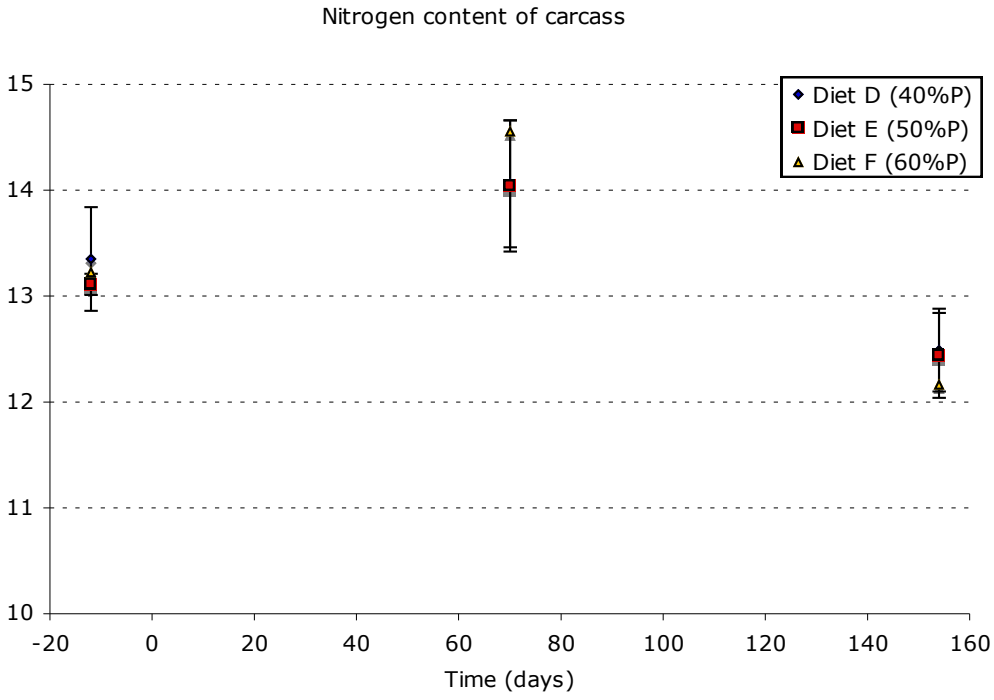



Figure 3.20 Nitrogen content of juvenile carcass (dry weight) (error bars are ± 1 SD).

It could be seen that nitrogen levels in adult muscle (dry weight) (Figure 3.19) remained constant throughout the study, remaining stable between 14.5% and 15%. Figure 3.17 (protein) and Figure 3.19 (nitrogen) confirmed the stable levels of protein and consequently nitrogen in dried adult muscle. Statistical analysis of nitrogen in the adult flesh showed that differences within diets were significant over the whole study ($n = 7$, $F = 6.12$, $P < 0.05$) but differences between treatments were not significant ($n = 3$, $F = 2.84$, $P = 0.063$).

As the whole juvenile carcass was analysed, (Figure 3.20) nitrogen levels are lower (results falling between 12.5 % and 14.5 % of dried whole cleaned carcass) than nitrogen (Figure 3.19) recorded for adult cod. Again, this was most probably due to the whole skeleton being analysed resulting in increased ash levels in the juvenile samples reducing relative protein content as a percentage. Nitrogen levels observed in the juvenile flesh did not show significant differences within each diet over the whole study ($n = 9$, $F = 2.68$, $P = 0.075$). However, differences between the diets were significant ($n= 3$, $F = 146.82$, $P < 0.05$).

### 3.8.3 Protein in liver

The protein content of adult and juvenile liver samples (dry weight) collected from the sacrificed fish were measured to monitor the degree of dietary protein retained in the liver. The small juvenile livers were pooled for each treatment to give a sufficient sample size. However, in both cases, nitrogen content was not analysed due to the consistency of the material and even when pooled, the quantity of liver available for analysis was inadequate. Protein content of adult liver is shown in Figure 3.21 and that of juvenile liver in Figure 3.22. The raw data for all proximate composition of adult and juvenile livers are shown in Appendices 13 and 14 respectively.

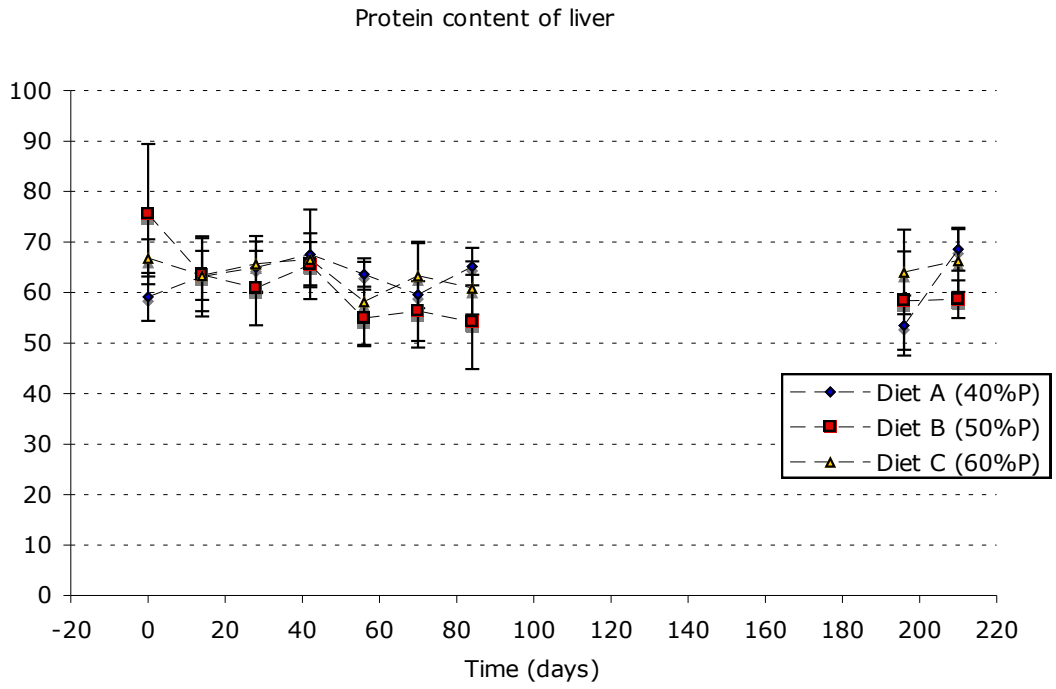



Figure 3.21 Protein content of adult liver (dry weight) (error bars are ± 1 SD).

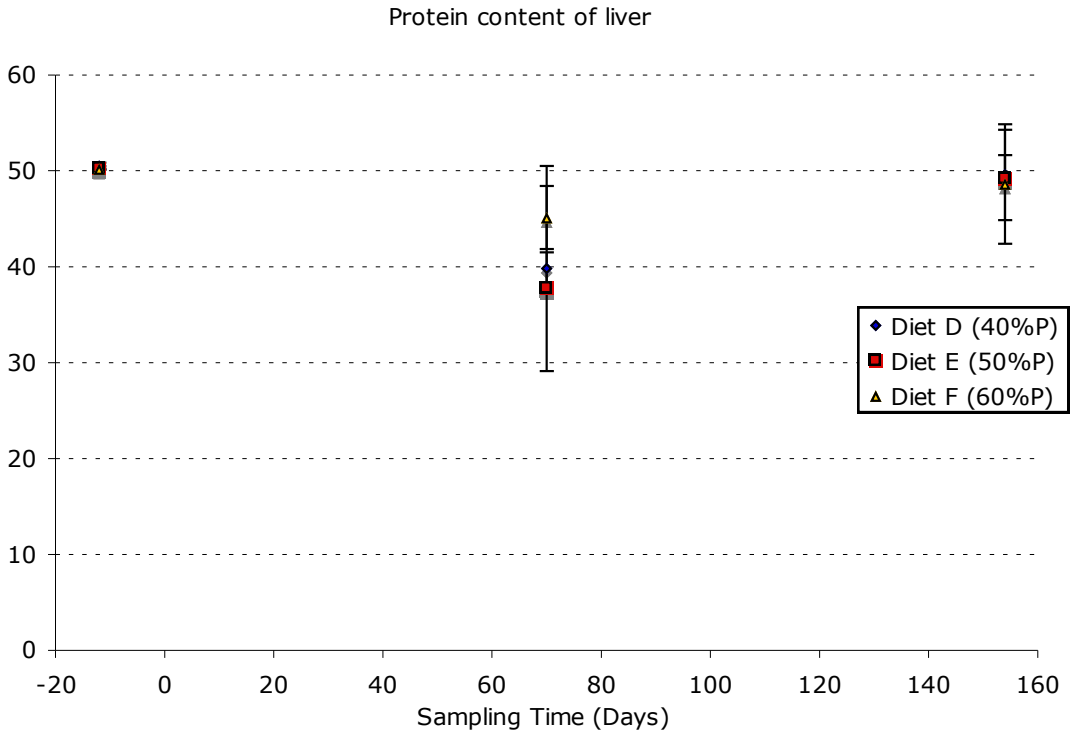



Figure 3.22 Protein content of juvenile liver (dry weight) (error bars are ± 1 SD).

Protein levels in the adult liver were variable over the course of the study with the majority of values falling between 60 % and 70 % of the dry weight samples. A slight fall in protein level occurred over the first 90 days of the study. This coincided with low lipid levels in the liver over the same period during which, the adult cod were re - conditioning following spawning. The highest lipid levels were observed between day 200 and day 220 (Appendix 13). Lipid content of the liver was primarily proportional to moisture content but to a lesser degree, a proportional relationship between lipid and protein content of the liver may be present. Analysis of the nitrogen content of the liver was not possible due to the consistency of the material and small sample size. However, given that protein is approximately 14 % ± 1 % nitrogen, nitrogen content of the liver can be calculated as between 8.4 % and 9.8 % of the dried sample. Such values were observed throughout the study, regardless of diet.

Protein content observed in the liver (dry weight) of the juvenile fish was much lower than that observed in the adult study. The majority of values were found to lie between 40 % and 50 %. However, the variability between samples was great and could be attributed to the consistency of the material and the small sample size permitting little or no replication. Again, analysis of the nitrogen content of the liver was not possible but was calculated as between 5.6 % and 7 % of the dried sample.

## 3.9 Discussion

### 3.9.1 Diets

It can be seen from the Tables 3.3 and 3.4 that proximate composition varied little for the three formulations throughout both investigations. Standard deviation is also small suggesting that there is little variation between the replicates. This shows accuracy as the single samples were sub-divided and analysed. It should be noted that the difference in the protein and lipid values stated by EWOS® to those found throughout the studies are due to differences in analytical methods. The present study analysed dry weight diet samples compared to the wet weight values stated by EWOS®. The EWOS® analysis methods provide accurate values as all the components are measured as '% wet weight'. The dry weight values obtained through analysis at the Institute of Aquaculture were also accurate as each component was recalculated using the moisture content and working back to 'wet weight' values. However, as a couple of calculations were undertaken in this method, it could be argued that the wet weight values calculated at the IoA are less accurate than the real values calculated by EWOS®.

Ingestion rates measured throughout the majority of the adult study were constant around 93 %. However, low ingestion rates at the beginning of the study could be explained by an error attributed to managerial concerns when calculating rations. Once this error was corrected, ingestion rates rose to approximately 93 %. Initial ingestion rates measured during the juvenile study were exceptionally low and could be explained by human disturbance of the investigated fish. The placement of the fine collecting mesh around the outflow on each sampling day disturbed the fish, greatly reducing appetite, as discussed by Kestemont & Baras (2002). Once the error was rectified (the mesh placed in each tank the night before sampling), ingestion rates rose sharply to approximately 90 %.

The drop in ingestion rates toward the end of the adult and juvenile tank studies could be explained by a decrease in appetite as the fish begin to mature and produce gametes (Ramnarine

*et al*, 1987). A reduction in feeding during the winter months is discussed in the same paper although whether this reduction in appetite is due to the winter-feeding or maturation remains unclear. Bjornsson & Steinarsson (2002) state that the optimal temperature for feeding decreases as the fish grow, suggesting the reduction in ingestion rates is primarily due to maturation in the adult study, but both factors may have a greater influence in the juvenile fish.

Extruded pellets are stable for longer periods of immersion. It became apparent in the tank studies that cod readily accepted food while it was suspended in the water column. It was observed that once food had settled on the tank bottom, it was hesitantly accepted only when appetite was greatest (morning feeding). Such behaviour may only have an impact on feeding in shallow tanks. Although the extruded pellets took a longer time to break down in water, nitrogen would be released over a longer period of time effecting ammonia concentration in the tank. However, in the current tank studies, the tanks were flushed following each meal, removing uneaten food in order to calculate ingestion rates. No such measurements could be made in the sea - cages at No Catch® Ltd., although noticeably, deeper cages will allow a longer period of access to the settling diet. As wild cod are pelagic and carnivorous, they will feed mainly in daylight hours relying on sight to hunt their prey. Consequently, it can be deduced that feeding during daylight hours could increase food uptake and growth, and optimise nutrient utilisation. Consequently, feeding regimes developed for cod need to be extremely accurate to exploit foraging behaviour maximizing feed intake, while keeping uneaten feed losses to an absolute minimum.

Extensive studies in salmonids show that feeding is affected by a number of abiotic factors such as photoperiod and temperature; physical factors such as wave and currents, and chemical factors which include oxygen, dissolved nitrogen compounds, pH and salinity. Fish have optimal ranges, sub - optimal ranges and lethal ranges for all of the chemical factors. Feeding is most likely to vary with the photoperiodic cycle, exhibiting an increase in feeding in spring and a decrease during autumn months (Eriksson & Alanara, 1992). The inverse relationship between

body fat content and feed intake explains a larger increase in appetite following periods when energy reserves are likely to be depleted such as those observed after a prolonged period of reduced feeding during the winter months.

Early studies with strictly carnivorous cod have shown a high capacity for protein hydrolysis (Lied & Njaa, 1982). Most protein digestion occurs in the stomach as a result of the action of pepsin, a product of pepsinogin breakdown through acid hydrolysis. The presence of food in the stomach stimulates the secretion of HCl resulting in the breakdown of pepsinogen to pepsin (De Silva & Anderson, 1995). Work undertaken by Kawai & Ikeda (1973) found that an increased content of fishmeal (high protein content with high digestibility) stimulated an increase in proteolytic enzyme action.

Dried diet and faecal samples collected throughout both tank studies were analysed for Yttirum (Section 3.4.3). As commented earlier, the Yttrium Oxide results show great inconsistency and variation with high standard deviations highlighting the wide range of results found. This is consistent in both the adult and juvenile diet samples questioning the accuracy of the results. The Yttrium Oxide results for both analysed diets were far lower than the inclusion rate as stated by EWOS®. Other work studying Yttirum has been undertaken on aquatic and terrestrial species; *Gadus morhua* (Hemre *et al*, 2003; a), *Salmo salar* (Carter *et al* 2003; Ward *et al* 2005), and mammals (Vhile *et al* 2007). Theses studies investigated diets incorporated with Yttrium Oxide over a range of concentrations from 0.01 g / kg up to 10 g / kg. At all levels, Yttrium Oxide recovery was far greater than that observed in the adult tank study at MERL.

Given the results of the current study, questions arose around the methodologies and the calculations used. Following a rigorous review of the collected results, methodology and calculations, it was concluded that the methodologies and calculations were correct. However, in further discussions regarding the viability of the results, it was suggested that the Yttrium Oxide digestion methods used (acid digestion on a hot plate) were dated, difficult to control (pers. comms. William Struthers) and should have not been recommended as a suitable method

of acid digestion. It has been since recommended that acid digestion should have been achieved through heating in a microwave system, being accurately controlled in terms of temperature and time. Consequently, the results obtained cannot be used to measure apparent digestibility observed in the juvenile and adult tank studies. However, nitrogen digestion was also measured and provided much more accurate results.

Dried diet and faecal samples collected throughout both tank studies were analysed for nitrogen (Section 3.3.3). Nitrogen digestion was calculated as the difference between nitrogen in the dried diet samples and nitrogen in the dried stripped faecal material. In both tank studies, nitrogen digestion was directly related to nitrogen (and associated protein) content of the diets. In the case of the adult study (with the exception of sampling on day 14) nitrogen digestion remains constant over the duration of the study. A mean value of 4.59 % (40 % protein), 5.50 % (50 % protein) and 6.40 % (60 % protein), and expressed as a percentage of the nitrogen content of the diet as 60.55 %, 60.92 % and 60.60 % respectively. In the juvenile study, the mean value was measured as follows: 4.54 % (40 % protein), 5.20 % (50 % protein) and 5.50 % (60 % protein), and a expressed as a percentage of the nitrogen content of the diet as 59.19 %, 56.90 % and 52.23 % respectively. Following an initial increase under each treatment (day 40 of the study), nitrogen digestion fell in all cases toward the end of the study.

When working with carnivorous species such as cod, the most efficient digestibility occurs when carbohydrate is present at very low inclusion (20 % - 30 %). No negative effects of nitrogen digestibility as a consequence of increased carbohydrate levels have been observed for cod (Hemre *et al*, 1990). Percival *et al* (2001) found that when analysing the apparent digestibility coefficient (ADC) for protein in Atlantic salmon, the dissection method of faecal collection produced lower values than those obtained by the stripping method. A similar study carried out on Atlantic cod (Hemre *et al*, 2003;b), found very small variations in ADC for dry matter, protein, lipid and starch, with a high correlation between the two methods for all entities examined. This supports the conclusion that sampling methods (dissection or stripping) did not

have an influence on digestibility results. For protein, the correlation between the two methods was weaker but still significant. ADC for protein varied from 76 % - 90 % with the average for all diets investigated at 85 % ± 5 % (protein content ranged from 36 % - 66 % in the investigated diets). These results were found to be in accordance with similar research for cod (Hemre *et al*, 1990; Lied & Njaa, 1982).

### 3.9.2 Growth indices

Growth of adult cod in tank systems over the study period was related to protein content of the diet with the greatest protein content promoting the greatest growth. From an initial mean weight of approximately 1250 g, it was however evident that the differences in the final weight promoted by each adult diet formulation, was not statistically significant. As protein is the most expensive component of aquafeeds (Pillay & Kutty, 2005; De Silva & Anderson, 1995; Barnabe, 1994), lower protein content would be economically beneficial to the producer and would also reduce nitrogen excretion (both particulate and dissolved) into the marine environment. As found in the current study, the final weights showed no significant difference in growth promoted by the 40 %, 50 % or 60 % protein diets suggesting that adult cod can be successfully fed on a 40 % protein diet, potentially reducing feed costs by 33 % and consequently reducing nitrogen impact upon the environment. However, further work is required on this topic as substitution of protein with carbohydrate could lead to an increase in carbon impact on the environment.

As discussed by De Silva and Anderson (1995), based on work undertaken by Hakanson (1986) and Kryvi (1989) a typical flow of nitrogen in salmonid pens shows that approximately only 25 % of the nitrogen ingested is retained within the fish. The remaining 75 % is released into the environment either as ammoniotelic nitrogen or nitrogen contained within solid wastes. This calculation was based on an FCR of 1.5. By reducing protein content of the diet through partial

replacement with another constituent such as carbohydrate may be possible but could lead to increased carbon pollution. Further work would be required to investigate commercial cod diet formulations that would maximise growth while keeping carbon and nitrogen pollution to a minimal or sustainable level.

All juvenile fish were approximately 110 g at the beginning of the acclimation period (2nd September, 2004) prior to the study commencing (14th September, 2004). It was evident that there was no significant difference in growth promoted by Diet E (50 % protein) and Diet F (60 % protein), with a mean final weight of approximately 380 g ± 95 g. Growth promoted by Diet D (40 % protein) was significantly lower with a mean final weight of 340 g ± 76 g suggesting that a higher than 40 % protein level may be required to promote optimal growth in juvenile cod. However, although higher protein levels are required for juvenile cod, the use of a 50 % protein diet as opposed to a 60% protein diet would potentially reduce feed costs by 17 % reducing nitrogen impact upon the environment in the juvenile stages of cod culture. This work supports other published work (Pillay & Kutty, 2005; Lambert & Dutil, 2000 and Foster *et al*, 1993). However, further work is required on this topic and could possibly investigate the interactions between protein content, pellet size and feeding regimes upon growth, FCE and nitrogen excretion associated.

As well as protein content of the diet, pellet size and feeding regimes, stocking density may also have an impact on growth. As cod preferentially eat prey swallowed whole (Clark *et al* 1995; dos Santos *et al*, 1993), it is imperative that cod should be fed the largest possible pellet size in order to minimise energy exerted when foraging and to potentially minimise the number of uneaten pellets, consequently reducing nitrogen pollution. The ingestion of stones to act as gastroliths, which aid digestion of large prey items, has been observed in wild cod. The provision of 'gastroliths' in cod aquaculture may be considered to aid digestion when investigating the use of the largest possible pellet sizes. The provision of broken mussel shells to act as gastroliths has been observed in the culture of Sheeps heads sea bream (*Puntazzo*

*puntazzo*) in the Mediterranean. However, *P. puntazzo* have large teeth that can easily break up the shell so an alternative may need to be considered when investigating the use of gastroliths in cod aquaculture.

When discussing feeding regimes, Lambert and Dutil (2000) observed that feeding adult cod to satiation three or five times weekly promoted greater growth than those fed to satiation twice weekly. However, at greater stocking densities, those fed to satiation five times weekly showed better growth than those fed three times weekly. It can also be concluded that juvenile cod require a higher protein diet fed frequently. As the fish grow protein levels in the diets and feeding frequency may be lowered while maintaining growth.

FCR varied greatly throughout the adult tank study. Initial FCR was high, possibly due to a number of factors such as sampling error (Section 3.6.1), reconditioning and also acclimation to the new diets. Following discussions with my site supervisor, (Dr. Bill Roy) it was concluded that a 12-day acclimation period would be sufficient. However, the majority of FCR were between 1 and 2 as these factors were rectified. All outliers were possibly due to an accepted hand feeding error (during the early stages of the study) and more locally to rapid fluctuations in water temperature and turbidity. Such fluctuations occurred during times of adverse weather. Literature states that hand feeding may result in higher FCR than automated feeding (De Silva & Anderson, 1995). This could be seen in the comparatively lower FCR observed following day 120 (when automated feeders were installed on the tanks). Rapid fluctuations in temperature can inflict stress upon the fish while turbidity is deemed to have the same negative effect on feeding (Kestemont and Baras, 2002).

The FCR calculated throughout the juvenile study also fluctuated over the 5 - month study period, but were generally lower than those observed throughout the adult study. The majority of values fell between 1 and 2.5. Initial FCR were high but this can be linked to an error in the sampling regime, disturbing the fish, which reduced appetite and FCE (Kestemont & Baras,

2002). Once amended, FCR dropped to approximately 1. A steady increase toward the end of the trial could possibly be linked to rapid fluctuations in water temperature and turbidity, which was observed in the adult study during periods of adverse weather. Precocious maturation of the juvenile cod (observed in the sacrificed fish), combined with the reduction in feeding during winter months (Karlsen *et al*, 1995; Ramnarine *et al*, 1987) can also explain the reduction in FCR observed in the latter stages of the juvenile study.

FCR of Atlantic salmon culture in Norway was approximately 2.25 in 1974 (Enell & Ackefors, 1992). Through the development of aquafeeds and the improvement in feeding regimes and techniques, this value has dropped to 1.2 - 1.3 (Enell & Ackefors, 1992), 1.2 (Rosenthal *et al*, 1995) and 1.17 Levings (1997). FCR values provided by No Catch® Ltd. remained constant at 1.2 showing that a low FCR is achievable with Atlantic cod, particularly with exclusive automated feeding regimes. With potential progression as observed with improving FCR in Atlantic salmon culture, it may be possible to further reduce the FCR when feeding Atlantic cod.

Johnson *et al* (1993a) studied the effect of protein sparing in Atlantic salmon on the FCR and nitrogen loading. They concluded that increasing lipid levels from 22 % to 30 % had no effect on growth but reduced FCR from 1.07 to 0.96, which in turn resulted in a reduction in ammonia excretion by 38 %. However, protein sparing to such a degree is not feasible with Atlantic cod as excess lipid is readily laid down in the liver.

SGR observed at the beginning of the adult investigation were low (0.2 % bw/day) as energy is directed toward reconditioning following spawning (De Silva & Anderson, 1995). As the fish regained condition, significant growth resumed around day 100 of the study (mid - October 2004), and SGR increased for each treatment to a peak of between 0.5 and 0.8 % bw/day, around day 150 of the study (early - December 2004). However, as the fish began to mature, energy was directed from growth to the production of gametes (De Silva & Anderson, 1995)

and the SGR fell to approximately 0.1 % bw/day by the end of the study. It could be seen that the initial SGR for juvenile cod was high (approximately 1 % bw/day) up until day 80 (early December), when the SGR dropped to approximately 0.4 % bw/day as the cod began to mature and more energy was redirected. Precocious maturation was observed in both male and female juvenile fish that were sacrificed toward the end of the study. Juveniles grow faster than adult fish and consequently, as the fish grow, SGR falls (Bjornsson & Steinarsson, 2002; Tucker, 1998; De Silva & Anderson, 1995). Such a phenomenon can be observed through comparing the current studies, which generally shows a higher SGR observed in the juvenile cod compared to the adult cod.

Stocking densities can also effect the SGR of adult cod in tank systems as discussed by Lambert & Dutil (2000) who found that increasing stocking denisites from 2 $kg/m^3$ up to > 40 $kg/m^3$ results in a drop in the SGR from 1.08 % to 0.66 %. Such findings may not be directly comparable to the current work, as stocking density of the adult tanks remained constant around 0.04 $kg/m^3$ from week to week (any increase in weight of the population was 'removed' every fourteen days with the sacrifice of three random fish). There is no significant difference in SGR promoted by the three diet formulations throughout either of the tank studies confirming the viability of using a 40 % protein diet for adult cod and a 50 % protein diet for juvenile cod.

### 3.9.3 Condition indices

K - Factor represents the relationship between the length and weight of a fish. For a given length, a heavier fish will have a greater K - Factor than a lighter fish. An increase in condition was observed over the course of the adult tank study. However, as the fish completed spawning approximately 30 days prior to the commencement of the study an increase in condition was not observed until approximately day 90 (early October, 2004) of the 210 - day investigation. As the fish regain condition, a greater proportion of ingested energy is anabolised and the increased

weight gain coincides with an increase in condition (Barnabe, 1994). Over the study period, condition increased from approximately 1.17 ± 0.17 for all diets investigated, to give final values of 1.56 ± 0.22 (40 % protein), 1.59 ± 0.25 (50 % protein) and 1.57 ± 0.26 (60 % protein). In the current study, the K-Factor of the juvenile fish increases over the full range of the 154 day study from a mean value of approximately 1.07 ± 0.11 for all diets investigated, to give final values of 1.20 ± 0.12 (40 % protein), 1.21 ± 0.10 (50 % protein) and 1.23 ± 0.11 (60 % protein). The standard deviation observed in each juvenile treatment was greater than that observed in the adult study suggesting greater variation within the juvenile fish. The condition of juvenile fish is higher in hatchery - reared juvenile cod than in wild counterparts (Grant *et al*, 1998). This is due to early feeding of high - energy diets in captivity (Grant *et al*, 1998). Again, such results confirmed that the 40% protein diet for adult cod and the 50 % protein diet for juvenile cod promoted optimal growth while maintaining condition and reducing feed costs and nitrogenous environmental impacts.

HSI is a morphological index as studied by Jensen (1979) and is a ratio of the weight of the liver expressed as a percentage of the total weight of the fish and gives an indication of the condition of the fish. Juvenile and adult cod develop large fatty livers when fed high - energy diets in captivity (Grant *et al*, 1998). In the adult study, an increase in HSI following spawning was evident under each treatment, with the high lipid diet (14 % lipid) promoting the greatest increase. The HSI observed throughout the juvenile trial was higher for each formulation (when compared to the adult study), and remained constant throughout but there was a distinct difference between the diet formulations relative to lipid content of diet. Such results agree with Morais *et al* (2001) and Foster *et al* (1992) who found that the HSI was strongly dependent on growth rate and lipid content of diet with a higher lipid content promoting a higher HSI. Such a phenomenon was particularly evident in the current juvenile study.

In cod, the majority of lipids are stored in the liver (Pillay & Kutty, 2005; Morais *et al*, 2001; Lambert & Dutil, 1997; Hemre *et al*, 2003;b and Jobling *et al*, 1991). When using similar sized fish of a similar condition, the higher the lipid content of the diet results in a greater degree of lipid deposition in the liver, resulting in a greater HSI. Such a trend could be observed in the adult study where the final HSI promoted by Diet A (14 % lipid) was greater than those promoted by Diet B (12 % lipid) and Diet C (10 % lipid). The relationship between lipid in the diet and HSI was more apparent in the juvenile study and a more distinct difference between the diets could be observed. This defining trend shows that Diet D (14 % lipid) promoted the greatest HSI (mean of 7.8 %) compared to 7 % promoted by Diet E (12 % lipid) and 6 % by Diet F (10 % lipid) over the 5 - month period. Morais *et al*, (2001) found that a relatively low protein and high lipid content (48 % protein & 16 % lipid) promoted greatest growth and FCE. However, one of the objectives of that study was to investigate if lipid could be used in protein sparing, while the second objective was to study if the deposition of more lipid induced in the liver could be used in enhancing the production of cod liver oil. The current at studies at MERL support Morais *et al* (2001) in that HSI is directly related to the lipid content of the diet. While protein sparing through increased lipid in the diet may reduce nitrogen excretion, the prolonged use of such a formulation may result in large livers in the cultured fish, which could be detrimental to the commercial value of the flesh (Jobling, 1993; Jobling, 1988).

### 3.9.4 Condition parameter relationships

As the K-Factor is a relationship between length and weight, and the Hepatosomatic Index is the relationship between the liver (wet weight) and total body wet weight, it is difficult to define a direct relationship between the two. The HSI and K-Factor and governed by the life history of the species and will be greatly influenced by the onset of

maturity, followed by spawning, then subsequent reconditioning prior to the onset of the second spawning season.

For a population of juvenile fish of a similar age kept under the same conditions it can be assumed that fish with a high HSI would likely have a high K-Factor and similarly, those with a low HSI would have a low condition factor. However, as there will be variations within the individuals of a population, any relationship between the HSI and K-Factor would be impossible to ascertain.

Trying to achieve a relationship with adult fish is made much more difficult due to the onset of maturation. Such a scenario results in a constant fluctuation of the weight of the fish and the weight of the liver of that particular fish. In *G. morhua*, all excess energy is stored in the liver and can be readily liberated when required. In the wild, this would occur during times when food is scarce and the production of gametes. In an aquaculture scenario, the fish are fed continuously, so liberation of stored energy will only occur during the production of gametes. During this period, the relative weight of the liver and the weight of each fish will fluctuate but the timing of which will not be the same, making any direct relationship between the HSI and K-Factor almost impossible to identify. Another problem would be that the individual fish mature at different rates and some may not mature at all. It is widely reported that female fish direct a lot more energy toward gamete production than male fish and therefore, HSI : K-Factor ratios are again different between the species.

In conclusion, there are too many variables, particularly within populations of mature fish to identify and create a plausible relationship between the HSI and the K-Factor.

### 3.9.5 Impact of sexual maturation on the growth and condition of adult and juvenile cod

It was observed that throughout both tank studies that as well as diet formulation, growth (weight gain, FCR and SGR) and condition (K - Factor and HSI) are influenced by the reproductive cycle. This is particularly evident in the adult tank study. Energy is partitioned between maintenance (catabolism), growth (anabolism) and reproduction. Energy ingested is primarily catabolised (maintenance) then anabolised (growth) or stored as energy (DeSilva & Anderson, 1995). In Atlantic cod, the liver is the primary store of energy (Pillay & Kutty, 2005; Hemre *et al*, 2003;b ; Morais *et al*, 2001; Lambert & Dutil, 1997; and Jobling *et al*, 1991). However, as the fish mature, energy is directed from anabolism to the production of gametes and spawning (De Silva & Anderson, 1995). The production of gametes requires a phenomenal expenditure of energy (Rideout & Burton, 2000; Karlsen *et al*, 1995), and the annual reproductive cycle can have a detrimental impact on the growth and condition of cod. The relative partition between growth and reproduction varies between species and the progression of gametogenesis. It has been found that there is an optimal protein level for reproductive success, which is related to optimal level for growth of a given species. When the dietary level of protein is optimal, a greater proportion of the population spawns (De Silva & Anderson, 1995).

Following spawning, energy stores are exhausted and the fish are in poor condition (Bjornsson & Steinarsson, 2002). This is exhibited in the early stages of the adult study with poor FCR, SGR and K-Factor, as well as low HSI. It can be seen that the adult fish required at least 120 - 130 days before FCR, SGR and Condition improved and the HSI increased (since spawning was completed approximately 30 days prior to the study commencing). Such observations can be related to gonad size (Rideout & Burton, 2000). Rideout & Burton (2000 state that gonad size of

wild male cod was largest in April and smallest in October, with a large increase throughout November.

It was observed that FCR and SGR decrease toward the end of both the adult and juvenile tank studies. This was due to the onset of maturation in both the juvenile and adult fish (observed from the fish sacrificed every fourteen days). The increase in K - Factor and HSI of the fish in both studies appeared to slow toward the end of the studies and would presumably begin to drop during the final stages of maturation, supporting observations made by Bjornsson & Steinarsson (2002). However, both of the current tank investigations were terminated before such a scenario could be noted. In their work studying reproductive energy in first - time spawning cod, Karlsen, *et al* (1995) found that females invest more energy in spawning than males. It could be deduced that male cod may require a shorter re - conditioning period and an exclusive male population would possibly be beneficial in terms of commercial production. Through using a higher lipid diet (14 - 16 % lipid), it can be shown that such a formulation could be beneficial in increasing the accumulation of lipid in the liver, thus increasing the HSI and reducing the reconditioning period following spawning. Such a regime would also feed costs (less protein) for up to 120 days following spawning. Reverting to a higher protein (and consequently, lower lipid) diet following reconditioning, should be considered to maximise anabolism and promote optimal gamete development.

### 3.9.6 Tissue composition

Protein (and nitrogen) levels retained in the fish remained constant over the course of both studies. As protein is 14 % ± 1 % nitrogen, both protein and nitrogen content was analysed to confirm actual levels. Protein and nitrogen of the flesh and liver of juvenile and adult fish were analysed. Adult flesh (dry weight) averaged approximately 93 % protein and 14.5 % nitrogen regardless of formulation. Juvenile carcass (whole cleaned/dry weight) fish exhibited an average protein content of 80 % protein and 13 % nitrogen regardless of treatment. The lower levels

exhibited in the juvenile fish are due to the whole carcass being analysed (skeleton inclusion increases ash levels considerably).

There are documented relationships between lipid and water, and protein and water in fish tissue and the relationships can be seen in Appendices 13 (adult cod) and 14 (juvenile cod). However, such relationships were only observed in the liver sampled from the sacrificed fish throughout both studies. The inverse relationship between moisture and lipid of the liver was best exhibited in the liver samples collected throughout the adult study. The large number of samples collected and the change in condition of the fish throughout the 7 - month study highlighted the phenomenon that increasing lipid levels (increase in condition) resulted in a decreasing moisture content (as discussed by Bulow, 1970). No such observation was evident in the adult flesh or in the flesh or liver samples collected throughout the juvenile study. Although not entirely relevant to the nitrogen budget, this work was undertaken out of interest and the aforementioned relationships can be seen in Appendices 13 (adult cod) and 14 (juvenile cod).

The relevant results discussed in this chapter such as diet composition, ingestion rates, digestibility, growth and condition parameters were taken forward to Chapters 7 (modeling) and 8 to help produce nitrogen flux models associated with the culture of juvenile and adult Atlantic cod in tank systems.

# Chapter 4

## DISSOLVED NITROGENOUS WASTE PRODUCTION ASSOCIATED WITH THE COMMERCIAL CULTURE IN TANK SYSTEMS

# DISSOLVED NITROGENOUS WASTE PRODUCTION ASSOCIATED WITH THE COMMERCIAL CULTURE IN TANK SYSTEMS

## 4.1 Introduction

Chapter 4 investigates the daily excretion rates of dissolved nitrogenous wastes associated with the commercial culture of adult and juvenile Atlantic cod in tank systems. Through the collection of a series of water samples (see below), daily profiles of ammonia, nitrite and nitrate were compiled for the adult and juvenile cod fed the three diet formulations. The juvenile and adult tank designs were the same as those described in Chapter 3 and water sampling ran concurrently with the tank growth trials, over a 7 - month period for the adult study (July 2004 - February 2005) and over a 5 - month period for the juvenile study (September 2004 - February 2005). As discussed in Chapter 3, both studies investigated three diet formulations (40 %, 50 % and 60 % protein), with lipid content varied to 14 %, 12 % and 10 % lipid respectively to keep the diets iso - energetic. A series of water samples were collected at 9.00 am (0 mins), 9.30 am (30 mins), 11.00 am (120 mins), 12.30 pm (210 mins), 3.45 pm (395 mins) and 4.30 pm (450 mins) throughout an 8 - hour period every fourteen days. The sampling strategy was designed so that water samples would be collected over the course of the day, during times of feeding and in periods of no feeding. It was thought that such a strategy would give an indication of nitrogenous waste excretion in relation to feeding.

## 4.2 Methods

### 4.2.1 Feeding regime

The adult fish were fed approximately 0.5 % bw per day; the ration was adjusted according to appetite. Feeding was by hand between July 2004 and November 2004 (two 15 - min. meals at 9 am and 4 pm). Automated feeders were introduced to the tanks altering the feeding regime for

the remainder of the study (November 2004 - February 2005). The introduction of automated feeders altered the feeding regime to two smaller 15 - min. meals beginning at 9 am and 4 pm with the remainder of the ration being fed equally throughout the day.

The juvenile fish were fed at 1.5 % bw per day throughout the trial; the ration was adjusted according to appetite. Feeding was by hand throughout the duration of the study (September 2004 - February 2005) and was offered at two distinct 15 - min. meals at 9 am and 4 pm.

### 4.2.2 Collection of water samples

Water sampling began prior to the morning meal at 9 am (0 min) then at 30 min., 120 min., 210 min., 395 min. (immediately prior to the meal at 4 pm) and finally at 450 min. In both adult and juvenile studies, single water samples were collected from the inflow, from within the tank and from the outflow of each tank. This approach allowed the adult diets to be studied in duplicate (2 tanks per diet) and the juvenile diets to be studied in triplicate (3 tanks per diet). Inflow samples were collected at 0 min. and 450 min., while the 'in - tank' and outflow samples were collected at all sampling times. When creating the ammonia profiles for each sampling date the mean inflow values were subtracted from the 'in - tank' and outflow values in order to remove background dissolved nitrogenous waste levels and make the profiles more accurate. Water samples were collected using sterile, sample - washed, labeled Universal pots. Following each sampling interval, all samples collected were filtered. Ideally, replicate samples from each tank would have been collected. However, due to time constraints when sampling and the initial provision of inadequate filtration equipment, this was not possible.

### 4.2.3 Filtration & analysis

Immediately following each sampling time interval, all samples collected at that time interval were filtered through 'Fisherband' Glass Microfibre filter paper 'MF200' in a Millipore vacuum

filter. The samples were then frozen (- 20 °C ± 1 °C) prior to transportation in cool boxes to the Institute of Aquaculture for analysis. The samples were later thawed and analysed for ammonia, nitrite and nitrate using a Digital Calorimeter (Bran & Luebbe, auto Analyser 3). The results were converted to µg/L/ kg biomass so the final profiles would be directly comparable.

### 4.2.4 Statistical analysis

The data was not transformed and differences between treatments (diets) on ammonia concentration in the water samples were analysed using a two - way ANOVA implementing Minitab™ version statistical software (Ryan & Joiner, 1994). In all cases, where $P < 0.05$, the statistical difference was considered significant.

## 4.3 Results

Ammonia is the most significant dissolved waste product of finfish aquaculture. (Pillay & Kutty, 2005; Halver & Hardy, 2002; Tucker, 1998; DeSilva & Anderson, 1995 and Jobling 1994). For the purpose of the thesis, only ammonia is discussed in this chapter with all ammonia nitrite and nitrate values shown in Appendices 15, 16 and 17. Nitrite and nitrate are less toxic than ammonia and it was considered that understanding the ammonia profile would be of utmost importance to the study. With the breakdown of ammonia to nitrite and nitrate in a constant state of fluctuation, it can be seen in Appendices 15, 16 and 17, that there is no direct relationship between the three dissolved nitrogenous wastes. It can also be seen in these Appendices that there are no defined trends in the daily profile of nitrite and nitrate for either the juvenile or the adult cod. Consequently, through highlighting the defined profiles in ammonia production, the most significant dissolved nitrogenous waste associated with commercial finfish culture can be accurately shown and discussed. However, this opens the door for future work to

study the relationships between the three dissolved nitrogenous wastes as a result of feeding different diet formulations under different regimes.

Prior to the set-up of the studies, it was decided that 6 tanks would be allocated by MERL to the adult study, and 9 tanks would be allocated to the juvenile study. Consequently, no further tanks were available to be utilised as controls. It was however deemed acceptable that sampling the inflow water to each tank would act as a control for each particular tank. The inflow values were subtracted from the in-tank values and the outflow values to eliminate any background dissolved nitrogenous wastes in the systems, providing more accurate results.

Figures 4.1 and 4.2 were compiled from daily profiles produced for adult cod under a manual feeding regime. All raw data is shown in Appendix 15. Figures 4.3 and 4.4 were compiled from daily profiles produced for adult cod throughout the automated feeding regime. All raw data is shown in Appendix 16. Figures 4.5 and 4.6 were compiled from daily profiles produced from samples collected throughout the juvenile study under an exclusive manual feeding regime. All raw data for the juvenile study is shown in Appendix 17. The arrows shown on each of the following Figures; 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6, represent the times when the cod were manually fed. In Figures 4.3 and 4.4, the cod were also fed two smaller meals at 9 am and 4.15 pm with the remainder of the ration being fed throughout the day.

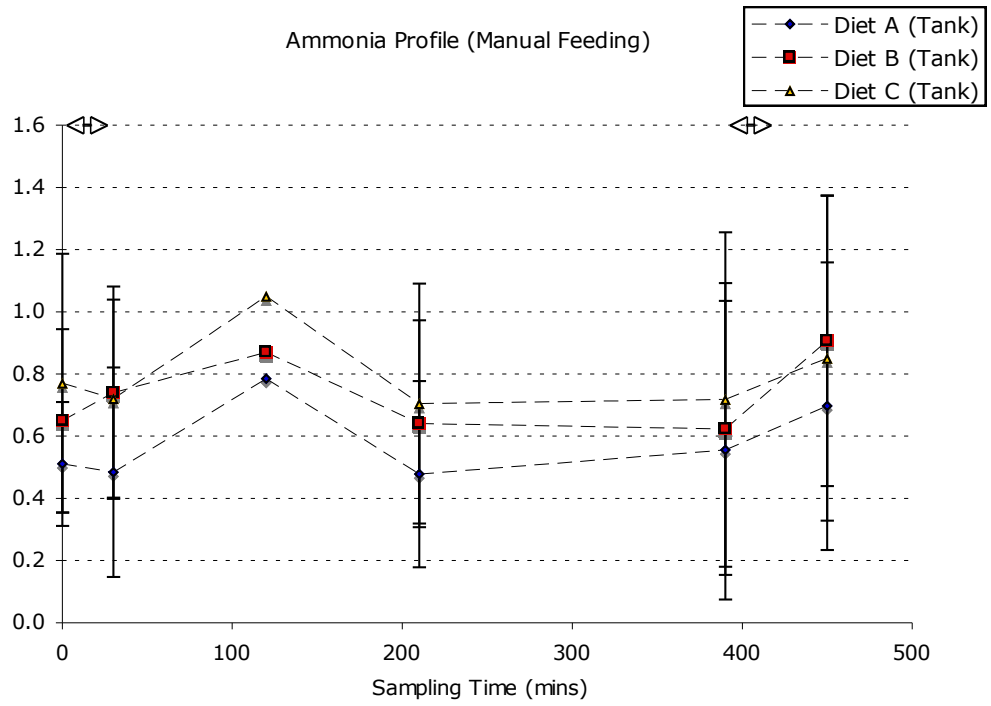



Figure 4.1 Ammonia profile in tank water under manual feeding (error bars are ± 1 SD). No error bars are shown at 120 min. as only a single sample was collected.

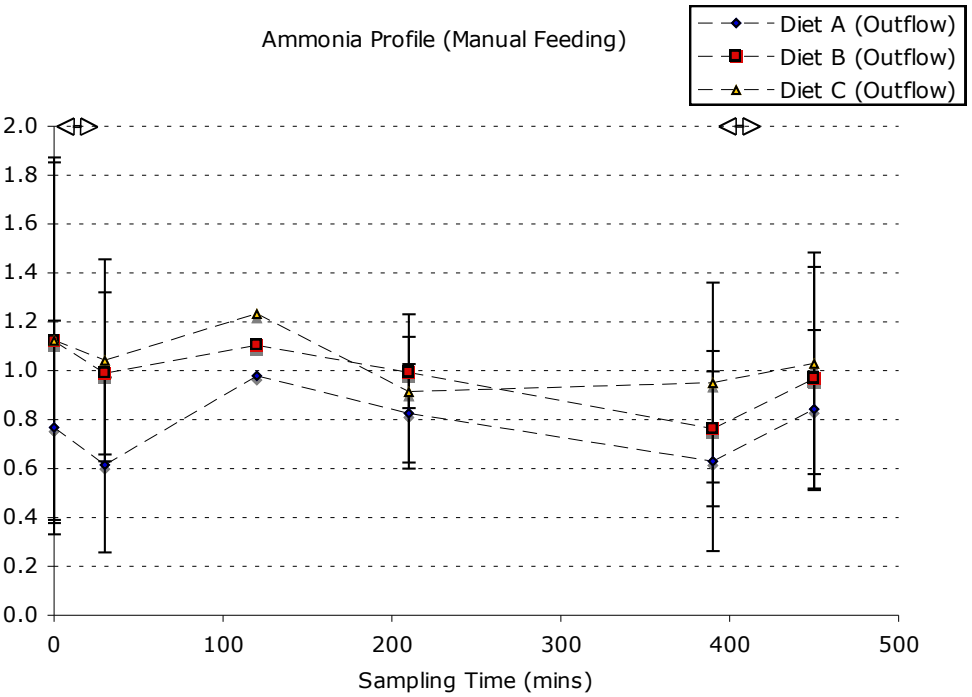



Figure 4.2 Ammonia profile in outflow water under manual feeding (error bars are ± 1 SD). No error bars are shown at 120 min. as only a single sample was collected.

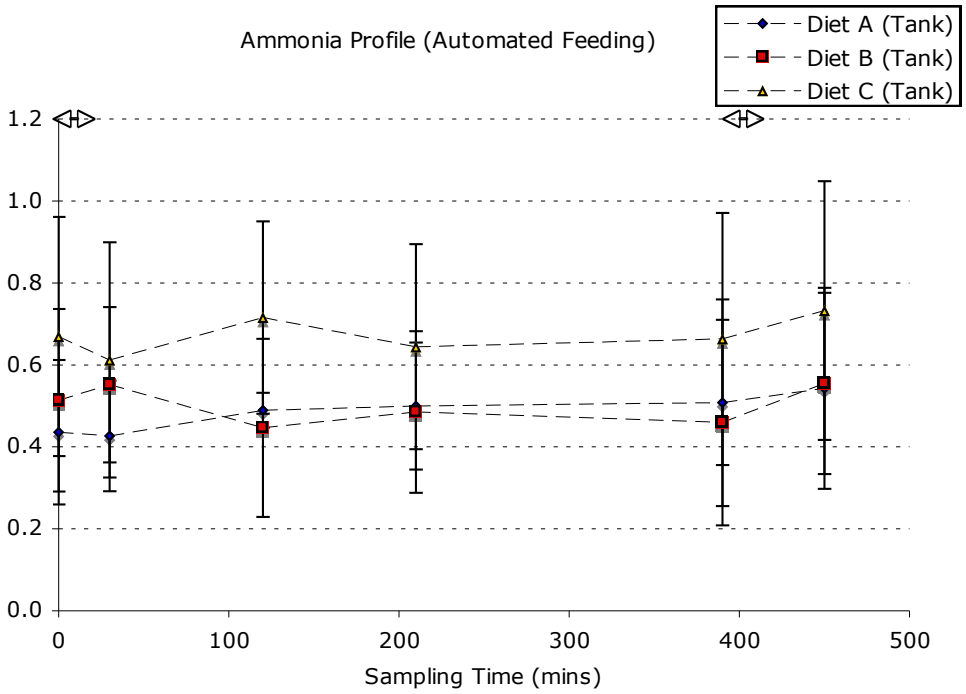



Figure 4.3 Ammonia profile in tank water under automated feeding (error bars are ± 1 SD).

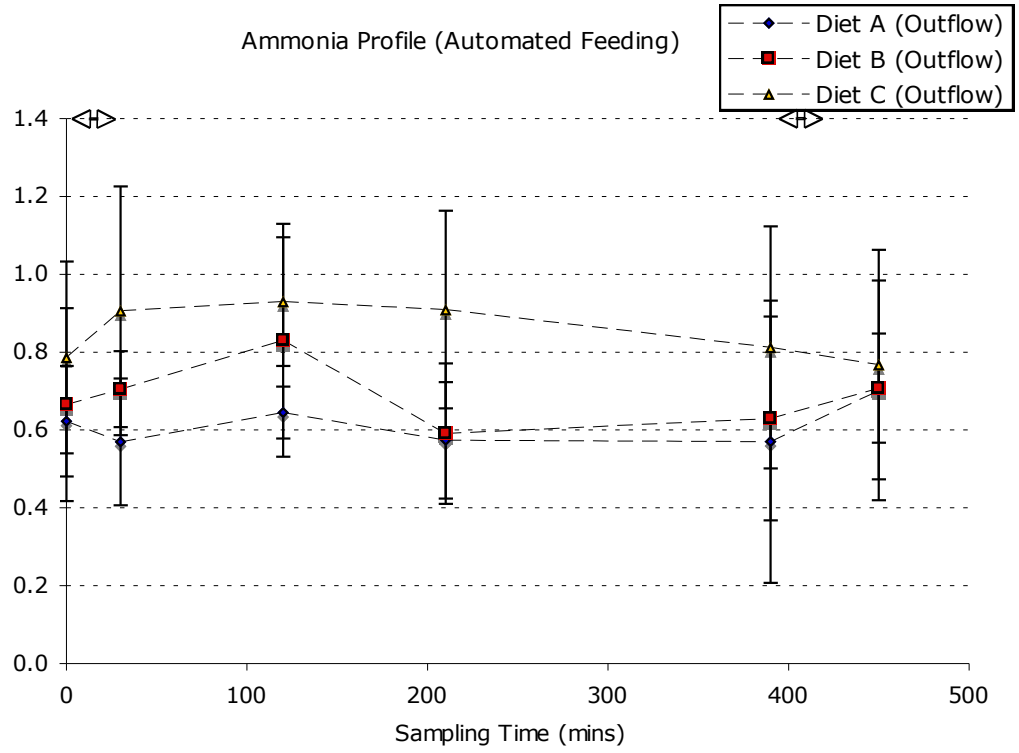



Figure 4.4 Ammonia profile in outflow water under automated feeding (error bars are ± 1 SD).

From Figures 4.1, 4.2, 4.3 and 4.4, it can be seen that ammonia excretion is related to feeding periods (shown by the arrows in afore - mentioned Figures). The volume and degree of excretion is dependent on the feeding regime and protein content of the diet, with the concentration of ammonia varying according to the sample source (in - tank or outflow).

From Figure 4.1, a minimal drop in ammonia concentration in the tank water was observed during and immediately following the first feeding (a 15 - min. meal commencing at 0 min.). Thereafter, an increase with all diets was noted, rising to a post-prandial-peak at 120 min. (105 min. following cessation of feeding). From a base concentration (0.5 µg/L/kg – 0.7 µg/L/kg), a mean rise of approximately 0.3 µg/L/kg biomass to the post-prandial-peak was recorded. Following this peak, ammonia levels drop for each diet and remain constant between 210 min. and 390 min. After the 15 - min. afternoon meal commencing at 420 min. ammonia levels rose at least until the final samples were collected at 450 min. A mean rise of approximately 0.2 µg/L/kg biomass was recorded between 390 min. and 450 min. It can be seen in Figure 4.2 that the daily ammonia profile measured in the outflow water follows a similar trend to the tank water. It can also be noted that the post-prandial-peak, although occurring at 120 min., is not as clearly defined as the post - prandial - peak measured in the tank water as was the rise in ammonia following the afternoon meal.

From both Figures 4.1 and 4.2, it is evident that trends observed of ammonia concentration in the tank and outflow water was related to protein (and associated nitrogen) content of the diets. Although in both cases, the excretion rates follow the same trend regardless of diet, it can be seen that the greater the protein (and associated nitrogen) content of the diet, the greater the ammonia level recorded at each sampling time. It can be observed that the mean ammonia concentration measured in the outflow water samples were generally higher (approximately 0.2 µg/L/kg biomass) than those recorded in the tank water. The trend observed (Figures 4.1 and 4.2) in the mean value for each treatment at each sampling time is mirrored in the error bars related to that mean value, confirming that a trend does exist. However, due to the range of the

error bars and the degree of overlap of the error bars, the difference between treatments and between sampling times over the 8 - hour period were not significant in the tank water ($n = 18$, $F = 1.10$, $P > 0.05$) or in the outflow water ($n = 18$, $F = 2.01$, $P > 0.05$).

From Figures 4.3 and 4.4, it can be seen that under an automated feeding regime, the ammonia concentration in both the tank and outflow samples remained comparably stable over the course of a sampling day. A small post - prandial - peak showed at 120 min. (105 min. following the cessation of a small meal) and a lesser increase in ammonia concentration following the afternoon meal was also observed. Such a trend can be observed in both tank and outflow samples. This was particularly evident in tank water (Figure 4.3) but was less pronounced in the outflow water (Figure 4.4), with only Diets A (40 % protein) and B (50 % protein) following a similar trend to each other. In general terms, Diet C (60 % protein) promoted greater ammonia levels compared to Diets A (40 % protein) and B (50 % protein), which were similar to each other over the duration of the sampling period. That said, differences in ammonia concentration promoted by each diet were not significant in the tank water ($n = 18$, $F = 2.39$, $P > 0.05$) or the outflow water ($n = 18$, $F = 1.36$, $P > 0.05$).

Figures 4.3 and 4.4 shows that ammonia levels promoted by each diet in the tank water and outflow water respectively, vary by approximately 0.1 µg/L/kg biomass over the course of the sampling day. Mean outflow values for each treatment were higher (between 0.1 µg/L/kg biomass and 0.2 µg/L/kg biomass) than those measured for the respective treatments recorded in the tank water samples. A post -prandial - peak at 120 min. was observed in both the tank and outflow samples. However, the peak was less pronounced as was the increase following the afternoon meal, than that recorded under a manual feeding regime (Figures 4.1 and 4.2 respectively).

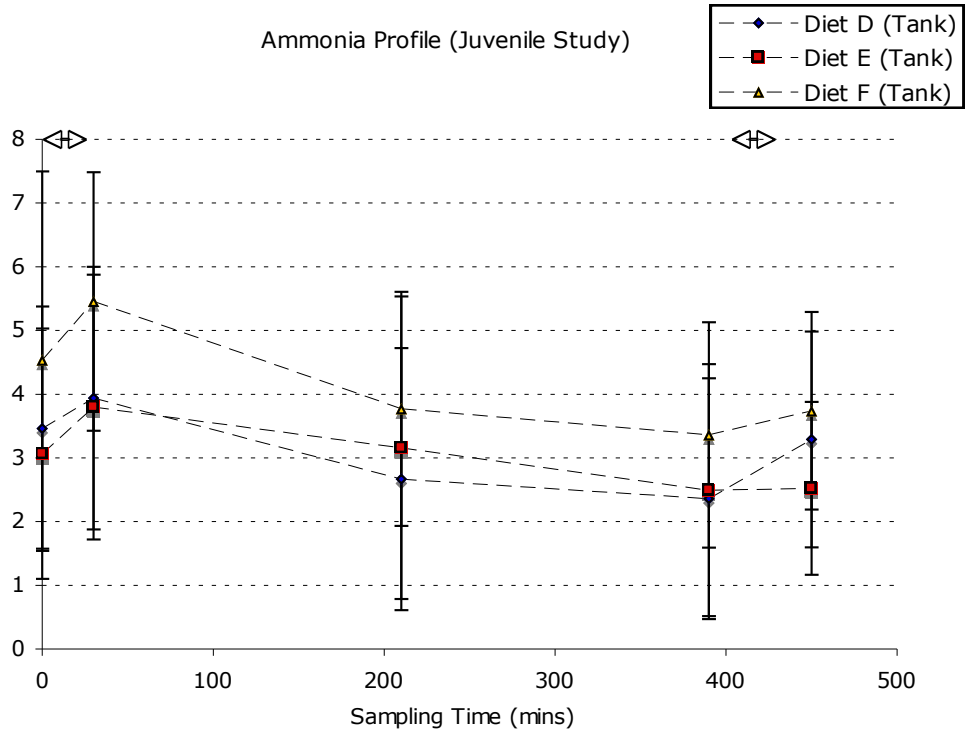



Figure 4.5 Ammonia profile in tank water throughout the juvenile study (error bars are ± 1 SD).

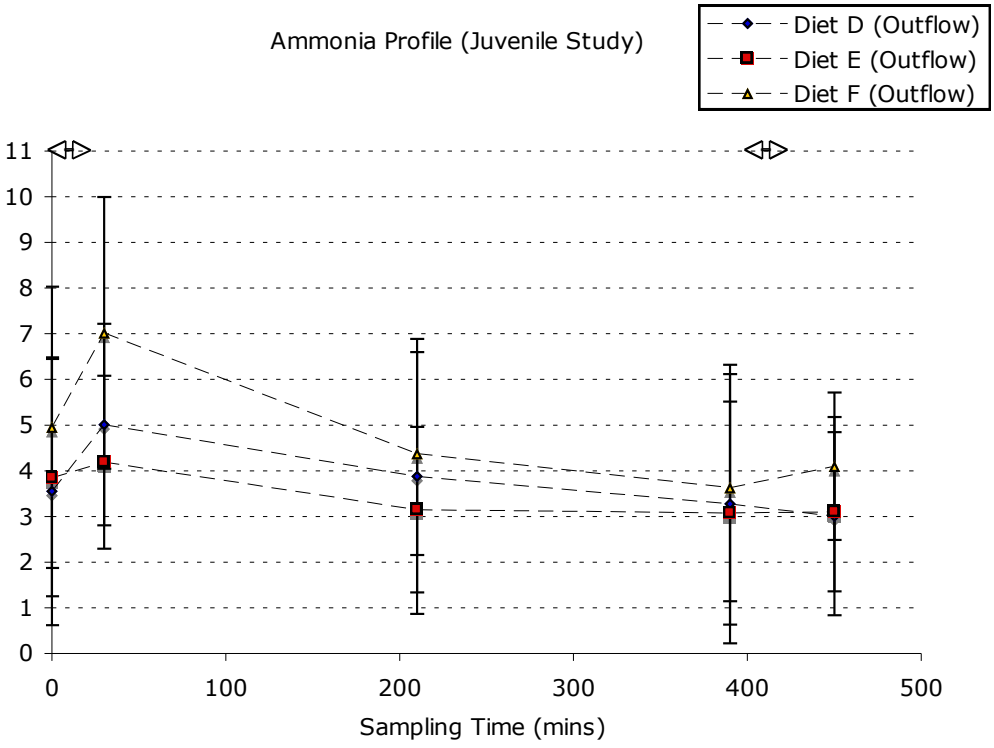



Figure 4.6 Ammonia profile in outflow water during the juvenile study (error bars are ± 1 SD).

The ammonia profile produced throughout the juvenile tank study showed a 'post - prandial - peak' at 30 min. (approximately 15 min. following the cessation of the morning meal). The increase to this 'peak' was recorded by approximately 1 µg/L/kg biomass for each diet. Following this 'peak' ammonia levels promoted by each diet formulation fell over the course of the day until 390 min. Following the afternoon ration, ammonia levels remained constant (50 % protein) or rose by approximately 0.5 µg/L/kg biomass with the other diets.

Although the outflow water profiles showed a similar trend to that observed in the tank water samples (Figure 4.5), it can be seen that the ammonia concentration in the outflow samples were between 0.25 µg/L/kg biomass and 0.75 µg/L/kg biomass higher than those observed in the tank water samples. Although the ammonia concentration promoted by each diet formulation followed a similar trend over the course of a sample period, it was evident that Diet F (60 % protein) promoted a greater ammonia level in tank water than Diet D (40 % protein) and Diet E (50 % protein).

The trend observed in the mean value for each treatment at each sampling time was mirrored in the error bars related to that mean value, confirming that a trend does exist. However, due to the range of the error bars and the degree of overlap of the error bars, the difference between treatments and between sampling times over the 8 - hour period were not significant in the tank water ($n = 15$, $F = 1.31$, $P > 0.05$) and in the outflow water ($n = 15$, $F = 2.41$ , $P > 0.05$).

## 4.4 Discussion

### 4.4.1 Ammonia profiles

The level of ammonia concentration in tank systems was dependent upon the feeding regime, protein (and associated nitrogen) content of the diet and the source from where the water was sampled (in - tank or outflow).

As discussed earlier in section 4.3, ammonia excretion can be directly related to the feeding regime. Under a manual regime, a post - prandial - peak was observed at 120 min., as was an increase in ammonia concentration following the afternoon feeding. A similar trend was observed in the outflow water, although the post - prandial - peak at 120 min. was not as pronounced as that observed in the tank water samples. Under an automated feeding regime, a smaller post - prandial - peak was observed at 120 min. and the increase following the afternoon meal was similarly less pronounced than that observed in tank water under a manual feeding regime. By comparing Figure 4.1 to Figure 4.3, it is evident that ammonia concentration noted throughout the sampling period in tank water is generally lower (c. 0.15 µg/L/kg biomass) for each treatment under an automated feeding regime. Similarly, comparing Figure 4.2 to Figure 4.4, a comparable difference is observed with ammonia values recorded under a manual feeding regime approximately 0.2 µg/L/kg biomass higher than that produced by the respective diet under an automated feeding regime.

By comparing Figure 4.1 to 4.5, it can be observed that although the values are vastly different, the trends in ammonia production associated with both juvenile and adult cod is similar in tank systems under a manual - feeding regime. It can be seen that in each study, a post-prandial peak occurs around 105 minutes following the cessation of the morning meal. It would have been beneficial to collect water samples over a greater number of time intervals throughout the sampling day. However, this was not possible due to time constraints but such a scenario clearly gives rise to future studies.

Given the post - prandial - peak recorded in Figure 4.1 at 120 min., it can be deduced from Figure 4.5 that a greater post - prandial - peak would occur at a later time than that recorded at 30 min. The difference in the sampling regimes (time intervals) between the studies was due to the inadequate filtration system (10 min. to filter a single sample). As the collected samples had to be filtered immediately, this did not allow a sampling interval at 120 min. in the juvenile study. However, the steep gradient, which can be observed under all treatments between 0 min and 15 min. (Figure 4.5) suggests that the following ammonia concentration would rise to a peak beyond the 15 min. sampling interval. As at the foot of the previous page, time constraints would not allow as comprehensive a sampling regime in either tank trial as the author would have liked. Consequently, this again gives rise to future research.

When comparing the ammonia values collected in tank and outflow water between adult cod (manual feeding) and juvenile cod, it was evident that levels recorded throughout the juvenile study were far greater than those observed in the adult study. These elevated levels observed throughout the juvenile study are due to a number of reasons:

1. Juvenile fish exhibit a far greater metabolic rate than adult fish.
2. The flow-through rate of water was far less in the juvenile tanks (12 L/min) compared to the adult tanks (90 L/min), resulting in a lower water turnover, consequently increasing the concentration.
3. Tank volume was much smaller in the juvenile study allowing a lower dilution of dissolved nitrogenous wastes within the juvenile tank water samples, thus increasing the concentration.

### 4.4.2 Feed intake

Feed intake by individual fish could not be evaluated during the present tank studies and therefore cannot be discussed in this thesis. It is however, important to understand feed intake, how different environmental factors can cause it to vary, and the impacts it can have on FCR and nitrogen loading.

Feed intake may vary considerably between days (Juell, *et al*, 1993) and seasons (Jobling & Baardvik, 1991). The causes of such variation are largely unknown but it is most likely that several biological and environmental factors contribute. Temperature is a major influence of metabolism and food intake (Jobling, 1997). The generation in tanks of moderate water currents may result in improved feed distribution and better feeding conditions (Christian & Jobling, 1990). FCR is also integral to nitrogen loading of the water. Marou (as cited in Seymour and Bergen, 1991) states that an increase in FCR from 1 to 1.5 can lead to a 70 % increase in nitrogen loading. There is much potential here for future study into the interactions of such biological and environmental factors and how they affect feeding, FCR and nitrogen loading.

Commercial aquafeeds generally use fish - meal as a major component (Enell, 1995), as it exhibits high digestibility rates in carnivorous fish (De Silva & Anderson, 1995) which consequently reduced solid waste discharge. With the aquaculture industry looking for alternatives to fish -meal (and there - by reducing the exploitation of the fisheries harvested to produce fish-meal), potential substitutes are likely to promote lower digestibility (Pillay & Kutty, 2005). Substitutes such as plant meal and plant oil exhibit very different nutritional profiles from fishmeal and fish oil. The use of substitutes would produce inferior digestibility compared to fishmeal and fish oil (which contain similar nutritional profiles to those required by carnivorous fish). In turn, this would reduce digestibility, increasing the particulate discharge connected to any aquaculture facility. Although this data is not directly relevant to the current study, it should be thoroughly considered when planning future investigations into the topic.

### 4.4.3 Gut evacuation

Gut evacuation was not measured during the current studies due to time constraints but mainly the consistency of the faecal material. The moisture content of the faecal material was so high (c. 80 %) that dispersion of the material into the water column was immediate upon excretion. However, it is important to discuss the process of gut evacuation and the impact that gut evacuation rates may have upon the daily profile of dissolved nitrogenous wastes excretion associated with the commercial culture of Atlantic cod.

Even under favourable environmental conditions, there may be considerable variation in feeding activity between days. This may be related to gastric evacuation time as it has been suggested that the return of appetite depends upon stomach fullness. Daily fluctuation in feeding may be induced by changes in the volume of food in the stomach and the time required for emptying. Such a phenomenon can be affected by the quality of the aquafeeds used. Studies suggest that gastric emptying follows two independent trends: a straight line as observed in *Gadus* spp. (Jones, 1974); or an exponential curve observed in *Salmo trutta* (Elliot, 1972) and *Oncorynchus* spp. (Brett & Higgs, 1970). Brett & Higgs (1970) superimposed a chart of the rate of gut evacuation over a chart showing the rate of ammonia excretion. At the peak levels of ammonia excretion, it was deduced that the stomachs of the fish were half full, concluding that the post - prandial - peak is entirely a product of feed intake. It has been suggested that the appetite of a fish returns as the stomach empties and as the gut is evacuated. Consequently, it would be expected for a given species, fish of different sizes feeding at regular intervals will voluntarily regulate feed intake so that digestion is complete prior to the next feeding. Such a phenomenon would require an acclimation period to feeding regimes and diet

As the partially digested mixture enters the upper intestine the nature of gastric excretions and muscular extractions are affected by a series of nervous and hormonal feedback mechanisms, which are induced partially in response to the distention of the intestine (Jobling, 1995). Receptor cells are sensitive to chemical stimuli such as the presence / absence of certain amino

acids and free fatty acids. The length of time that food remains in the stomach varies considerably with larger particles requiring a greater period of time to be broken down. The retention of large indigestible particles such as bone and exoskeletal material congregate in the antrum region and may contribute to grinding of the more friable components of the meals, thus aiding digestion. It is found that cod in the wild preferentially eat large prey swallowed whole. They actively ingest small stones to act as gastroliths, which aid digestion of the large food particles. Once digestible material has evacuated the stomach, the pylorus will remain open to allow large indigestible solids to be removed from the stomach. In a series of experiments with Atlantic cod, it was found that regular feeding of small rations delayed emptying of the undigested solids and emptying of this material did not commence until nearly all nutrients had been removed from the food.

Gastric evacuation times vary from a few hours to a number of days depending on factors such as species, water temperature, meal size and physical characteristics of the food. In general, gastric evacuation times decrease with an increase in temperature, but will increase with an increase in ration size and feed particle size. This was shown in a study on Atlantic cod fed differing ration sizes of whole and minced herring (dos Santos *et al,* 1993), which can be seen in Table 4.1 below.

Table 4.1 Comparison of rations used in a digestibility study (dos Santos *et al,* 1993).

| | No. of Rations | Weight of ration | Total weight |
|---|---|---|---|
| 1 | 1 | 4 | 4 |
| 2 | 1 | 8 | 8 |
| 3 | 1 | 16 | 16 |
| 4 | 1 | 32 | 32 |
| 5 | 1 | 48 | 48 |
| 6 | 4 | 4 | 16 |
| 7 | 2 | 8 | 16 |
| 8 | 8 | 4 | 32 |
| 9 | 2 | 16 | 32 |

Treatments 4, 5 and 9 took the longest to digest, suggesting that particle size and number of individual rations influence digestion rates with a large ration requiring a longer period for digestion. When fed whole herring, approximately 50 % of the ration was evacuated within 24 hours. When the herring was minced, a ration of the same weight, approximately 80 % of the ration was evacuated within 24 hours.

Intestinal length will effect gut retention time and the absorption of nutrients of the partially digested mix. Atlantic salmon (*Salmo salar* L.) has an intestine length to body length ratio of 0.75 – 0.85. Atlantic cod (*Gadus morhua* L.) has an intestine length to body length ratio of 1 – 1.5. This suggests that the proportionately longer intestine of cod will increase gut retention time and absorption of nutrients.

Due to the wide scope of the tank studies at MERL, it was not possible to measure gut evacuation rates of cod in order to assess its impact upon the daily ammonia water concentration profiles. However, given the fluid consistency of cod faecal material and the rapid post - prandial - peak, it is likely that gut evacuation and the liberation of ammoniacal nitrogen contributes to this observation. Similar observations were made by Herlin (2003) who found that Atlantic cod faecal material has a high moisture content and therefore high leaching rates, exhibiting rapid dispersal once ejected from the fish. It is important to understand the relationship of gut evacuation relative to feeding regimes, diet formulation, pellet size and number of influential biological and environmental factors in order to encompass the entire aquaculture system as to how such factors contribute to dissolved nitrogenous waste production on a temporal and spatial basis. Again there is much scope for further work to investigate the influence of such factors on the timing and degree of gut evacuation.

### 4.4.4 Ammonia toxicity

Rasmussen & Korsgaard (1996) found that ammonia and the ammonium ion influence feeding when present in moderate concentrations. High water turnover rates are required to maintain optimal water conditions, maintaining high oxygen concentration and reducing the potential for accumulation of dissolved wastes such as ammonia.

Ammonia is the main end product of nitrogen metabolism in teleosts, most of which are highly sensitive to ammonia toxicity (Halver & Handy 2002; Tucker, 1998; Lovell, 1998; De Silva & Anderson, 1995; Pillay & Kutty, 1995, and Handy & Poxton, 1993). Ammonia exists as ammonia $NH_3$ and ammonium ion $NH_4^+$. The equilibrium that exists between these two states of ammonia, and thus the toxicity of ammonia is affected by environmental parameters such as temperature, pH, salinity and oxygen levels. pH has a strong influence on ammonia toxicity (Baranabe, 1994). Acute toxicity of ammonia has been investigated in a number of species but there are few studies of chronic exposure on the growth of marine fish. A study to investigate the impact of chronic ammonia exposure on the growth of juvenile cod was undertaken by Foss *et al* (2004). They concluded that when exposed to an ammonia concentration greater than 0.06 mg/l, feed intake of the juvenile cod was significantly reduced. However, even fish exposed to the highest concentration of ammonia (0.17 mg/l) still maintained a high SGR (> 1 % bw/day). The growth reduction is attributed to a decrease in food intake. Fish exposed to differing ammonia concentrations displayed an acclimatory response and by the end of the 96 - day study, differences in SGR between the exposed populations and the control population was not significant.

Carnivorous fish have an excretion efficient system of excretion of nitrogen from protein metabolism (Halver & Handy 2002; Tucker, 1998; Lovell, 1998; De Silva & Anderson, 1995; and Pillay & Kutty, 1995). At low temperatures, (Pillay & Kutty, 1995) suggest that diets containing over 40 % protein, results in stress due to excess excretion of ammonia from the

gills. This is a very general statement as ammonia toxicity is influenced by a numbers of factors, including water exchange, stocking density and fish size.

$NH_4OH$ is rare in the natural environment and is only a transition stage in the degradation of organic nitrogenous compounds, which are oxidised to nitrite and nitrate. Nitrates are utilised by plants and constitute the starting point of primary production in marine aquatic ecosystems; primary production is limited by their absence. Marine fish appear to be less sensitive to ammonia than freshwater fish with critical levels of ammonia; salmonids (0.02 mg/l), other freshwater fish (0.01 mg/l) and marine fish (0.05 mg/l). Maximum levels for long-term exposure of ammonia before FCR and growth are inhibited range from 0.06 mg/l (*O. tshawytsha*), 0.4 mg/l – 0.14 mg/l (*O. mykiss*) and 0.011mg/l (Turbot).

### 4.4.5 Protein content of commercial cod aquafeeds

Ackefors & Enell (1994) state that nitrogen content of commercial salmonid aquafeeds has been reduced through protein sparing and as a result, for every tonne of fish produced, discharges of nitrogen are approximately 53 kg. Manufacturing criteria in the production of commercial feeds is important when reducing feed-derived wastes. Extruded pellets have a lower settling velocity and a higher water stability than non-extruded pellets (leaching of dissolved wastes from uneaten food occurs over a longer period of time). At present, nitrogen content of commercial salmon diets is approximately 7 % resulting in lower volumes of nitrogenous compounds such as ammonia being excreted. This is compared to a nitrogen content in cod diets of approximately 8%. Ammonia concentration of the blood is related to protein content of the diet and rises between 3 and 8 hours following a meal. However, this value varies depending upon species, protein content of the diet and protein quality in the diet. Ammonia concentration in the blood is greater in freshwater species than marine species but urea concentration of the blood can be up to 6 times higher in marine fish (Halver & Hardy, 2002).

Daily profiles of the dissolved nitrogenous wastes (ammonia, nitrite and nitrite) measured throughout both of the tank-based studies and discussed in this chapter were taken forward to Chapters 7 (modeling) and 8 to help compile nitrogen flux models for different sized cod cultured in tank systems, both fed a range of diet formulations under different feeding regimes.

# Chapter 5

## DIETS & GROWTH PARAMETERS STUDIED IN COMMERCIAL CAGE SYSTEMS

# DIETS & GROWTH PARAMETERS STUDIED IN COMMERCIAL CAGE SYSTEMS

## 5.1 Introduction

The cage - based studies were undertaken at the No Catch® Ltd. organic cod site located in Vidlin Voe on the east coast of Shetland. The aim of the fieldwork was to investigate the effects of diets on growth, condition and tissue composition (discussed in Chapter 5), as well as the daily production of dissolved nitrogenous wastes (discussed in Chapter 6), and the production of particulate wastes (discussed in Chapter 7) associated with the commercial culture of juvenile and adult Atlantic cod (*Gadus morhua* L.) grown in a commercial cage site. Three sampling trips to study adult cod were made to the production site in Vidlin Voe in September 2005, November 2005 and February 2006. A single trip to study juvenile cod held at the nursery site in Vidlin Voe was made in April 2006.

The mouth of Vidlin Voe was to the northeast of the nursery and production sites (Figures 6.1 and 6.2), exposing the site to a dynamic environment, especially during winter months. Compared to other areas in the U.K., the tidal range in Shetland is narrow, a matter of feet between high and low tide (www.wxtide.co.uk). Water temperatures rise to approximately 13 ºC during summer months and fall to approximately 8 ºC during the winter (data supplied by No Catch® Ltd.). Data obtained from hydrographic studies carried out by No Catch® Ltd. and is shown in Appendix 18.

The production cages were used for the ongrowing of cod prior to harvest. The site consisted of 17 Polar Cirkels cages each measuring 30 m in diameter, which were moored in approximately 30 m depth of water. The nursery site consisted of two cage blocks (both blocks consist of 14 cages that each measure 7 m by 7 m), which were moored in approximately 15 m of water. The nursery cages were used for rearing juvenile cod prior to transfer to the production cages.

All feeding at No Catch® Ltd. was controlled by computer. Consequently, all data concerning feed input, feeding regime, cage biomass, size of fish and all growth parameters in the sampled cages at the production site, and the nursery site were held on computer and supplied for the relevant sampling days (Appendix 19).

The diets used by No Catch® Ltd. were supplied by Biomar® in Grangemouth. A well - boat was used to transfer the diet from Lerwick to the feed barges at Vidlin. Transfer time from Grangemouth to Vidlin was approximately two days. Deliveries varied in size and frequency according to changes in feeding rates but are ordered so that the diet would be on the barge for no longer than two weeks prior to feeding. All feeding was controlled by computer and distributed from the feed barges using air pressure, through floating pipes to each cage.

## 5.2 Adult Atlantic cod in cage systems

### 5.2.1 Adult stock

The cod that were sampled from the production site originate from Machrihanish Marine Farm (MMF) and were spawned from wild broodstock between October 2003 and December 2003. The advanced spawning at MMF was induced through photoperiod manipulation. The vaccinated cod arrived at Vidlin in September 2004, each weighing approximately 30 g. After 14 months following transfer to the sea, the cod had reached roughly 1300 g and were maturing to spawn in early 2006.

### 5.2.2 Adult diet & feeding regime

The adult fish were fed at a rate of 0.5 % b/w per day over two extended meals on a commercial adult cod diet as a 12 mm pellet. Each meal lasted approximately 3 hours during which each cage was fed an equal volume of the ration at 5 - minute intervals over the 3 hour duration. The approximate protein content of the diet fed to the adult fish was 52 %. The timing of meals and

ration size were determined by biomass and feeding behaviour, which was influenced by daylight hours and water temperature. Longer daylight hours and favourable temperatures during summer months permitted increased feeding compared to winter months.

Approximately 60 % of the daily ration was fed in the morning meal. The timing and rationing of both meals was primarily determined by day – length. Rations and meal duration were monitored daily and amended if required. The feed barge moored at the production site (positioned in place of a Polar Cirkel cage) supplied feed to all the production cages. Air pressure carried each pre - determined ration of feed from the feed barge via floating pipes to the cages, where the pellets were deflected off a small metal sheet to disperse the pellets across the cage, allowing all fish access to food.

## 5.3 Juvenile Atlantic cod in cage systems

### 5.3.1 Juvenile stock

The juvenile cod investigated came from two different sources. Those in cage 6 were hatched at Nu Fish® Ltd. (a hatchery owned and operated by No Catch® Ltd.) in mid - August 2005 and were stocked in February 2006, weighing approximately 32 g by April 2006. Those in cages 14 and 16 were hatched at MMF in April 2005 and transferred to Vidlin between October and November 2005, weighing approximately 75 g in April 2006.

### 5.3.2 Juvenile diet & feeding regime

The juvenile fish were fed approximately 1.5 % b/w per day on a commercial juvenile diet containing approximately 57 % protein as a 3 mm pellet. The timing of meals and ration size were determined by biomass and feeding behaviour, which was influenced by daylight hours and water temperature. Longer daylight hours and favourable temperatures during summer months permitted increased feeding compared to winter months. The juvenile cod were fed five

meals every day; each meal contributed to 20 % of the daily ration. Rations and meal duration were monitored daily and amended if required. During each meal, all the cages were fed a small ration every 5 minutes, giving each cage up to 70 individual rations of 200 g over the course of each meal. A second feed barge is moored between the two cage blocks at the nursery site, supplying feed to all the nursery cages on both cage blocks. Air pressure carried the feed from the feed barge via a floating pipe to each cage. The pipe reaches the centre of each cage where the air pressure fires the feed off a small metal sheet dispersing the pellets across the cage, allowing all fish access to food.

## 5.4 Sampling regime & analysis

During each sampling trip, a sample of each diet was collected from the respective feed barge (therefore representative of the current feeding) and frozen (- 20 $^{\circ}$C ± 1 $^{\circ}$C) prior to analysis for proximate composition (Section 2.4.1). Following each sampling trip to Vidlin, twelve fish were randomly caught from one of the sampled cages by the staff at No Catch® Ltd. and sent on ice to the Institute of Aquaculture for analysis of growth (Section 2.5.1) K - Factor (Section 2.5.4), HSI (Section 2.5.5) and tissue composition (Section 2.6). FCR data and SGR data were supplied by No Catch® Ltd.

## 5.5 Results - diets

### 5.5.1 Proximate composition

Table 5.1 shows proximate composition of the commercial adult diet fed at the production site and Table 5.2 shows the composition of the juvenile commercial diet fed at the nursery site. All raw data used to compile Tables 5.1 and 5.2 are shown in Appendix 20.

Table 5.1 Proximate composition of 12 mm Biomar® diet (With the exception of the moisture and energy values, all measurements are % dry weight).

| | | Sept. 2005 | Nov. 2005 | Feb. 2006 |
|---|---|---|---|---|
| Component | Biomar® | IoA | IoA | IoA |
| | | | | |
| Moisture (%) | | 13.20 | 11.5 | 11.6 |
| Ash (%) | 14.0 | 12.22 ±0.0 | 13.3 ± 0.1 | 12.5 ± 0.1 |
| Lipid (%) | 14.0 | 14.14 ± 0.0 | 14.5 ± 0.1 | 14.7 ± 0.1 |
| Protein (%) | 57.0 | 50.9 ±0.9 | 52.9 ± 0.1 | 52.5 ± 0.0 |
| Energy (kJ) | | 20.7 ±0.0 | 20.8 ± 0.0 | 20.7 ± 0.1 |
| Nitrogen (%) | 9.1 | 8.62 | 8.5 | 8.9 |

Table 5.2 Proximate composition of 3mm Biomar® diet. (With the exception of the moisture and energy values, all measurements are % dry weight).

| | | Apr. 2006 |
|---|---|---|
| Component | Biomar® | IoA |
| | | |
| Moisture (%) | | 10.1 |
| Ash (%) | 14.0 | 14.3 ± 0.0 |
| Lipid (%) | 14.0 | 14.7 ± 0.2 |
| Protein (%) | 57.0 | 58.4 ± 0.5 |
| Energy (kJ) | | 20.6 ± 0.1 |
| Nitrogen (%) | 9.1 | 9.6 |

It can be seen from the Table 5.1 that composition values are similar for the diet formulation throughout the adult study. Only a single trip was made to investigate the juvenile site so only a single sample of diet was collected and analysed (Table 5.2) therefore it was not possible to examine fluctuations in proximate composition of the juvenile diet over a period of time.

Subtle differences in the adult study may be attributed to differences between batches of diet. It was also noted that the IoA analysis provided similar results in composition as those found by Biomar®. Standard deviation was also small suggesting that there was little variation between

the replicates. This showed accuracy as the single samples were sub-divided and analysed. It could be seen that although lipid content between the juvenile and adult diets was similar, higher protein content was used for juvenile cod. This suggests that the inclusion rate of protein in the Biomar® diets is optimal for juvenile cod. Similar results were found in the juvenile tank studies where a higher protein (50 % - 60 %) diet promoted the greatest growth and condition in the fish.

## 5.6 Results - growth parameters

As stated in Section 5.4, twelve fish were sacrificed following each sampling trip to Vidlin Voe. Lengths and weights were taken allowing the K - Factor to be calculated. The HSI was also calculated giving a further indication of the condition of the fish. No Catch® Ltd. provided FCR and SGR (Appendix 19). The results obtained from the adult study are shown in Table 5.3 with the juvenile results shown in Table 5.4. All raw data is shown in Appendix 22.

Table 5.3 Growth parameters (± 1 SD) of adult cod measured over the 3 sampling trips.

| Parameter | Sept. '05 | Nov. '05 | Feb. '06 |
|---|---|---|---|
| Weight * | 1330 ± 197 | 1853 ± 340 | 2185 ± 829 |
| Length * | 466 ± 31 | 515 ± 31 | 563 ± 63 |
| K-Factor * | 1.31 ± 0.18 | 1.34 ± 0.07 | 1.6 ± 0.20 |
| Food Conversion Ratio | 1.2 | 1.2 | 1.2 |
| Specific Growth Rate | 0.4 | 0.35 | 0.23 |
| Hepatosomatic Index* | 10.38 ± 1.39 | 9.37 ± 1.04 | 8.77 ± 2.52 |

*Denotes mean value calculated from the twelve sacrificed fish.

Table 5.4 Growth parameters (± 1 SD) of juvenile cod measured over the single sampling trip.

| Parameter | April '06 |
|---|---|
| Weight * | 58 ± 14 |
| Length * | 172 ± 12 |
| K-Factor * | 1.12 ± 0.09 |
| Food Conversion Ratio | 1.5 |
| Specific Growth Rate | 0.87 |
| Hepatosomatic Index * | 9.68 ± 1.04 |

*Denotes mean value calculated from the twelve sacrificed fish.

The above parameters show the fish in the commercial study to be in a better condition than the fish in the tank studies.

## 5.7 Results - proximate composition of tissue

### 5.7.1 Proximate composition of adult tissue

Tissue (muscle and liver) sampled from the twelve sacrificed fish (September 2005, November 2005 and February 2006), were analysed for proximate composition (Section 2.6.2). Proximate composition of adult muscle is shown in Table 5.5 while the proximate composition of adult liver is shown in Table 5.6. Raw data of proximate composition of adult muscle is shown in Appendix 23 and the proximate composition of adult liver is shown in Appendix 24.

Table 5.5 Proximate composition (% ± 1 SD) of adult muscle.

| Component | Sept. 2005 | Nov. 2005 | Feb. 2006 |
|---|---|---|---|
| | | | |
| Moisture | 78.59 ± 0.19 | 78.44 ± 0.21 | 77.97 ± 0.66 |
| Ash | 1.37 ± 0.05 | 1.28 ± 0.13 | 1.38 ± 0.11 |
| Lipid | 0.31 ± 0.05 | 0.31 ± 0.07 | 0.21 ± 0.03 |
| Protein | 19.93 ± 0.55 | 19.93 ± 0.49 | 20.37 ± 0.11 |
| Nitrogen * | 14.8 ± 0.04 | 14.76 ± 0.16 | 14.94 ± 0.37 |

* Nitrogen is a percentage of protein (wet weight).

Table 5.6 Proximate composition (% ± 1 SD) of adult liver.

| Component | Sept. 2005 | Nov. 2005 | Feb. 2006 |
|---|---|---|---|
| | | | |
| Moisture | 22.51 ± 0.32 | 24.01 ± 0.13 | 27.16 ± 4.70 |
| Ash | 4.03 ± 0.02 | 4.03 ± 0.08 | 5.97 ± 0.10 |
| Lipid | 70.04± 0.23 | 64.70 ± 0.68 | 59.91 ± 2.02 |

It can be seen that proximate composition of the adult muscle remained constant throughout the investigation. Analysis suggests that differences between sampling trips were not significant for protein ($n = 12$, $F = 3.01$, $P = 0.063$) or nitrogen ($n = 12$, $F = 1.37$, $P = 0.269$). The slight drop in lipid content in February was due to the liberation of energy stores from muscle during gametogenesis.

Analysis of nitrogen content of the liver was not possible due to the consistency of the dry weight material. Accurate protein content of the liver was also difficult to measure due to the consistency of the dry weight material. Consequently, protein and nitrogen are not discussed. The larger standard deviation observed for moisture and lipid content of the liver in February is due to increased variability as fish are in different stages of gametogenesis and possibly precocious spawning. Over the course of the investigation, relative lipid content of the liver dropped and moisture content increased, showing the inversely proportional relationship

between moisture and lipid. It can be seen that as lipid levels in the liver fell, the relative moisture content of the liver increased.

### 5.7.2 Proximate composition of juvenile tissue

Tissue sampled (cleaned carcass and liver) from the twelve juvenile fish sampled in April 2006, were analysed for proximate composition (Section 2.6). Proximate composition of the cleaned carcass and pooled[+] juvenile liver are shown in Table 5.7, with all raw data recorded in Appendix 25.

Table 5.7 Proximate composition (% ± 1 SD) of juvenile tissues.

| Component | Carcass | Liver |
|---|---|---|
| | | |
| Moisture | 80.63 ± 0.35 | 37.01 ± 1.01 |
| Ash | 2.84 ± 0.28 | 0.61 ± 0.13 |
| Lipid | 0.57 ± 0.10 | 50.18 ± 0.48 |
| Protein | 18.02 ± 1.06 | |
| Nitrogen | 12.45 ± 0.16 | |

* Nitrogen is a percentage of protein (wet weight).

[+] The liver samples were too small to analyse individually and were randomly pooled into three groups, which were analysed for proximate composition.

## 5.8 Discussion

As little other work has been undertaken in this area, much of the discussion is related to other studies and considers factors that may influence the results found in the currents studies.

### 5.8.1 Diets

Proximate composition of the adult diet varies little for throughout the three sampling trips. Subtle differences may be attributed to differences between batches of diet. Standard deviation is also small and there was little variation between the replicates, which showed accuracy, as the single samples were sub - divided and analysed. It could be seen that although lipid content between the juvenile and adult diets was similar, higher protein content was used for juvenile cod. Similar findings were made in the juvenile tank study (a higher protein content is required) suggesting that the commercial Biomar® diet for juvenile cod is accurate.

Being pelagic and carnivorous, wild cod feed mainly in daylight hours relying on sight to seek their prey. Consequently, it can be deduced that feeding during daylight hours only would increase food uptake and growth, and optimise nutrient utilisation. Ingestion rates could not be measured during the cage studies at No Catch® Ltd. However, in a commercial situation under an automated species - specific feeding regime, ingestion rates of 95 % are used as a feeding control.

A reduction in feeding during the winter months is discussed by Ramnarine *et al*, (1987) although whether this reduction in appetite is due to the winter conditions or the onset of maturation remains unclear. In a study of adult fish, Bjornsson & Steinarsson (2002) state that the optimal temperature for feeding decreases as the fish grow, suggesting that the reduction in ingestion rates are primarily due to maturation. The reduction in winter - feeding and precocious maturation of larger juveniles may have a greater influence on the ingestion rates associated with the juvenile fish. Extensive studies in salmonids show that feeding is affected by a number

of abiotic factors such as photoperiod and temperature; physical factors such as wave and currents, and chemical factors which include oxygen, dissolved nitrogen compounds, pH and salinity. Feeding is most likely to vary with the photoperiodic cycle, exhibiting an increase in feeding in spring and a decrease during autumn months (Eriksson & Alanara, 1992). The inverse relationship between body fat content and feed intake explains a larger increase in appetite following periods when energy reserves are likely to be depleted such as those observed after a prolonged period of reduced feeding during the winter months. Consequently, feeding regimes developed for cod need to be extremely accurate. Factors such as season and the development of sexual maturity need to be accounted for to exploit differing foraging behaviour, while keeping uneaten feed losses to an absolute minimum.

As faecal material was not collected from the Atlantic cod studied at No Catch® Ltd., nitrogen digestibility could not be analysed. However, from studies undertaken at MERL, nitrogen digestibility is related to nitrogen content of the diet. With adult cod in tank systems, nitrogen digestibility (as a percentage of nitrogen ingested) remains constant around 60 % regardless of protein content. However, it was observed with juvenile fish, that there was a significant decrease in nitrogen digestibility with an increase in nitrogen content of the diet.

With a carnivorous species such as cod, the most efficient digestibility occurs when carbohydrate is present at very low inclusion (20 % - 30 %). No negative effects of nitrogen digestibility have been observed for cod as a consequence of increased carbohydrate levels (Hemre *et al*, 1990). Percival *et al* (2001) found that when analysing the apparent digestibility coefficient (ADC) for protein in Atlantic salmon, the dissection method of faecal collection produced lower values than those obtained by the stripping method. A similar study carried out on Atlantic cod (Hemre *et al*, 2003;b), found very small variations in ADC for dry matter, protein, lipid and starch, with a high correlation between the two methods for all entities examined. This supports the conclusion that sampling methods (dissection or stripping) did not have an influence on digestibility results. For protein, the correlation between the two methods

was weaker but still significant. ADC for protein varied from 76 % - 90 % with the average for all diets investigated at 85 % ± 5 % (protein content ranged from 36 % - 66 % in the investigated diets). These results were found to be in accordance with similar research for cod (Hemre *et al*, 1990; Lied & Njaa, 1982).

### 5.8.2 Growth & condition indices

Growth of adult cod in cage systems was steady over the 3 sampling trips to the production site. The Biomar® diet fed at No Catch® Ltd. was of a similar composition to Diet B fed at MERL (50 % protein: 12 % - 14 % lipid). However, due to different feeding regimes, condition factors, stocking densities and environments, growth was not directly comparable.

As protein is the most expensive component of aquafeeds, lower protein content would be economically beneficial and would reduce nitrogen excretion (both particulate and dissolved) into the marine environment. Replacing protein with carbohydrate may be possible but would lead to increased carbon pollution. Future work would be required to investigate commercial cod diet formulations that would maximize growth while keeping carbon and nitrogen pollution at minimal or sustainable levels. When discussing feeding regimes, Lambert and Dutil (2000) observed that feeding adult cod to satiation three or five times weekly promoted greater growth than those fed to satiation twice weekly. However, at higher stocking densities above 40kg/$m^3$, those fed to satiation five times weekly showed better growth than those fed three times weekly. In a commercial situation, optimising growth is essential. As discussed in Chapter 3, it appeared that higher protein levels are required for juvenile cod. This phenomenon was mirrored at No Catch® Ltd. where a high protein diet (57 %) was fed to the juvenile cod. Foster *et al* (1993) found that growth rates of juvenile fish were greater when the fish were fed every day as opposed to alternate days. The current work combined with the observation of Lambert & Dutil (2000) and Foster *et al* (1993) suggest that juvenile cod require a high protein diet fed

frequently to maintain maximum growth. As the fish grow, protein levels in the diets and feeding frequency may be lowered while maintaining growth. As cod preferentially eat prey swallowed whole (Clark *et al* 1995; dos Santos *et al*, 1993), it is imperative that cod should be fed the largest pellet size possible in order to minimise energy exerted when foraging. However, further work into formulations, pellet size and feeding regimes is required for both juvenile and adult fish to maintain optimal growth throughout the culture cycle.

Food Conversion Ratio noted for the adult cod remained constant at 1.2 between September 2005 and February 2006. The FCR recorded for the juvenile cod was 1.5 (April 2006). The constant values recorded were attributed to automated feeding, which is altered on almost a daily basis exploiting the feeding behaviour of the cod.

Specific Growth Rates were higher in juveniles (SGR of 0.87 measured in April 2006) compared to the adult cod (0.4 in September 2005, 0.35 in November 2005 and 0.23 in February 2006). As both the juvenile and adult cod were fed consistently throughout each day, SGR is related to size of fish (Bjornsson & Steinarsson, 2002). A decreasing SGR between September 2005 and February 2006 can be attributed to an increase in fish size over the period. A decrease in SGR is mirrored by a decrease in the HSI over the same period (possible links between the two parameters are discussed at the end of Chapter 3). As feeding rates and Food Conversion Ratios remain the same over the course of the study, it may be concluded that the maturing fish direct ingested energy, and also liberate energy stores from the liver, toward the production of gametes.

The fish in the cage study were in better condition than the fish studied in the tank investigations. This was most probably due to a continual growth pattern at No Catch® Ltd. promoted by a more consistent diet formulations and feeding regimes (fish at MERL were subject to different investigations, feeding regimes and feed formulations). All of which would have a detrimental impact of body and tissue morphology as well as condition and HSI.

Similarly, the HSI measured in the fish sampled from No Catch® Ltd. were consistently higher than the HSI measured in both tank studies (10.38 % ± 1.39 % (Sept. 2005), 9.07 % ± 1.04 % (Nov. 2005) and 8.77 % ± 2.52 % (Feb. 2006)). In the juvenile study, the HSI was measured at 9.68 % ± 1.04 %. The higher HSI values can be attributed to a more consistent feeding regime and diet compared to the experimental diets and regimes studied at MERL. A falling HSI observed in the adult study at No Catch® Ltd can be attributed to maturation and the liberation of energy stores for the production of gametes. The onset of maturation results in a decrease in growth rate as energy is directed from anabolism to the production of gametes. It would be beneficial to the industry to delay maturation of adult cod so that energy is directed toward anabolism (growth) for a larger proportion of the year. The use of photoperiod to delay maturation would increase growth rates and reduce the growout period. Such a scenario gives rise to future work looking photoperiod with a combination of different diets and regimes to delay maturation and reduce the reconditioning period following maturation.

### 5.8.3 Tissue composition

Protein (and nitrogen) levels retained in the fish remained constant over the course of the cage investigations. As protein is approximately 14 % ± 1 % nitrogen, both protein and nitrogen content was analysed to confirm actual levels. Protein and nitrogen of the flesh and liver of juvenile and adult fish were analysed. Dry weight adult flesh averaged approximately 93 % protein and 15 % nitrogen. Dry weight juvenile carcass (whole cleaned) fish exhibited an average protein content of 80 % protein and 12.5 % nitrogen. The lower levels exhibited in the juvenile fish were due to the whole carcass being analysed (skeleton inclusion increases ash levels considerably). Such results are similar to those results obtained from the tank studies, and the standard deviation observed throughout all studies were small, suggesting that the proximate composition of the adult cod flesh and juvenile carcass in the tank and cage studies are similar.

There are documented relationships between oil and water, and protein and water in fish tissue Foster *et al*, 1992). However, such relationships were only observed in the liver sampled from the sacrificed fish in the adult cage studies, mirroring the relationship observed in adult liver sampled throughout the tank study. Moisture, ash, lipid, protein and nitrogen measured in adult and juvenile tissue were much more consistent in the flesh than in the liver samples. This suggested that the liver composition (primarily lipid) was affected to a greater degree by changes in condition and maturation. As all excess lipid in cod is deposited in the liver, changes in condition and maturation will effect the lipid content of the liver (lipid liberated for energy) and consequently, HSI and lipid content of the liver can be indicative of the condition of the fish.

Although the fish grow in weight and length throughout the investigation, the HSI and SGR values fell, a phenomenon that may be linked to reduced winter feeding and the onset of sexual maturation (Ramnarine *et al*, 1987) and also due to both increased catabolism of ingested energy and through the liberation of energy stores for the production of gametes.

The relevant results discussed in this chapter such as diet composition, estimated ingestion rates, growth and condition parameters were taken forward to Chapters 7 (modeling) and 8 to help produce nitrogen flux models associated with the culture of juvenile and adult Atlantic cod in commercial cage systems.

# Chapter 6

## DISSOLVED NITROGENOUS WASTE PRODUCTION ASSOCIATED WITH THE COMMERCIAL CULTURE OF ATLANTIC COD IN SEA-CAGES

# DISSOLVED NITROGENOUS WASTE PRODUCTION ASSOCIATED WITH THE COMMERCIAL CULTURE OF ATLANTIC COD IN SEACAGES

## 6.1 Introduction

The cage - based studies were undertaken at the No Catch® Ltd. organic cod site located in Vidlin Voe on the east coast of Shetland. One of the aims of the fieldwork was to investigate the production of dissolved nitrogenous wastes associated with the commercial culture of juvenile and adult Atlantic cod (*Gadus morhua* L.) grown in a commercial cage site. This chapter discusses the daily excretion rates of ammonia, nitrite and nitrate associated with the commercial culture of Atlantic cod in cage systems. The collection of water samples used to study dissolved nitrogenous waste production was undertaken concurrently with the work carried out when studying diets, growth, condition and tissue composition as discussed in Chapter 5. Three sampling trips to study adult cod were made to the production site in Vidlin Voe in September 2005, November 2005 and February 2006. A single trip to study juvenile cod held at the nursery site in Vidlin Voe was made in April 2006. Details of both sites can be seen in Figure 6.1 and 6.2.

Water samples were collected from three cages at 45 - min. intervals over the course of three consecutive days on each trip. The sampling strategy was designed so that water samples were be collected over the course of each 8 - hour day during times of feeding and when not being fed. It was considered that such a strategy may give an indication of nitrogenous waste excretion rates in relation to feeding.

## 6.2 Site Layout

Vidlin Voe is located on the east coast of Shetland. The mouth of the Voe is to the North East exposing the voe to a dynamic environment. Figure 6.1 shows an aerial view of the Voe and surrounding area. Tables 6.1 and 6.2 give the GPS co-ordinates of the nursery site and production site in Vidlin Voe.

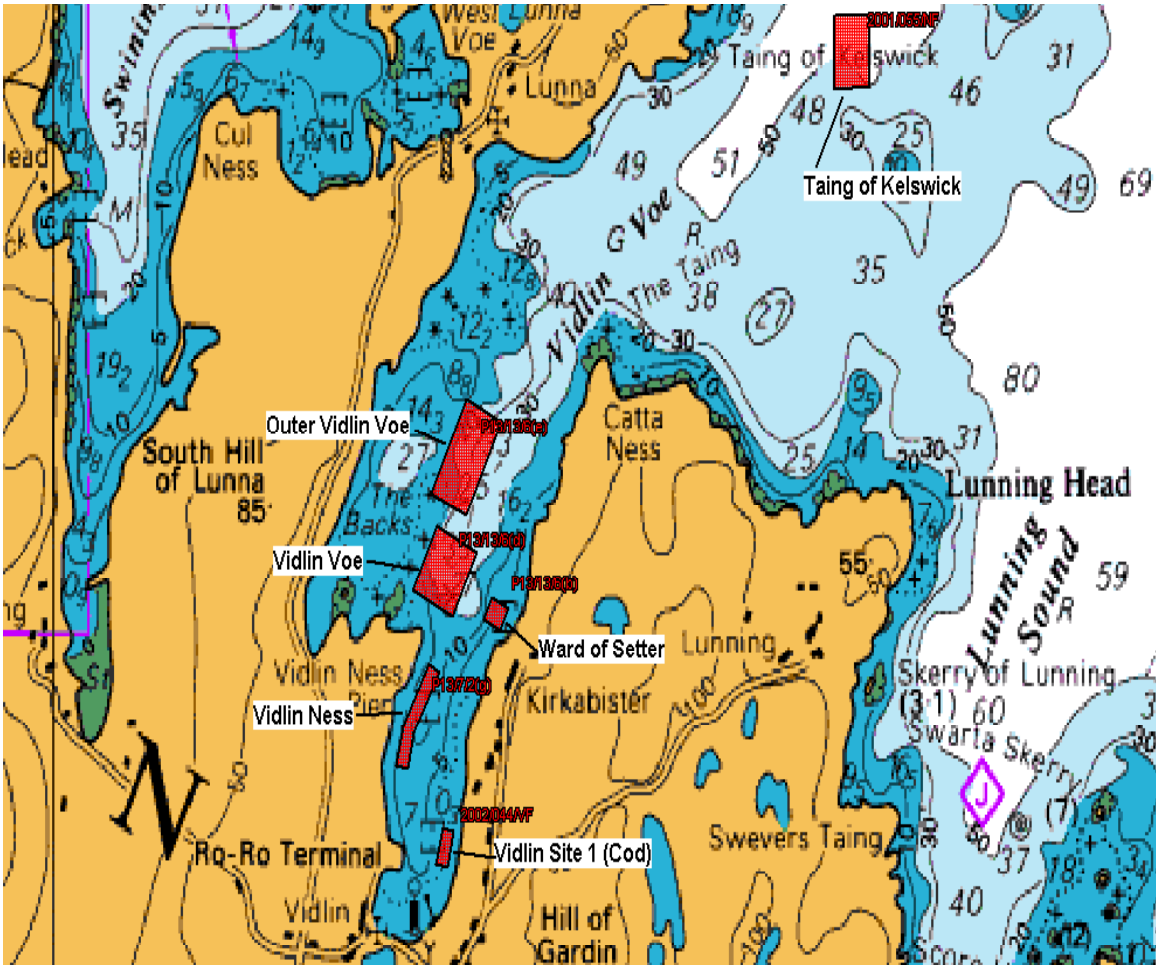



Figure 6.1 Bathymetric map of Vidlin Voe.

On Figure 6.1, the production site is represented by the two red shaded blocks in the centre of the Voe and the nursery site by the narrow red shaded area closer to the head of the Voe and the Western shore (marked as Vidlin Ness).

Table 6.1 GPS co-ordinates of the four corners of the nursery site in Vidlin Voe.

| | Northings | Westings | HU |
|---|---|---|---|
| NW | 60º 22.698 N | 1º 07.766 W | 48100 / 66343 |
| NE | 60º 22.696 N | 1º 07.746 W | 48118 / 66337 |
| SW | 60º 22.665 N | 1º 07.798 W | 48071 / 66280 |
| SE | 60º 22.661 N | 1º 07.778 W | 48090 / 66271 |

Table 6.2 GPS co-ordinates of the four corners of the production site in Vidlin Voe.

| | Northings | Westings | HU |
|---|---|---|---|
| SW | 60º 23.182 N | 1º 07.467 W | 48363 / 67241 |
| SE | 60º 23.172 N | 1º 07.420 W | 48405 / 67227 |
| NW | 60º23.402 N | 1º 07.262 W | 48545 / 67652 |
| NE | 60º23.388 N | 1º 07.205 W | 48572 / 67628 |

## 6.3 Methods

Please note that throughout this chapter that the three cages utilised at the production site are three replicates and the reported cages numbers merely show the position of the cage for the author reference. The same scenario is true of the three cages utilized at the nursery site. Therefore, the three cages investigated at each site should be treated as three replicates. The three cages at both the production and nursery site held the same fish throughout each study. That is to say, no fish were added to, or harvested from these cages and the only fluctuation is fish numbers would be due to an accepted mortality rate.

### 6.3.1 Collection of water samples

Duplicate water samples were collected from each of the three adjacent cages every 45 min. from approximately 9 am to 4.30 pm over the course of three consecutive days during each sampling trip. The timing and number of samples collected were determined by daylight hours, weather conditions and to a lesser degree, feeding times. The feeding of the adult cod occurred over two meals a day with a set number of equal rations being fed over a set period of time i.e. each meal. The juvenile cod were fed five meals per day; a set number of equal rations being fed over a set period of time.

In the adult study, the same three cages were sampled on each trip. These cages were located at the northeast end of the production site. In the juvenile study, water samples were collected from three cages on the 'inner' cage block of the nursery site. Water samples were collected using a weighted plastic pipe measuring 5 m in length and 2 cm in diameter. A stopper was placed in one end of the tube in order to create a vacuum, after which the pipe was lowered into the cage to a depth of approximately 4 m. The stopper was then removed and the pipe filled with water before the stopper was replaced.

The pipe was retrieved and emptied into an acid - washed and sample - rinsed, labelled 500 ml polypropylene bottle and placed in a cool box until filtering. The sample bottle was filled and the excess water allowed to over - flow from the bottle. The position from where the sample was collected at each cage was recorded using handheld GPS. The positions are shown in Table 6.3 below.

Table 6.3 GPS positions of collection points for water samples.

| Production Site | | | |
|---|---|---|---|
| | Northings | Westings | HU |
| Cage 1 | 60º 23.402 | 1º07.262 | 48545/67652 |
| Cage 10 | 60º 23.388 | 1º07.205 | 48598/67628 |
| Cage 11 | 60º 23.364 | 1º07.234 | 48572/67584 |
| | | | |
| Nursery Site | | | |
| | Northings | Westings | HU |
| Cage 6 | 60º 22.686 | 1º 07.765 | 48101/66319 |
| Cage 14 | 60º 22.688 | 1º 07.767 | 48099/66321 |
| Cage 15 | 60º 22.694 | 1º 07.763 | 48102/66332 |

### 6.3.2 Hydrography

Hydrographic details for Vidlin Voe are shown in Appendix 18. However, water samples were collected from a depth of 4 m and it can therefore be deduced that surface currents and wind speed will effect the movement of surface water and therefore the dispersal of dissolved nitrogenous wastes in the surface water. In turn, this could have an effect on the results obtained. On the following page, Figure 6.2 shows the current flow measured at the surface in Vidlin Voe, while Figure 6.3 shows the wind speed measured from the production site in Vidlin Voe.

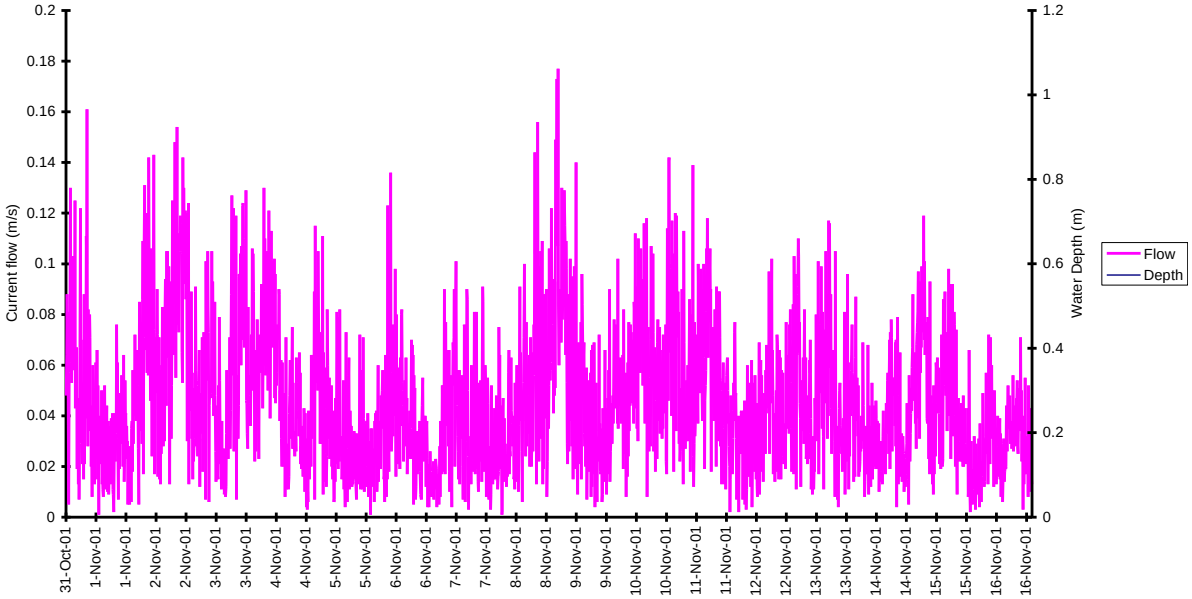



Figure 6.2 Current flow at surface in Vidlin Voe.

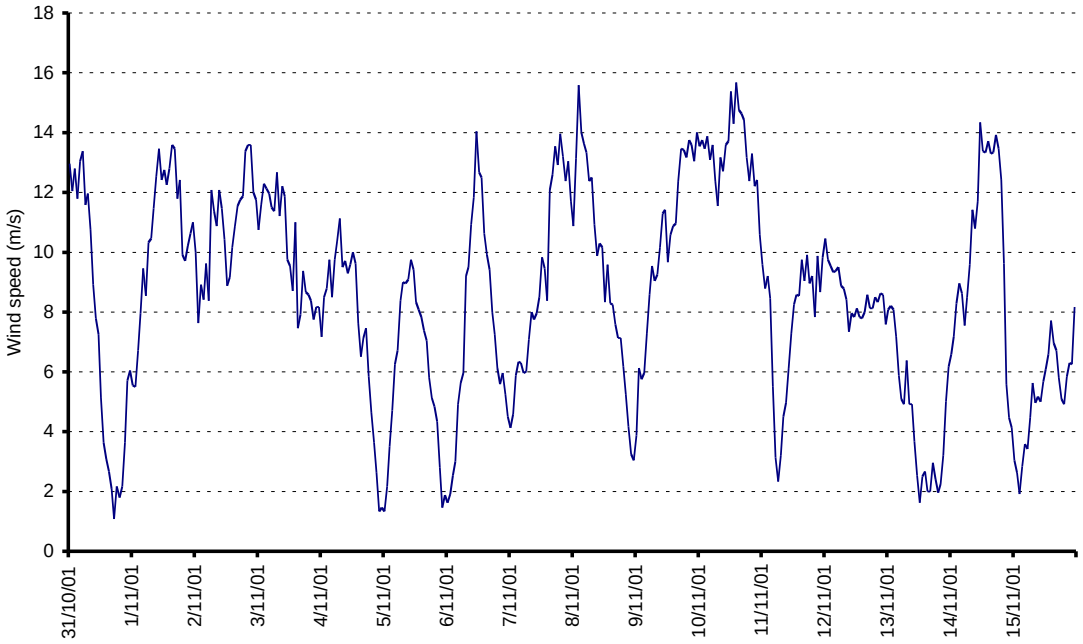



Figure 6.3 Wind speed measured from production site in Vidlin Voe.

When comparing Figure 6.2 (current flow) to Figure 6.3 (wind speed), it can be seen that there is a strong correspondence between wind speed and current speed. From Appendix 18, it can be seen that currents enter the Voe (South Westerly direction) along the seabed and leave the Voe (North Easterly direction) through the surface water. Large variation in wind speed and current speed will undoubtedly have a large influence on the temporal and spatial dispersion of dissolved nitrogenous wastes.

### 6.3.3 Filtration & analysis

Approximately 5 min after the samples were collected, they were filtered through 'Fisherband' Glass Microfibre filter paper 'MF200' in a Millipore vacuum filter. The samples were then frozen (-20 $^{\circ}$C ± 1 $^{\circ}$C) prior to transportation in cool boxes to the Institute of Aquaculture for analysis. The samples were later thawed and analysed for ammonia, nitrite and nitrate using a Digital Calorimeter (Bran & Luebbe, auto Analyzer 3). Due to different fish biomass values in each cage, the results were converted to µg/L/ tonne ww fish.

### 6.3.4 Statistical analysis

Statistical significance of differences in ammonia concentration in the water samples were analysed using a two-way ANOVA implementing Minitab™ version statistical software (Ryan & Joiner, 1994). In all cases, where $P < 0.05$, the statistical difference was considered significant.

## 6.4 Results - dissolved nitrogenous wastes

The dissolved nitrogenous wastes associated with the commercial culture of adult and juvenile Atlantic cod cultured at No Catch® Ltd. were analysed and represented graphically to monitor daily fluctuations in dissolved nitrogenous waste production in relation to feeding. As discussed earlier, there is a constant breakdown from Ammonia to Nitrite and Nitrate. A remit for the thesis was to quantify the production of dissolved nitrogenous wastes associated with commercial cod farming. As such, the water samples collected at No Catch® Ltd. were analysed for Ammonia, Nitrite and Nitrate. However, given that Ammonia is the most significant dissolved waste product of finfish aquaculture, (Pillay & Kutty, 2005; Halver & Handy, 2002; Tucker, 1998; DeSilva & Anderson, 1995 and Jobling 1994) and the sheer volume of results, only Ammonia profiles are discussed in the thesis with all dissolved nitrogenous waste values shown in Appendix 26.

The daily ammonia profiles for each sampling trip to the production site are shown in Figure 6.4 (September 2005), Figure 6.5 (November 2005) and Figure 6.6 (February 2006). The daily ammonia profiles for the sampling trip to the nursery site in April 2006 are shown in Figure 6.7. Weather conditions observed during each water-sampling day throughout both studies are shown in Appendix 27.

It should be noted that the cage numbers used in the figures are purely identifying the cage sampled (cages 1, 10 and 11 in the adult study and cages 6, 14 and 15 in the juvenile study). In each case the three cages are replicates of a single treatment and not three individual treatments. The only differing factors between the cages are fish size and biomass and consequently, the daily volume of feeding (See Appendix 19). The arrows in Figures 6.4 - 6.7 represent the times of feeding during the day.

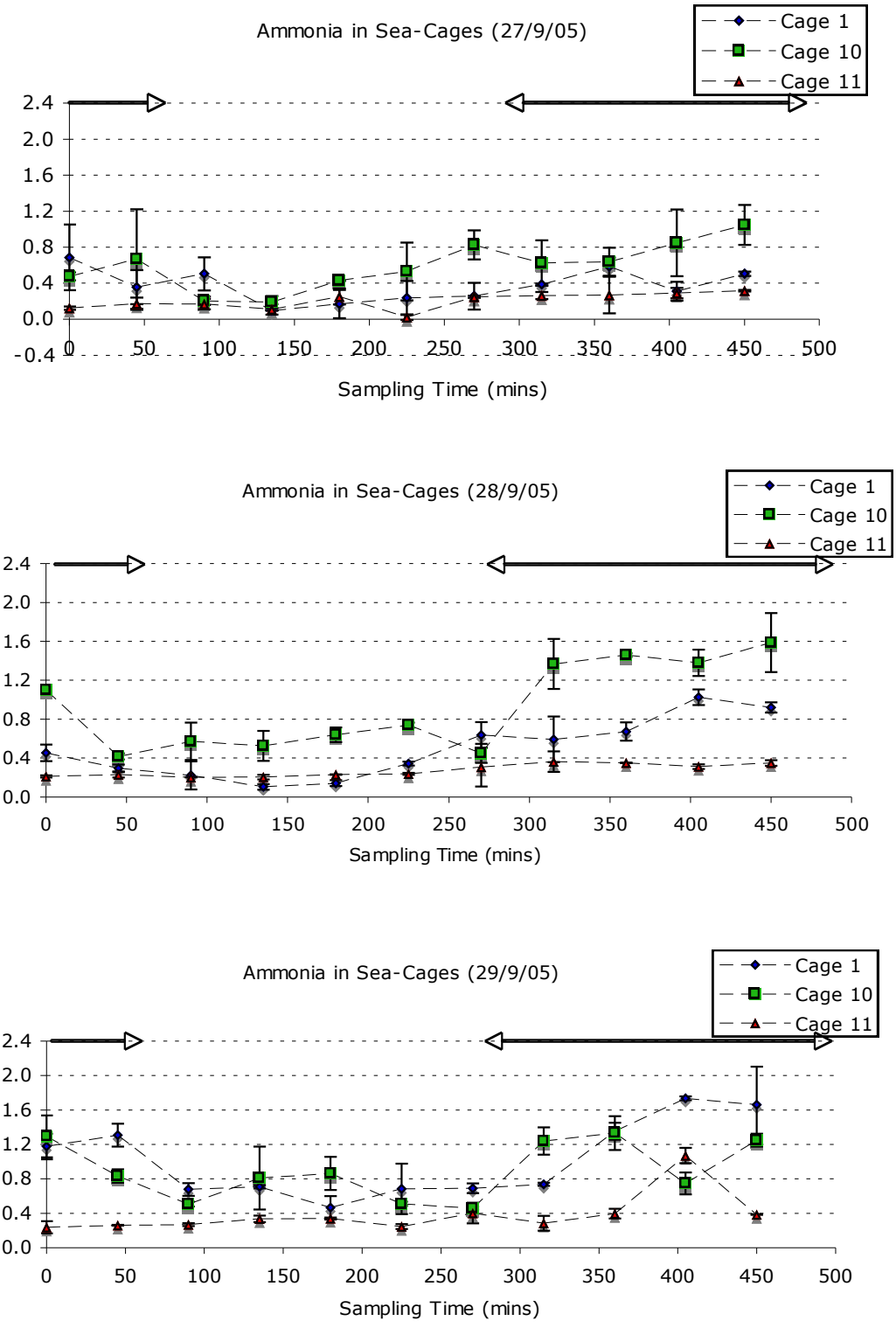



Figure 6.4 Daily ammonia profiles measured in September 2005 (error bars are ± 1 SD).

The arrows represent feeding times.

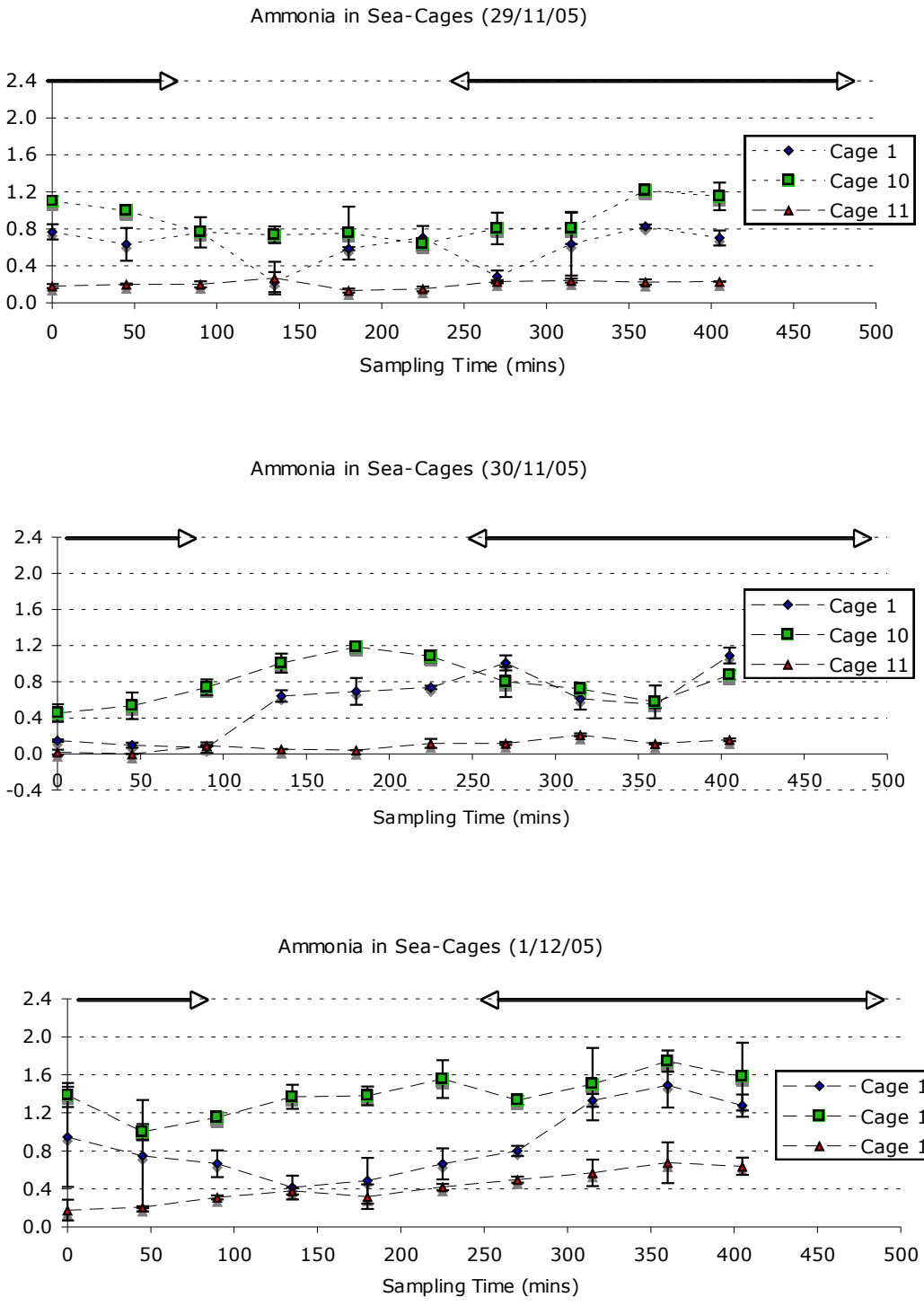



Figure 6.5 Daily ammonia profiles measured in November 2005 (error bars are ± 1 SD).

The arrows represent feeding times.

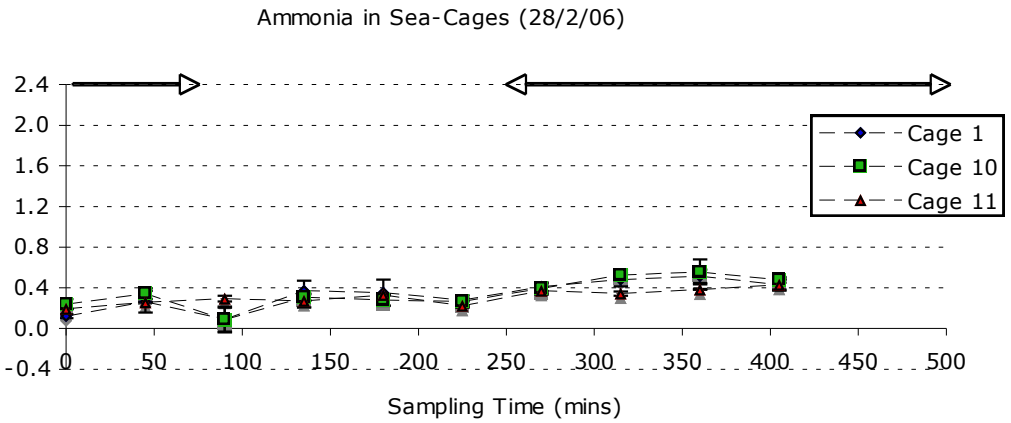



Figure 6.6 Daily ammonia profiles measured in February 2006 (error bars are ± 1 SD).

The arrows represent feeding times.

A relationship between feeding and ammonia concentration of the sampled water is observed throughout the three sampling days in September 2005 (Figure 6.4). A drop was noted following termination of the morning meal at 50 min., thereafter, ammonia levels remained at a lower level until the afternoon meal commenced at 270 min. An increase in ammonia concentration was then observed at 315 min. and levels remained elevated over the remainder of the sampling period. Statistical analysis of differences between cages for the three sampling days in September were shown to be significant ($n = 3$, $F = 26.48$ $P < 0.05$) and ($n = 3$, $F = 13.67$, $P < 0.05$) for the 27/9/05 and the 28/9/05 respectively. The relationship between feeding and ammonia levels in the three sampled cages in November 2005 (Figure 6.5) was less pronounced than that observed in September 2005. On the first sampling day in November, a similar trend in ammonia concentration to that noted during the sampling days in September 2005 was evident. The following day, an increase in ammonia concentration was recorded when the fish are not feeding followed by a drop in concentration prior to the afternoon meal. On the final sampling day in November, a slight decrease in ammonia concentration was observed upon cessation of the morning meal. Thereafter, an increase in ammonia concentration was evident over the remainder of the sampling period. Again, statistical analysis was carried out on difference

between cages sampled on each day during November 2005. Following a similar trend to September, differences between cages and between day are significant ($n = 3$, $F = 87.59$, $P < 0.05$) and ($n = 3$, $F = 26.74$, $P < 0.05$). This is to be expected due to changeable weather influencing many variables that would have an impact on the ammonia levels measured in each cage on each day. Due to adverse weather conditions over the targeted sampling period in February 2006, water sampling was curtailed to a single day (Figure 6.6). Unfortunately, this did not allow comparisons between the other two sampling trips. However, when statistical analysis was undertaken between the cages sampled in February, differences were found not to be significant ($n = 3$, $F = 0.22$, $P = 0.801$). Ammonia concentration observed in each cage on this single sampling day ranged from 0.1 µg/L/tonne biomass to 0.5 µg/L/tonne biomass. Each cage showed a slight increase in ammonia concentration over the sampling period. The complete range of ammonia levels throughout the sampling days in September 2005 and November 2005 all lie between 0.05 µg/L/tonne biomass and 1.6 µg/L/tonne biomass depending on the cage sampled and sampling time. Levels recorded in February 2006 were much lower ranging from 0.05 µg/L/tonne biomass and 0.5 µg/L/tonne biomass. This can be primarily attributed to the adverse weather in February 2006 (Force 9, North Easterly gale) increasing the dispersal rate of solid and dissolved wastes.

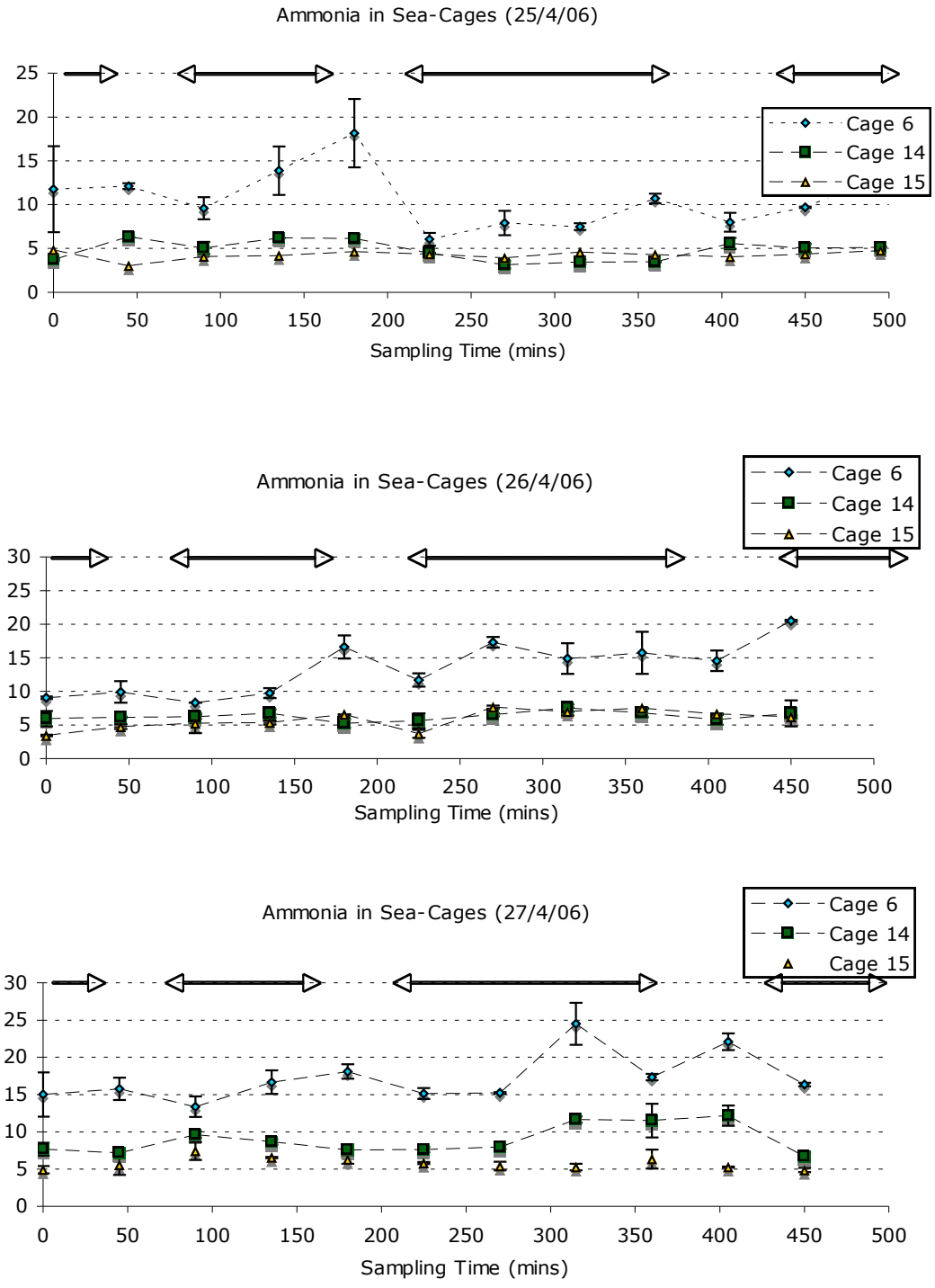



Figure 6.7 Daily ammonia profiles sampled in April 2006 (error bars are ± 1 SD).

The arrows are representative of feeding times.

As the juvenile fish were fed four meals over the sampling period (Figure 6.7) compared to the two meals offered to the adult fish in the production cages, the relationship between feeding and ammonia excretion was not so evident. It could be seen that in cages 14 and 15 in particular, ammonia concentration remained constant around 5 µg/L/tonne biomass over the three sampling days. Ammonia concentration in cage 6 showed a greater degree of variation and was consistently higher than those values recorded in cages 14 and 15.

By comparing Figures 6.5, 6.6 and 6.7 (production site) to Figure 6.8 (nursery site), it was clearly evident that ammonia levels recorded in the nursery cages were far higher than those recorded in the production cages. Statistical analysis shows that differences between ammonia content of the sampled cages and of the cages between sampling days was significant ($n = 3$, $F = 129.53$, $P < 0.05$) and ($n = 3$, $F = 27.06$, $P < 0.05$) respectively. This shows the marine environment can be unpredictable which in turn can result in variable values of dissolved nutrients being recorded, even within adjacent cages over the course of a few sampling days when conditions appear to be similar.

## 6.5 Discussion

### 6.5.1 Ammonia profiles

A direct relationship between feeding and ammonia concentration of the sampled water is observed in September 2005. A drop is noted following termination of the morning meal at 50 min. There-after ammonia levels remains at a lower level until the afternoon meal commences at 270 min. An increase in ammonia concentration is observed at 315 min. and levels remained elevated over the remainder of the sampling period. The relationship between feeding and ammonia levels in the three sampled cages in November 2005 is less pronounced than in September 2005. On the first sampling day in November, a similar trend to that observed in September 2005 is evident. The following day, an increase in ammonia concentration is

recorded when the fish are not feeding followed by a drop in concentration prior to the afternoon meal. On the final sampling day in November, a slight decrease in ammonia concentration is observed upon cessation of the morning meal. Thereafter, an increase in ammonia concentration is evident over the remainder of the sampling period. Due to adverse weather conditions over the targeted sampling period in February 2006, water sampling was curtailed to a single day. Levels recorded in February 2006 are much lower and can be primarily attributed to the adverse weather (Force 9, North Easterly gale) increasing the dispersal rate of dissolved wastes.

As the juvenile fish were fed four meals over the sampling period compared to the two meals offered to the adult fish in the production cages, the relationship between feeding and ammonia excretion is not so evident. It can be seen that in cages 14 and 15 in particular, ammonia concentration remains constant over the three sampling days, but ammonia concentration in cage 6 showed a greater degree of variation and was consistently higher than those values recorded in cages 14 and 15.

### 6.5.2 Gut evacuation

Although immeasurable as previously discussed in Chapter 4, gut evacuation rates and impacts on ammonia production are discussed as they will have an impact on the ammonia profiles of dissolved nitrogenous waste production associated with the culture of juvenile and adult Atlantic cod in cage systems. Therefore, in order to understand the ammonia profiles, it is important to consider the timing of gut evacuation in relation to protein content of the diet and the feeding regime.

Even under favourable environmental conditions, there may be considerable variation in feeding activity between days. This may be related to gastric evacuation time as it has been suggested that the return of appetite depends upon stomach fullness. Daily fluctuation in feeding may be

induced by changes in the volume of food in the stomach and the time required for emptying. Such a phenomenon can be affected by the quality of the aquafeeds used. Studies suggest that gastric emptying follows two independent trends: a straight line as observed in *Gadus* spp. (Jones, 1974); or an exponential curve observed in *Salmo trutta* (Elliot, 1972) and *Oncorynchus* spp. (Brett & Higgs, 1970). Brett & Higgs (1970) superimposed a chart of the rate of gut evacuation over a chart showing the rate of ammonia excretion. At the peak levels of ammonia excretion, it was deduced that the stomachs of the fish were half full, concluding that the post - prandial - peak is entirely a product of feed intake. It has been suggested that the appetite of a fish returns as the stomach empties and as the gut is evacuated. Consequently, it would be expected for a given species, fish of different sizes feeding at regular intervals will voluntarily regulate feed intake so that digestion is complete prior to the next feeding. Such a phenomenon would require an acclimation period to feeding regimes and diet.

The length of time that food remains in the stomach varies considerably with larger particles requiring a greater period of time to be broken down. The retention of large indigestible particles such as bone and exoskeletal material congregate in the antrum region and may contribute to grinding of the more friable components of the meals, thus aiding digestion. It is found that wild cod preferentially eat large prey swallowed whole and actively ingest small stones to act as gastroliths, which aid digestion of the large food particles.

In a series of experiments with Atlantic cod, it was found that regular feeding of small rations delayed emptying of the undigested solids and emptying of this material did not commence until nearly all nutrients had been removed from the food (dos Santos *et al,* 1993). Gastric evacuation times vary from a few hours to a number of days depending on factors such as species, water temperature, meal size and physical characteristics of the food. In general, gastric evacuation times decrease with an increase in temperature, but will increase with an increase in ration size and feed particle size. This was shown in a study on Atlantic cod fed differing ration sizes of whole and minced herring (dos Santos *et al,* 1993). When fed whole herring, approximately 50

% of the ration was evacuated within 24 hours. When the herring was minced, a ration of the same weight, approximately 80 % of the ration was evacuated within 24 hours.

Intestinal length will effect gut retention time and the absorption of nutrients of the partially digested mix. Atlantic salmon (*Salmo salar* L.) has an intestine length to body length ratio of 0.75 – 0.85. Atlantic cod (*Gadus morhua* L.) has an intestine length to body length ratio of 1 – 1.5. This suggests that the proportionately longer intestine of cod will increase gut retention time and absorption of nutrients.

### 6.5.3 Ammonia dispersal in cage studies

Waves and water currents can also impact on feeding (Malakeh *et al*, 1998). In non-rigid cages such as those used in the production site at No Catch® Ltd. dissipate little wave energy. Excessive water flow in exposed sites may effect the ability of fish to capture food. There have been relatively few studies on feed intake by caged fish (Andrew *et al*, 2002 & Juell, 1995). Consequently, there is still a poor understanding of how feeding methods and feed rates interact with environmental factors and stocking densities to determine food intake, growth and production. This, in turn influences production efficiency and environmental impacts. Recent research has focused on salmonids and suggest that a marked improvement of FCR, waste losses to the environment and production economics can be achieved through a better understanding of feeding behaviour (Andrew *et al*, 2002; Beveridge & Kadri, 2000 & Jobling, 1995).

Rensel (1989) measured dissolved nitrogen 'upstream' of a cage site and also from the centre of the cage site. A value of 0.003 mg/l measured at the 'upstream' sampling site increased to 0.023 mg/l at the centre of the cages site. Maximum levels were observed during slack tide. Weston (1986) reported an ambient level of dissolved nitrogen 0.3 mg/l – 1.9 mg/l while Brooks *et al* (2002) quotes values of 0.09 mg/l. Both measurements were recorded from commercial salmon sites.

Literature indicates that the concentration of dissolved inorganic nitrogen is very low on the perimeter of salmon farms and is essentially immeasurable at a distance greater 9 m of the said perimeter. Gowen *et al* (1988), Rensel (1988) and Parametrix® (1990) were in agreement that the quantity of dissolved inorganic nitrogen added by even several commercial operations would have no measurable effect on phytoplankton density.

Gowen *et al* (1988) when studying a large salmon farm on Scottish sea-loch with very restricted water exchange concluded that the farm had no measurable effect on phytoplankton density. Similar findings were made by Smith (1975). Pridmore & Rutherford (1992) made similar observations and concluded that in the Pacific Northwest, salmon farming (*Onchorynchus* spp.) had little or no effect on ambient levels of either dissolved nutrients or phytoplankton density. All literature is in agreement, that with the exception of a few shallow and poorly flushed sites, the potential for sea-cage enhancement of phytoplankton populations is remote or non-existent. It has been shown that Atlantic salmon retain approximately 28 % of nitrogen and 30 % of phosphorus in modern feeds McVeigh *et al* (2002) as cited in Stickney & McVeigh (2002). Improved diets and FCR have reduced waste output from of salmon farms from 9.5 kg phosphorus and 78 kg nitrogen per tonne of fish produced, to 7 kg and 49.3 kg respectively. It is only necessary to keep nutrient levels below a threshold limit for harmful algal blooms or levels that result in the reduction of dissolved oxygen.

### 6.5.4 Polyculture

Commercial aquaculture, whether it is Atlantic salmon or Atlantic cod, has impacts upon the environment. Polyculture is seen as a method of reducing such impacts as well as providing other marketable aquaculture products.

The use of marine polyculture involving marine plants can reduce enrichment of nitrogen and phosphorus released from commercial finfish culture. *Porphyra* species (red algae known as

Nori) is a valuable commodity, which is sold to an almost exclusive Japanese market and had a global value of approximately US$2 billion in the early 1990's.

Work undertaken by Fei (1998) found that Nori responds to elevated levels of dissolved nitrogen and phosphorus by absorbing more into its tissues. Experimental work on polyculture of *Porphyra* species with Atlantic salmon in Maine is underway. It has been suggested that the placement of macroalgal farms close to marine fish farms will provide an optimal location and may help balance nitrogen levels in the ecosystem. However, a high constant supply of nutrients is required to sustain the macroalgae and reduced levels of nutrients (particularly during summer months) may lead to fertilisation of the macroalgae. Such a scenario suggests that polyculture may only be a viable option on large-scale commercial farms.

Shellfish are very efficient at filtering water and an individual bivalve can filter between 1 litre and 4 litres of seawater per hour. Mussel farms are shown to increase water clarity by converting phytoplankton (held in suspension) into rapidly settling organic particles (faecal waste), which can increase sedimentation rate threefold (Kaspar, *et al*, 1985). However, further work into shellfisheries management and understanding of the positive (increasing water clarity) and the negative (increasing sedimentation) aspects of bivalve culture are required in order to model such relationships with commercial finfish culture.

Daily profiles of the dissolved nitrogenous waste (ammonia, nitrite and nitrite) production obtained throughout both of the cage-based studies and discussed in this chapter were taken forward to Chapter 7 (modeling) and Chapter 8 to help compile nitrogen flux models for different sized cod grown in commercial cage culture systems.

# Chapter 7

## MODELLING

# MODELLING

This chapter discusses the modelling of waste generated by commercial cod farming using data from previous chapters for parameterising. Further data presented in this chapter is used for calculating physical parameters and validation of the model. Models are useful for regulation when assessing the direction and severity of effluent deposition from a marine cage site into its environment. As yet, there are no deposition / dispersion models used specifically for commercial cod culture and the data concerning discharge consents granted by SEPA to the industry use the increased protein level of cod diets and retro - fit the associated nitrogen data into DEPOMOD models, created for Atlantic salmon culture.

The model developed here through the spreadsheet of the salmon model CAPOT has several advantages over the DEPOMOD system. CAPOT utilises a spreadsheet in which data can be entered and the model run with great simplicity. CAPOT is much better as a flexible research tool as it is simple to supplement additional data and modify existing data to incorporate into the model allowing quicker development of the model. As yet, the model has not been validated for commercial cod culture but DEPOMOD and CAPOT models developed for commercial cod culture will be compared in this chapter. A full description of the CAPOT model, how the data is entered and how the model is run, is shown in Section 7.4.

Modelling allows the comparison of environmental impacts from different production scenarios (i.e. regulation based on husbandry). Such modeling would be useful in assessing the different environmental impacts using different feeding regimes such as 40% protein diet fed daily (similar regime to Atlantic salmon) compared to a 50% protein diet fed 2 times per week. However, the use of the spreadsheet model allows rapid modelling of any production scenario, which is useful to the regulatory body (SEPA), when granting accurate discharge consents specific to cod aquaculture.

## 7.1 Settling Velocities

Settling velocities of the six EWOS® diets investigated at MERL and the two Biomar® diets used at No Catch® Ltd were measured and recorded using the methods described in Section 2.4.2.

### 7.1.1 Diets investigated at MERL

Settling velocities were measured for the diets investigated in the adult and juvenile studies (Figure 7.1). All raw data is shown in Appendix 28. The four temperatures are representative of the water temperature at MERL at each collection date (13 ºC in June 2004, 15 ºC in September 2005, 10 ºC in November 2004 and 8 ºC in February 2005).

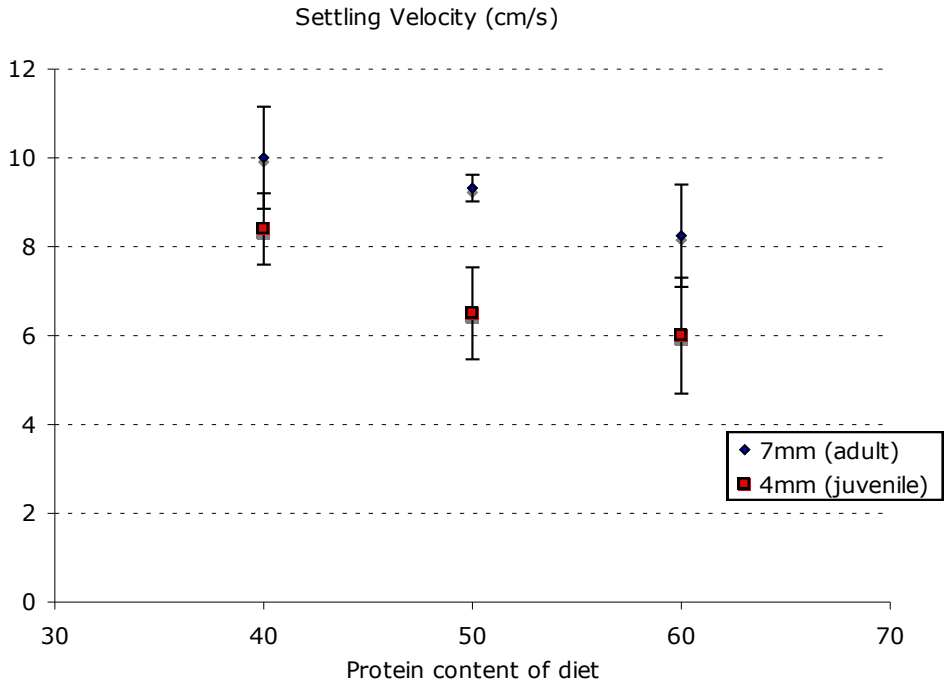



Figure 7.1 Settling velocities for the adult and juvenile EWOS® diets investigated.

(error bars are ± 1 SD).

It can be seen that for both the 7 mm pellet (adult) and 4 mm pellet (juvenile), a greater rate of protein inclusion (and lesser lipid), results in a slower settling velocity. Statistical analysis confirms that the differences between formulations for each pellet size, is significant ($n = 25$, $F = 21.5$, $P < 0.05$) for the adult diet and ($n = 25$, $F = 34.96$, $P < 0.05$) for the juvenile diet.

From Figure 7.1, it can be observed that settling velocities are generally faster with a larger pellet. As the settling velocities were only measured over 1 m in depth, the difference in settling rates between the formulations for each pellet size was not significant as the studies utilized tanks with a depth of around 1 m. Given the shallow depths of the tanks and the fact that the adult and juvenile cod rarely accepted food that had settled on the bottom of the tanks, the depth can have a major impact on ingestion rates and apparent food utilisation.

### 7.1.2 Diets investigated at No Catch® Ltd.

Settling velocities measured for the 12 mm Biomar® adult diet and the 3 mm Biomar® juvenile diet are shown in Table 7.3 and Figure 7.2 with the raw data contained in Appendix 29. The four temperatures are representative of the water temperature in Vidlin Voe at each collection date (13 ºC in September 2005, 10 ºC in November 2005, 8 ºC in February 2006 and 7.5 ºC in April 2006).

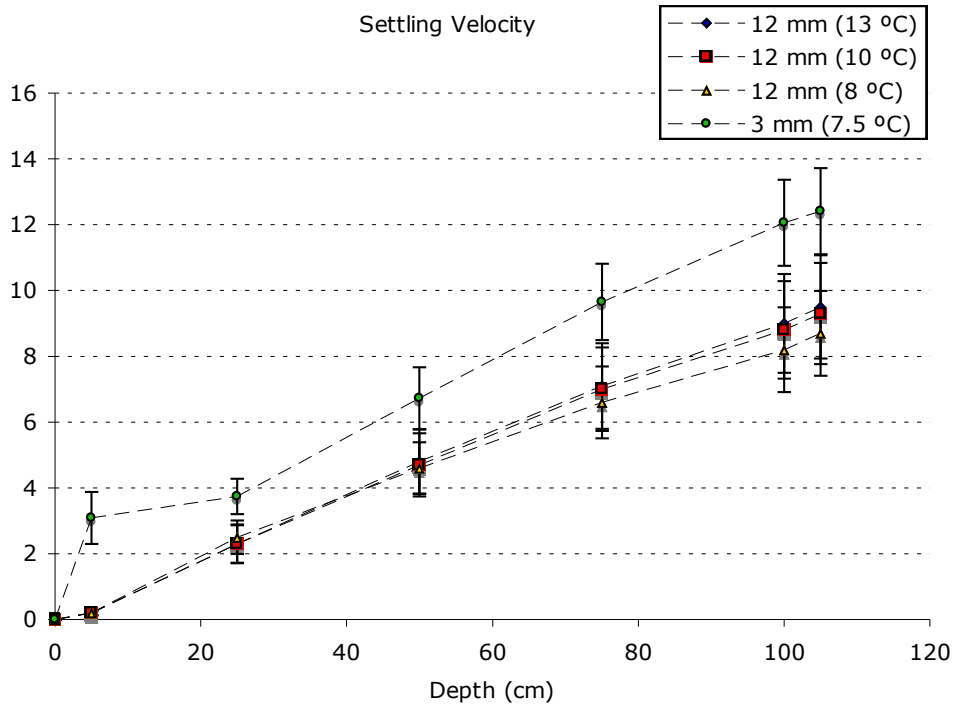



Figure 7.2 Settling velocities for 12 mm Biomar® and 3 mm Biomar® diets.

(error bars are ± 1 SD).

Figure 7.2 shows that the 3 mm pellet settles at a slower rate than the 12 mm pellet. Lipid can affect buoyancy, but from comparing Tables 5.1 and 5.2, it can be seen that lipid levels in the diets are the same (14 %), suggesting that pellet size is the primary factor affecting settling velocity. Temperature may have an effect on settling velocity as with the 12 mm pellet which exhibits an increasing settling velocity with a decreasing temperature. However, the difference is not significant over a depth of 1 m ($n = 25$, $F = 0.18$, $P = 0.973$). Understandably, the large difference in pellet sizes resulted in a significant difference in settling velocity ($n = 25$, $F = 27.24$, $P < 0.05$).

## 7.2 Leaching rates

Leaching rates of the six EWOS® diets investigated at MERL (3 formulations at 2 pellet sizes) and the 3 mm and 12 mm Biomar® used at No Catch® Ltd. were measured and recorded using the methods described in Section 2.4.3.

### 7.2.1 Diets investigated at MERL

For the purpose of the thesis, the ammoniacal nitrogen leached after 30 min. of immersion will be plotted against protein content of the diets using a mean temperature of 11 ºC. The leaching of ammoniacal nitrogen from the six EWOS® diets investigated at MERL are shown in Figure 7.3. All raw data for the leaching of the diets investigated in the adult study are shown in Appendix 30 and for the juvenile study are shown in Appendix 31.

It can be seen that the greater the protein (and associated nitrogen) content of the diet, the greater concentration of ammonia in the water following 30 min. of immersion at the different temperatures investigated.

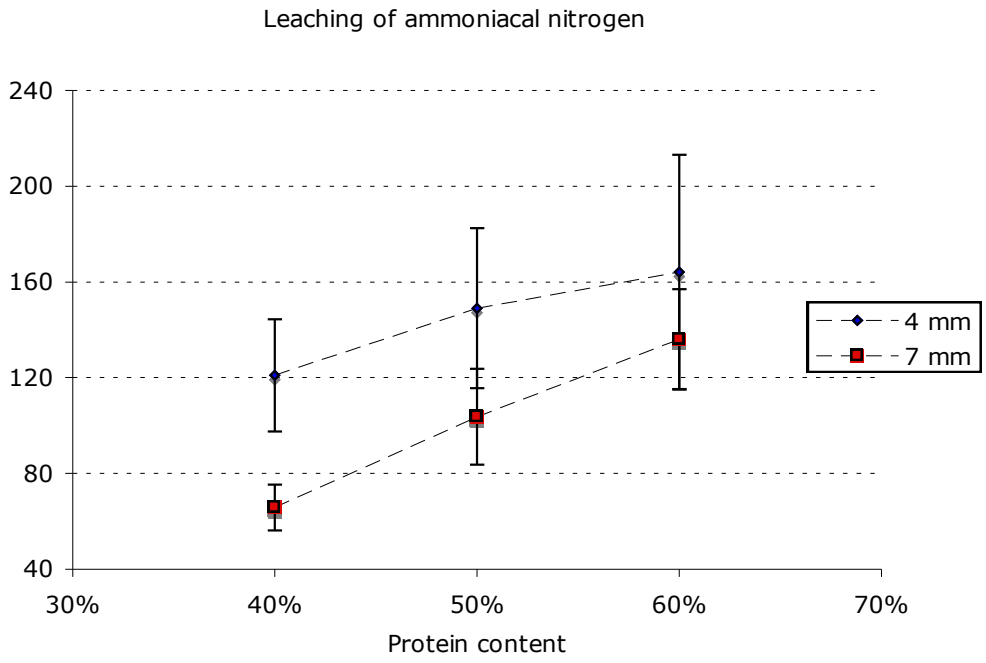



Figure 7.3 Leaching of ammoniacal nitrogen into water (µg/L) following 30 minutes of immersion in artificial seawater, of the diets investigated in the tank studies.

(error bars are ± 1 SD).

Statistical analysis carried out on the 7 mm pellet showed that temperature had no significant effect on leaching rates ($n = 5$, $F = 1.5$, $P = 0.291$). However, a significant result was obtained when analysing differences in protein content between the diets ($n = 3$, $F = 23.16$, $P < 0.05$). Statistical analysis carried out on the 4 mm pellet showed that a difference in temperature had significant effect on leaching rates ($n = 4$, $F = 17.45$, $P < 0.05$). A significant result was obtained when analysis the impact of protein content between the diets ($n = 3$, $F = 9.2$, $P < 0.05$). Given the higher surface area of the smaller pellets for a given weight (1 g investigated), such an observation would be expected due to the increased surface area allowing greater leaching rates. When comparing leaching rates of the 4 mm pellet to the 7 mm pellet investigated in the adult study, it can be seen that ammonia concentration in the water is consistently higher for the 4 mm

pellet than the 7 mm pellet regardless of protein content. As 1 g of each diet was analysed regardless of pellet size, the higher leaching rates of the 4 mm pellet will be due to a greater surface area of 1 g of the smaller pellet.

### 7.2.2 Diets investigated at No Catch® Ltd.

The leaching of ammoniacal nitrogen from the 12 mm adult diet is shown in Figure 7.4 with the temperatures representative of the water temperature in Vidlin Voe when the diets were sampled (13 ºC in September 2005, 10 ºC in November 2005 and 8 ºC in February 2006). The leaching of the 3 mm juvenile diet is shown in Figure 7.5. The 7.5 ºC temperature investigated is representative of the temperature in Vidlin Voe in April 2006. All raw data is shown in Appendix 32.

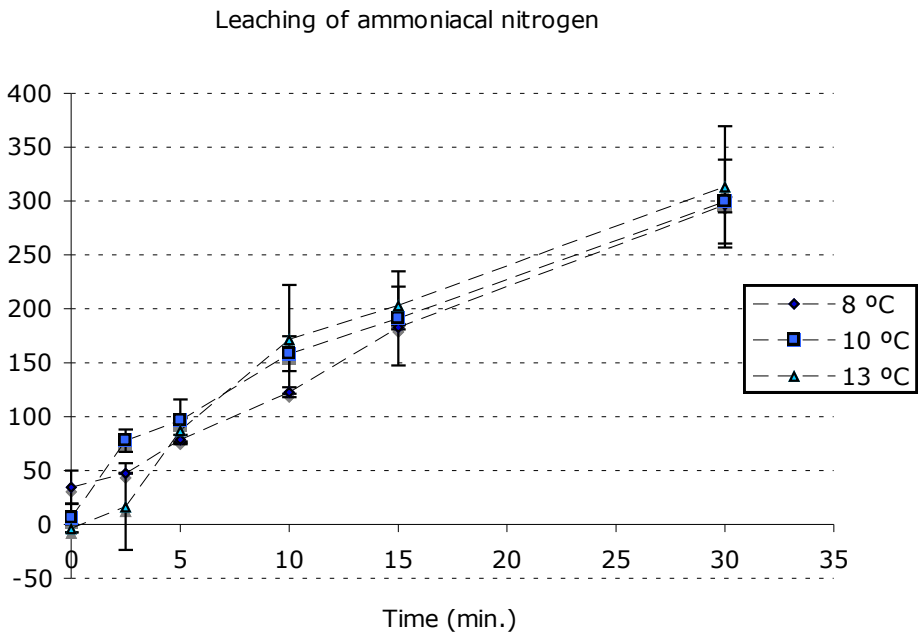



Figure 7.4 Leaching of ammoniacal nitrogen into water (µg/L) from 12 mm Biomar® diet.

(error bars are ± 1 SD).

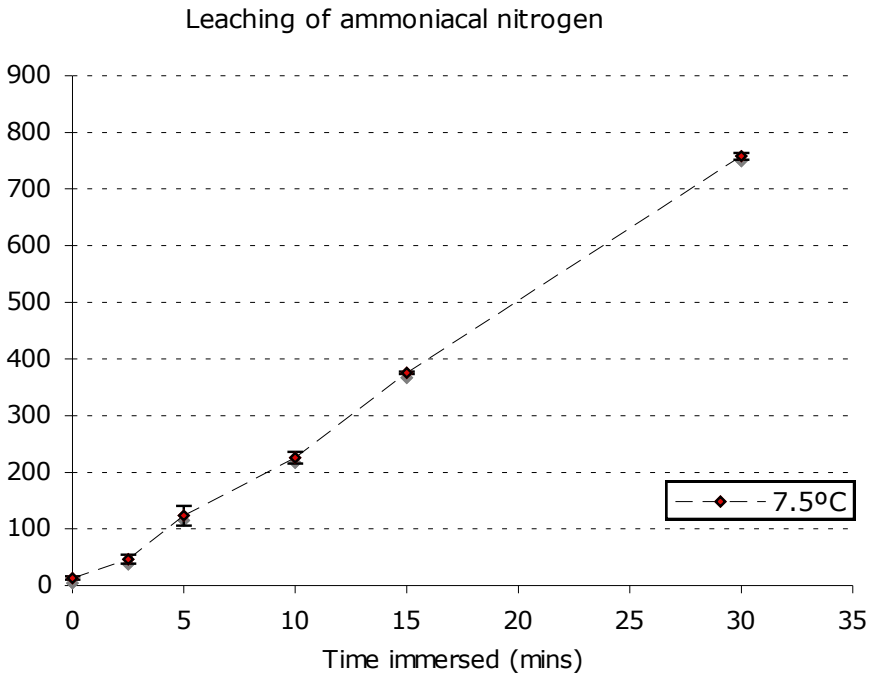



Figure 7.5 Leaching of ammoniacal nitrogen into water (µg/L) from 3mm Biomar® diet.

(error bars are ± 1 SD).

From Figures 7.4 and 7.5, it is apparent that the concentration of ammoniacal nitrogen into the water increases over the period of immersion. It was observed that for all temperatures (8 ºC, 10 ºC and 13 ºC), the initial leaching rate from the 12 mm pellet was higher in the first 5 min. (an increase of 15 - 20 µg/L per min.) and began to slow down after 10 min. to approximately 7 µg/L - 10 µg/L per minute. A final concentration of approximately 300 µg/L was observed. The increase in concentration of ammoniacal nitrogen from the 3 mm pellet remains constant around 20 µg/L per minute over the immersion period with a final concentration of around 760 µg/L. This is related to pellet size as 1 g of each diet was analysed (which equates to approximately 7 of the 3 mm pellet or a single 12 mm). The greater leaching exhibited by the 3 mm pellet is due to a greater surface area of the pellets in contact with the water. However, the temperature range was small, the immersion period short and the actual differences between leaching, as a result of temperature was not significant. ($n = 12$, $F = 3.02$, $P > 0.05$).

## 7.3 Deposition rates

A series of sediment traps (Figure 7.8) were deployed along transects around the nursery and production sites at Vidlin Voe, facilitated an investigation into particulate waste deposition associated with a commercial sea-cage operation. A control trap was used to take background levels into consideration. The cages sampled at the production and the nursery sites are shown in Figures 6.1 and 6.2. All raw data concerning deposition rates from the production and nursery sites in Vidlin Voe are shown in Appendix 33.

### 7.3.1 Hydrographic data

Hydrographic information collected at Vidlin Voe is shown in Appendix 18. Sediment samples were collected from approximately 3 m above the seabed. Dispersion is affected by currents at

all depths, influencing the movement of water and therefore feed and faecal material is subject to currents throughout the water column. This could have an effect on the results obtained.

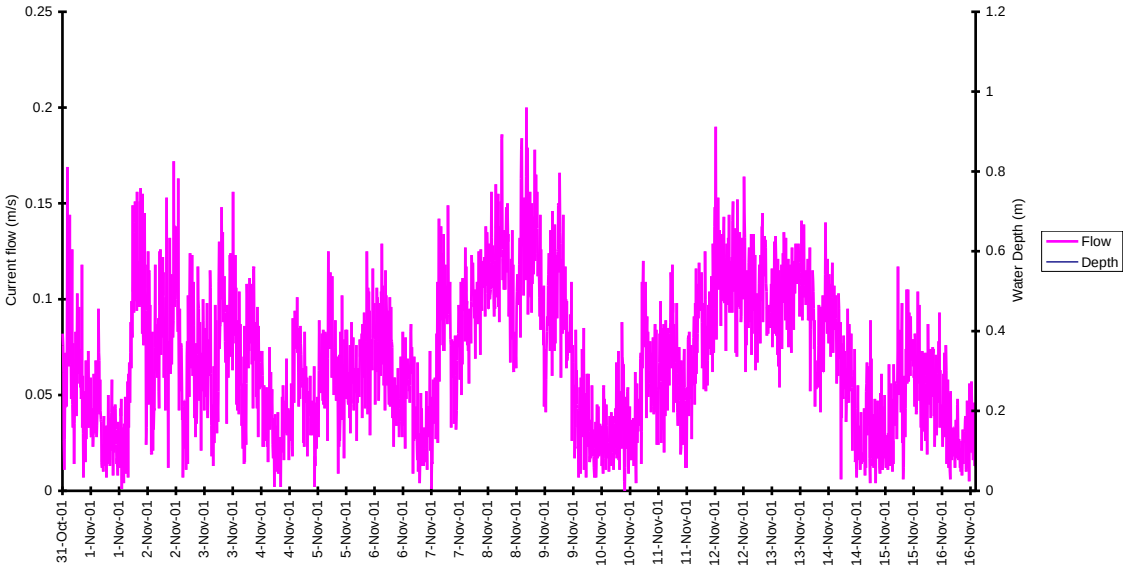



Figure 7.6 Current flow at seabed in Vidlin Voe

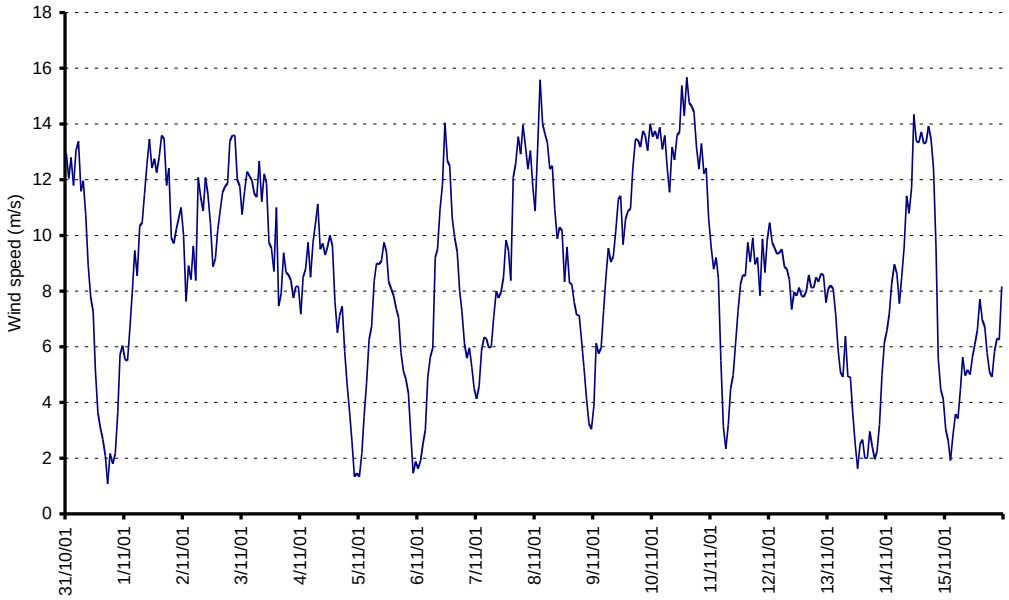



Figure 7.7 Wind speed measured from production site in Vidlin Voe.

When comparing Figure 7.6 (current flow) to Figure 7.7 (wind speed), it can be seen that there is a much weaker correlation between wind speed and current speed than that observed in the surface water. From Appendix 18 it can be seen that currents enter the Voe (South Westerly direction) along the seabed and leave the Voe (North Easterly direction) through the surface water. Large variation in wind speed and current speed will undoubtedly have a large impact on the temporal and spatial dispersion of particulate wastes.

### 7.3.2 Collection of particulate waste

Sediment traps were deployed around the production and nursery sites to measure sedimentation rates of discharged particulate waste material. A trap deployed at 60 m from the site along a transect perpendicular to the prevailing current would act as a control and permit background sedimentation rates to be taken into account. The combination of sedimentation rates and the hydrographic data would give an indication of the direction and severity of dispersion of particulate matter associated with the cage site. From the collected samples, total sedimentation rates as well as carbon and nitrogen sedimentation rates were calculated. An inorganic/organic ratio of the collected samples was determined and in turn, sedimentation rates of organic and inorganic carbon and nitrogen were calculated.

Each trap was deployed for a period of approximately 72 hrs. However, due to inclement weather conditions, this was not always possible. To negate this factor from the results, deposition rates were calculated as g DW/$m^2$/d so that deposition between sampling trips could be directly comparable.

### 7.3.3 Positioning and deployment of sediment traps

Hydrographic data supplied by No Catch® Ltd. (Figures 7.4 and 7.5 and in greater detail in Appendix 18) was studied to assess the optimal positions of deployment for the sediment traps.

In order to accurately assess the sediment production of the cage site, 3 sediment traps, each containing four replicate collectors (pots), were deployed at various distances from the cages (5 m, 15 m and 25 m) on a transect in the direction of the prevailing current and 3 sediment traps deployed along a transect perpendicular to the prevailing current (10 m, 30 m and 60 m). In the adult study, a seventh trap was deployed between two of the cages sampled for water (0 m). The transects run from the sampled cages at the northeast end of the production site. In the juvenile study, the transects ran from the northeast end of the cage block. Depths and positions references were taken of each trap during each trip, using a Hondex hand - held echo sounder (accuracy ± 0.5 m) and a Magellan hand - held GPS as required by SEPA. GPS positions of the sediment traps deployed around the production site are shown in Table 7.1 and positions of deployment around the nursery site are shown in Table 7.2.

Table 7.1 GPS positions of sediment traps deployed around the production site in Vidlin Voe.

| Number | Position | Northings | Westings | HU |
|---|---|---|---|---|
| 1 | Between Cages | 60º 23.378 | 1º 07.245 | 48561 / 67605 |
| 2 | 5m NE | 60º 23.381 | 1º 07.205 | 48595 / 67625 |
| 3 | 15m NE | 60º 23.391 | 1º 07.200 | 48603 / 67640 |
| 4 | 25m NE | 60º 23.407 | 1º 07.190 | 48610 / 67656 |
| 5 | 10m NW | 60º 23.381 | 1º 07.301 | 48510 / 67616 |
| 6 | 30m NW | 60º 23.391 | 1º 07.325 | 48489 / 67636 |
| 7 | 60m NW | 60º 23.408 | 1º 07.347 | 48457 / 67657 |

Table 7.2 GPS positions of sediment traps deployed around the nursery site in Vidlin Voe.

| Number | Position | Northings | Westings | HU |
|---|---|---|---|---|
| 1 | 5m NNE | 60º 22.712 | 1º 07.760 | 48105 / 66319 |
| 2 | 15m NNE | 60º 22.714 | 1º 07.745 | 481127 / 66369 |
| 3 | 25m NNE | 60º 22.719 | 1º 07.751 | 48114 / 66385 |
| 4 | 10m ESE | 60º 22.702 | 1º 07.728 | 48134 / 66339 |
| 5 | 30m ESE | 60º 22.691 | 1º 07.699 | 48160 / 66328 |
| 6 | 60m ESE | 60º 22.684 | 1º 07.637 | 48219 / 66323 |

Each trap (Figure 7.8) was lowered into position using a 25 kg weight tied 2 m 'below' the bottom of the trap, allowing the trap to sit approximately 2 m above the seabed. It was thought that such a distance would reduce the risk of contamination through disturbance of the seabed.

A further length of rope was tied to the top of the trap and two small buoys were tied on 2 m 'above' the trap to hold the trap upright in the water column. With the labelled sample pots attached to the bottom of each collector, the trap was slowly lowered into position. When the 25 kg weight landed on the seabed, extra rope was fed out and a large marker buoy was tied to the end of the rope to mark the position.

On retrieval, the traps were lifted onto the boat using a winch. After careful draining of excess water, the replicate sample pots were removed from the collectors and a lid screwed onto each. The labeled samples were then frozen (-20 $^{\circ}$C ± 1 $^{\circ}$C) prior to transportation in cool boxes to the Institute of Aquaculture for analysis.

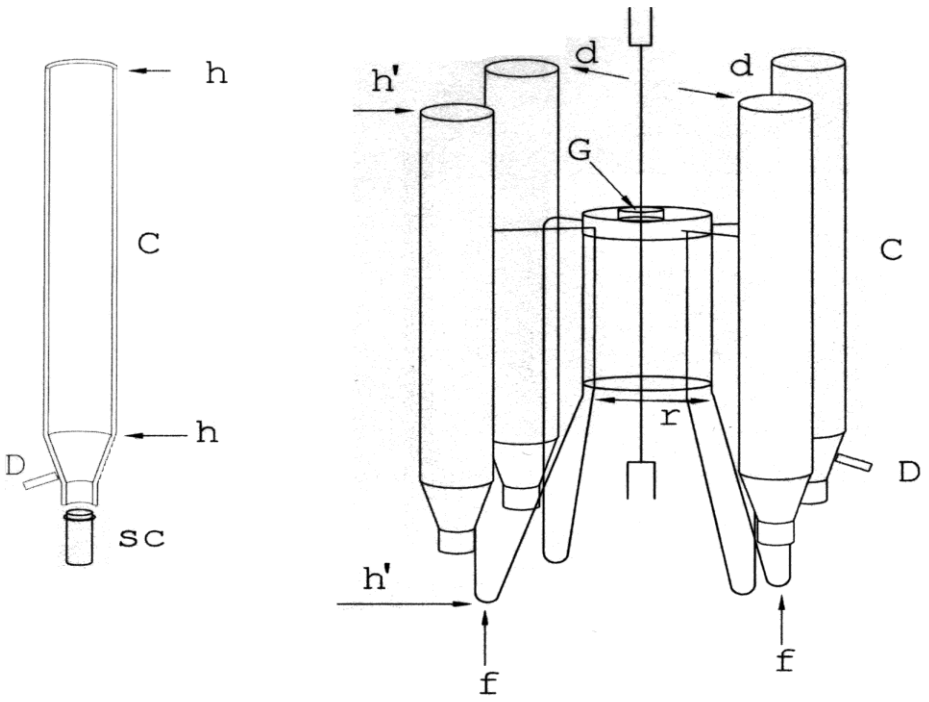



Figure 7.8 Detailed illustration of the sediment trap design (from Chen, 2000).

A cross-section of a single collection cylinder is shown on the left.

### 7.3.4 Analysis

Samples were thawed at room temperature and left undisturbed to ensure all sediment settled in the pots. The water was decanted off using a 10 mL glass pipette and discarded. Each pot was then filled with distilled water to remove any salt crystals in the sediment. The sediments were allowed to settle before the water was decanted off as before and discarded. This was repeated to ensure that the samples were thoroughly rinsed. The rinsed samples were then transferred to pre-weighed labelled foil trays and placed in a Gallenkamp Drying Oven at 60 $^{\circ}$C (± 1 $^{\circ}$C) for 48 hours then reweighed. The samples were dried at 60 $^{\circ}$C (± 1 $^{\circ}$C) to ensure that no organic matter would be burned off.

### 7.3.5 Calculation of deposition rates

The area of a single trap was calculated as 50 $cm^2$ (0.005 $m^2$), 8 cm in diameter, and the length of time the trap was submerged was used to calculate the deposition rate of sediment (as g $DW/m^2/d$). The sediment samples were analysed for carbon and nitrogen using the Perkin - Elmer Series II CHNS/O Analyzer 2400. This allowed total carbon and nitrogen deposition rates to be calculated in g $DW/m^2/d$.

After sub - samples for carbon and nitrogen analysis were taken, the remaining samples for each sediment trap for each sampling date were then pooled, weighed and heated in a Gallenkamp muffle furnace at 600 ºC for 12 hours to burn off all organic material. The ashed samples were then analysed in the Perkin - Elmer Series II CHNS/O Analyzer 2400, for inorganic carbon and nitrogen.

### 7.3.6 Organic deposition at the production site

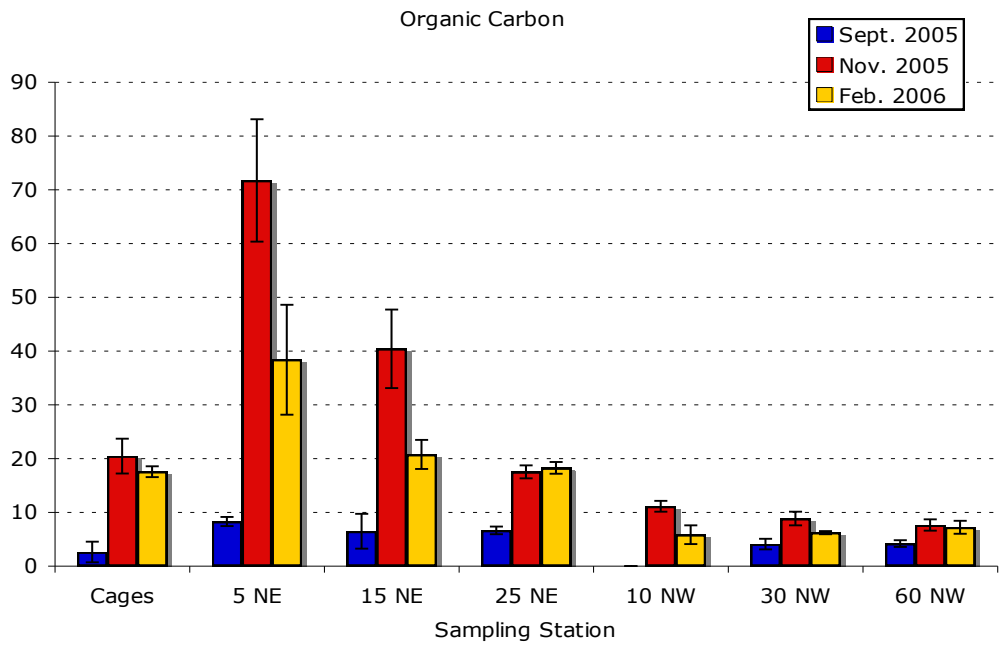



Figure 7.9 Deposition rates of organic carbon calculated over a 3-day collection period at the production site (error bars are ± 1 SD). Control trap is 60 NW.

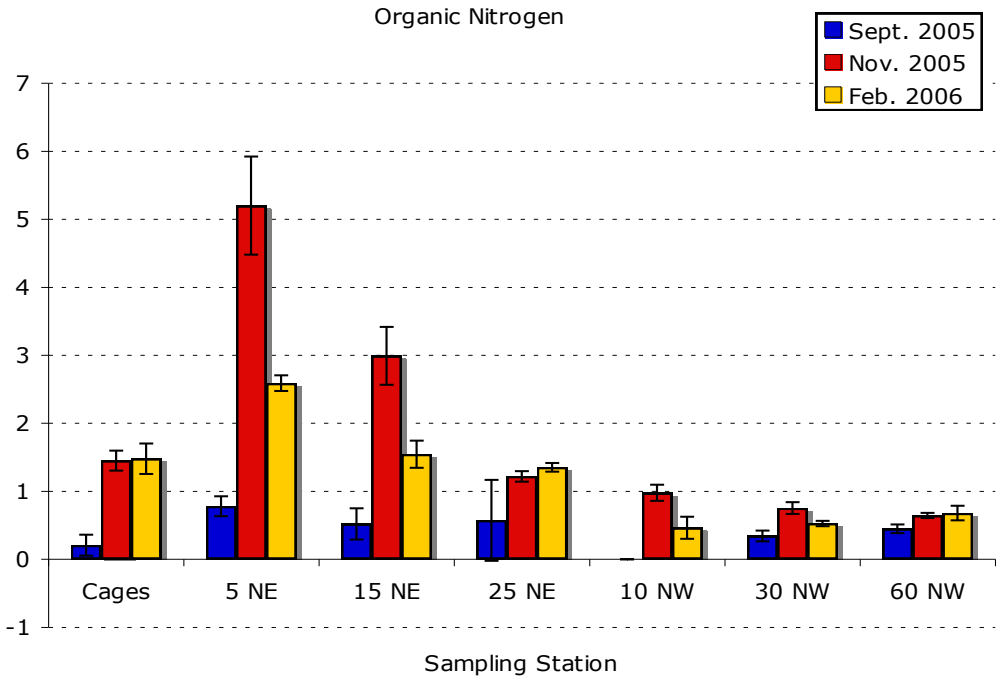



Figure 7.10 Deposition rates of organic nitrogen calculated over a 3-day collection period at the production site (error bars are ± 1 SD). Control Trap in 60NW.

The sedimentation rates of organic carbon and nitrogen decrease with an increasing distance from the production cages, and was also dependent along which transect the trap was deployed. Traps on the transect of the prevailing current had consistently higher sediment than those on the transect perpendicular to the prevailing current. Such a result suggests that the prevailing current plays and important part in the dispersal of organic nitrogen and carbon. Another result of note is the lower organic nitrogen and carbon in the trap deployed between the adult cages. This highlights the ease of dispersal, due of the high moisture content of faecal material produced by cod, and the role of currents in the dispersal of organic waste produced by the farm. Helrin (2003) also suggested that a high moisture content and high leaching rates of cod faecal material results in rapid dispersal of the material (and associated nitrogen content) once evacuated into the environment. The elevated values of organic sediment collected in the sediment traps on November 2005 and February 2006 was effected by the rough weather observed at Vidlin suggesting that these elevated values may be a result of resuspension. It was also noted that no feed pellets were recorded of the deployed sediment traps. Given the high quantities of feed input into the production cages, the fact that no pellets are collected, could suggest in part that the feeding regime is at an optimal level. However, it also indicates that the traps may have inadequate.

## 7.3.7 Organic deposition at nursery site

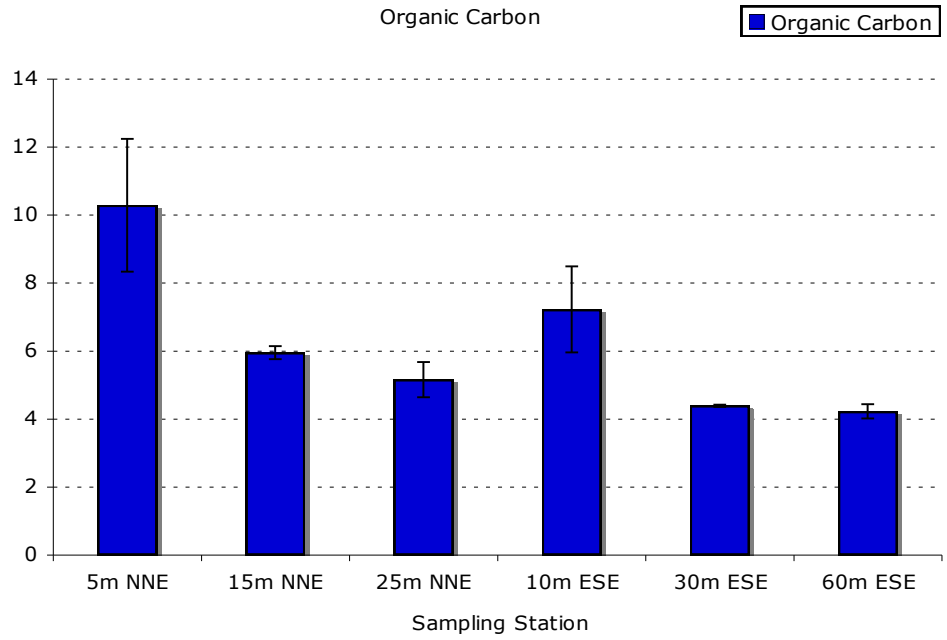



Figure 7.11 Deposition rates of organic carbon calculated over a 3-day collection period during the sampling trip to the nursery site in April 2006. (error bars are ± 1 SD).

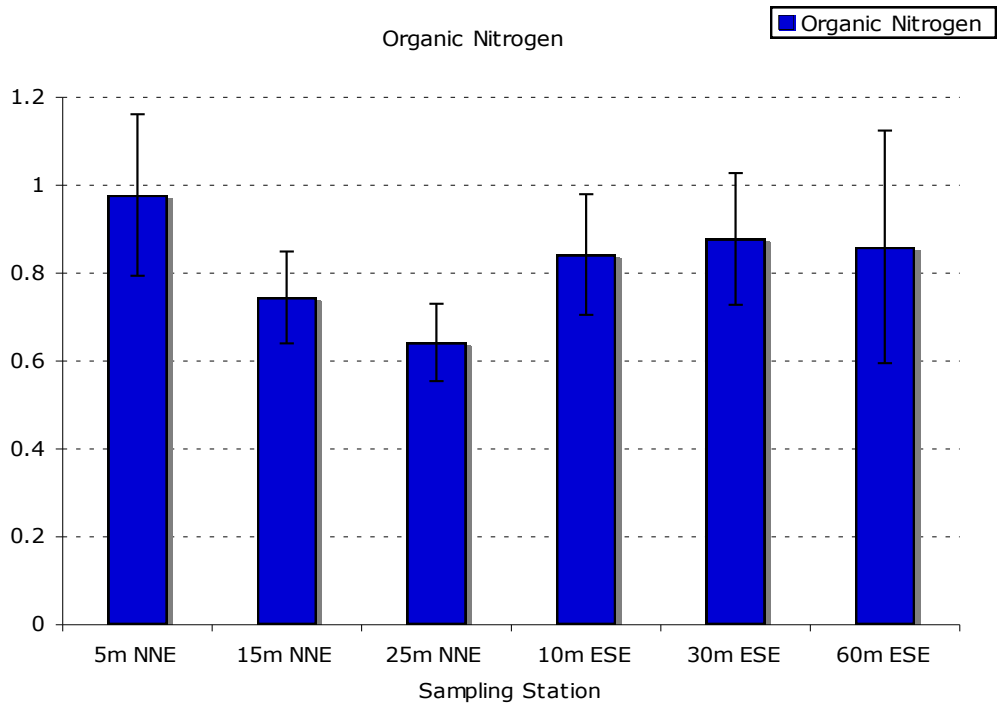



Figure 7.12 Deposition rates of organic nitrogen calculated over a 3-day collection period during over sampling trip to the nursery site in April 2006 (error bars are ± 1 SD).

At the nursery site, a similar trend in organic carbon deposition was observed to that of the production site, although the values were lower. Higher deposition rates are observed on the NNE transect (prevailing current) compared to that observed on the ESE transect (perpendicular to the prevailing current). It can also be seen that deposition rates decrease along both transects with an increasing distance from the cage site. The control value (60 m ESE) is similar to those results obtained at 30 m ESE and only slightly lower than those recorded at 15 m NNE and 25 m NNE. This suggests that carbon impact is local to the nursery site. However, disturbance at the control trap due to several return sailings of the ferry from Vidlin Harbour to the Outer Skerries may need to be considered in any future waste modeling.

A decrease in organic nitrogen deposition can be observed along the NNE transect. On the ESE transect, the lowest value is measured at the closest sampling station to the cages (10m ESE). The control station exhibits the third highest deposition value of organic nitrogen of all the sampling stations. If such background levels are subtracted from the results obtained at the other sampling stations actual results would be minimal if non - existent. Again, the disturbance at the control station may need to be a considered factor in any future work. The above results only give an indication of organic deposition around the nursery site as only a single sampling trip was carried out. Further work would either confirm the obtained results or perhaps give an insight into the elevated carbon and nitrogen levels observed along the ESE transect paying particular attention to the 60 m ESE sampling station.

## 7.4 Dispersion modelling using CAPOT

The Cage Aquaculture Particulate Output and Transport (CAPOT) model, estimates the amount and subsequent location of solid waste entering the marine environment from fish cultured in sea cages. The CAPOT model is the culmination of several studies by a number of investigators, the basic principles of which have been developed in two forms; 1) Spreadsheet based (Telfer, 1995) and 2) GIS - based models (Corner *et al*, 2006). The former was formulated mainly as a self - standing development tool to test ideas, which have subsequently been incorporated into the spatial modelling capabilities of IDRISI GIS software (Clark Labs, USA).

The CAPOT model has been simplified to be more 'user - friendly' than previously, and is now in a form that can be more widely used. The model uses production information, site - specific hydrographic data and a number of empirically derived measures or assumptions to create a nutrient budget of the amount of waste, and the form in which it enters the environment.

The movement of the waste after release was modelled using a sectoral system (12 sectors of 30 degrees on a 360 degree circuit) based on the speed and directions of water currents at the cage farm location. The waste / settlement associated with each cage is individually entered into an excel spreadsheet or 'layer' (in this case, 17 cages and therefore 17 'layers'). Once all the data was entered into the excel spreadsheet (Data Input worksheet), it was then plotted using Surfer $^{TM}$. Different production scenarios; with and without resuspension at different modelled periods (September 2005 and November 2005) were investigated by changing the values in the cells in the 'Data Input' worksheet, and re-plotting the resulting final text output in Surfer$^{TM}$. The fact that data in the 'Data Input' worksheet can be altered easily and quickly to model many different productions scenarios makes the CAPOT model a very useful tool.

The spreadsheet data plotted in Surfer$^{TM}$ produced contour models of organic carbon deposition ($g/m^2$/3days) around the production site, based upon the biomass and growth of adult cod at the production site at Vidlin. The values calculated in the model were compared to the observed values obtained and calculated from the sediment traps (Figure 7.19). Deposition rates were

calculated for three days as this is the duration that the sediment traps were deployed. Figure 7.13 shows a graphic representation of the production site in Vidlin Voe. The basis of this representation was used to formulate the suspended solid model for September 2005 (Figure 7.14), the carbon production model for September 2005 (Figure 7.15), the carbon production model for November 2005, (Figure 7.16), the carbon production model for September 2005, after resuspension (Figure 7.17) and the carbon production model for in November 2005, after resuspension (Figure 7.18). All models were compiled using hydrographical data, settling velocities, leaching rates and faecal inputs, and an FCR of 1.2.

Figures 7.15 to 7.18 contain a black line around the site, which represent a threshold limit of $2gC/m^2/day$. This level is significant, as stated by Cromey *et al* (2002) and Beveridge (2004), as a threshold concentration for organic carbon enrichment in temperate marine waters. At concentrations below this level, Carbon enrichment can be successfully bio - turbated showing no significant impact. However, at levels greater than $2g/C/m^2$ day, a significant depletion of oxygen in the sediments is evident Cromey *et al* (2002) and Beveridge (2004).

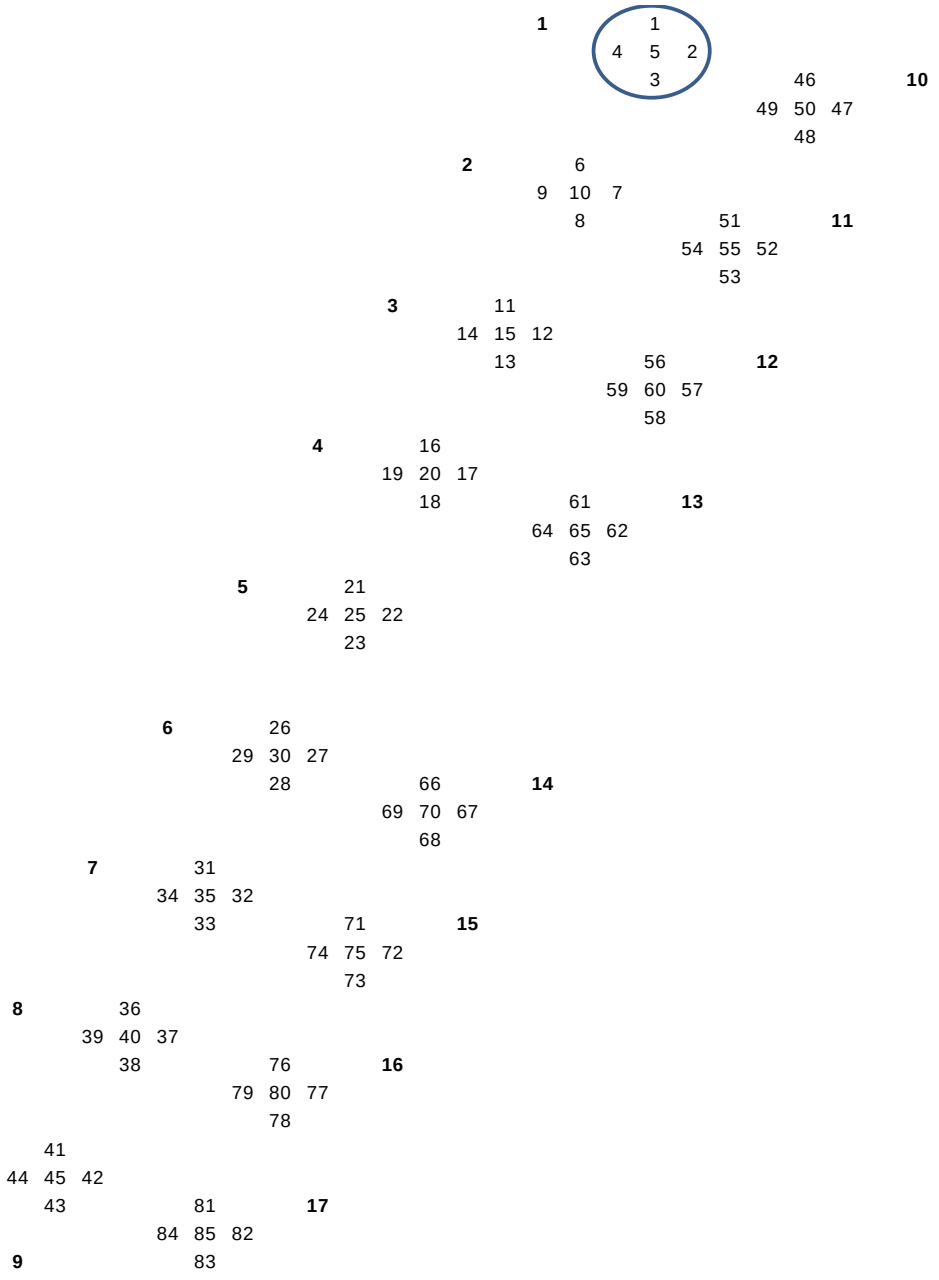



Figure 7.13 Model of production site in Vidlin Voe.

The numbers on the boundaries represent the cage numbers at the production site. The other values represent the biomass in the cages as provided by No Catch® Ltd.

Figures 7.14 through to and including 7.18 are based upon this site layout.

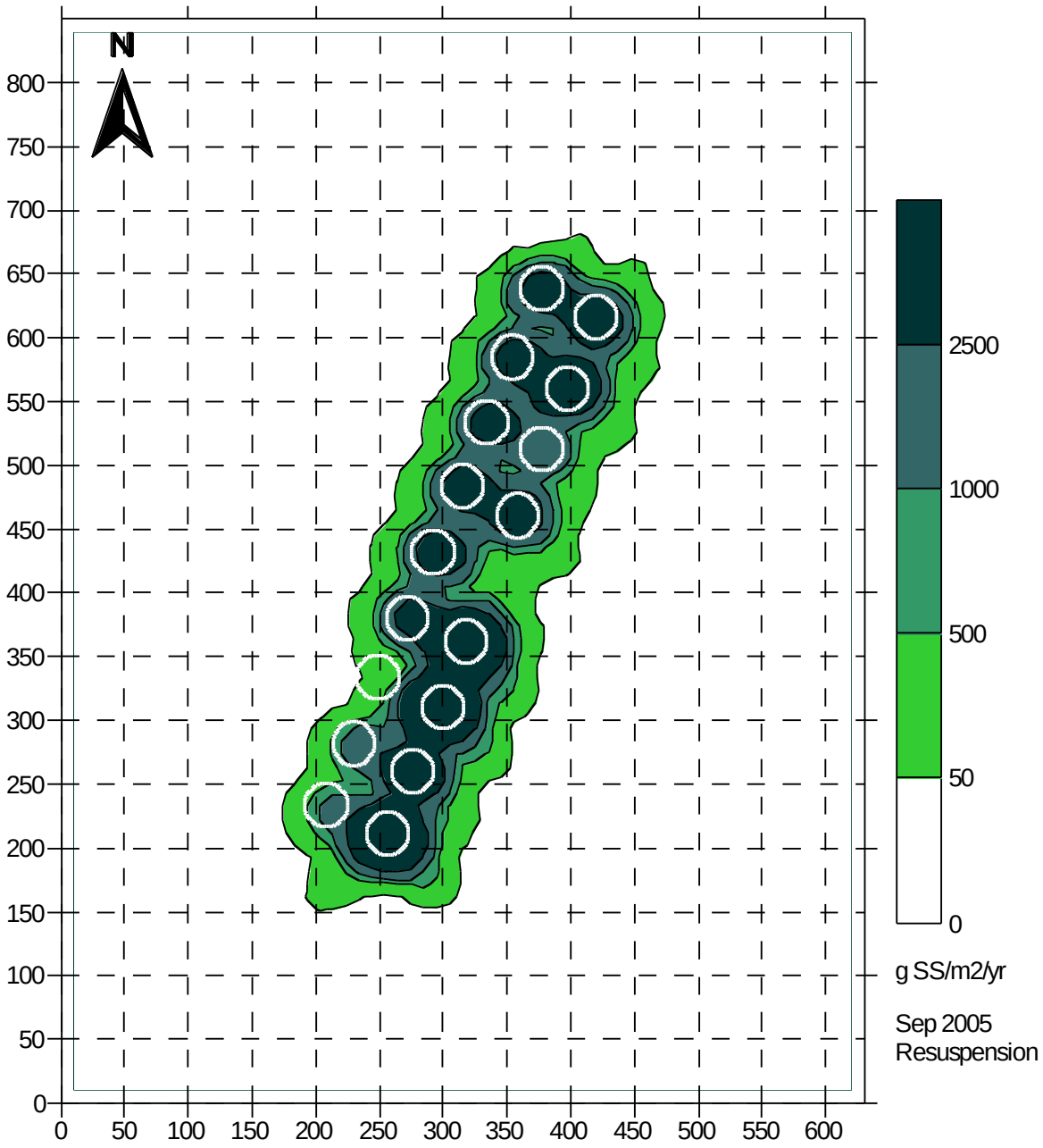
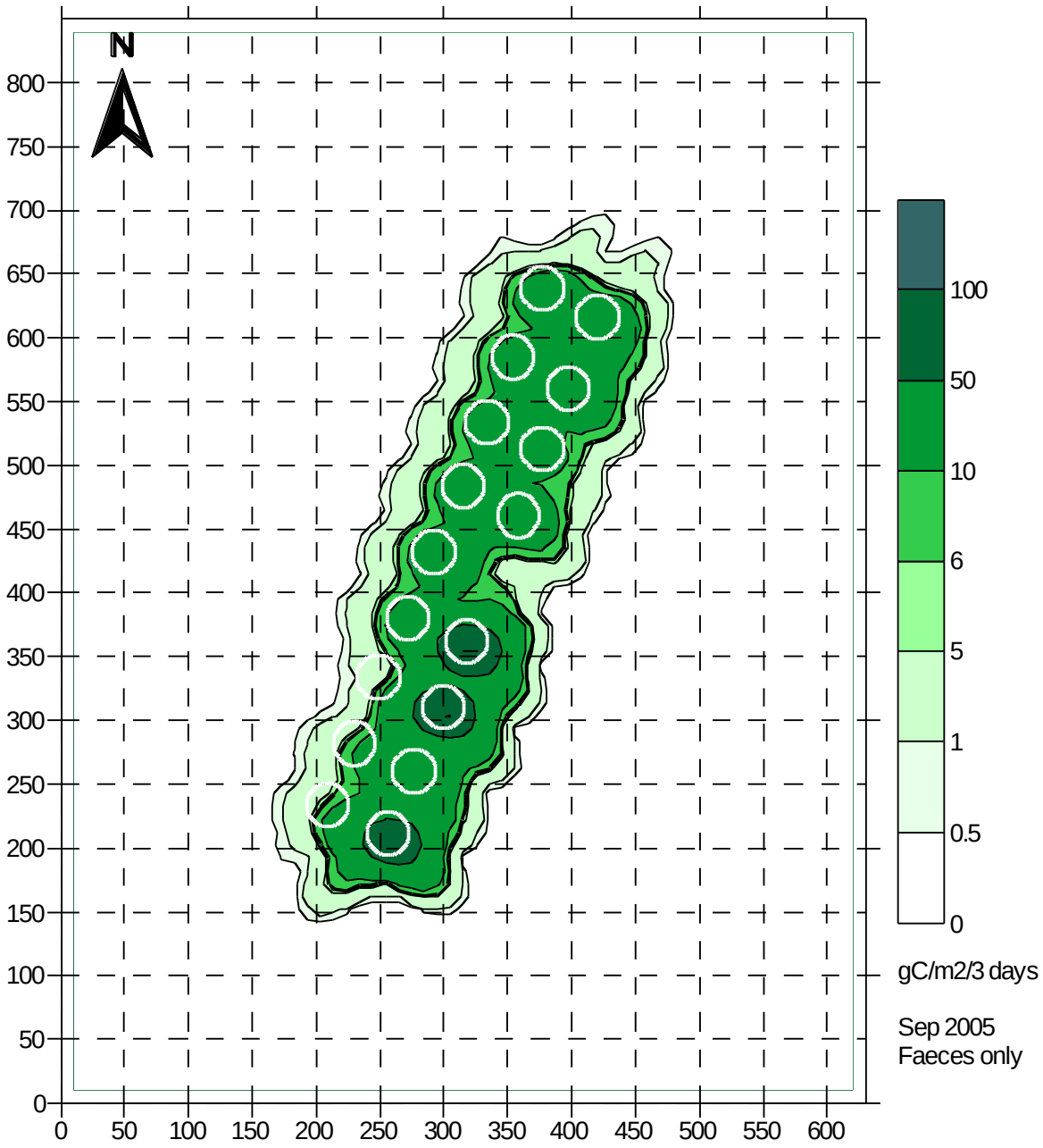
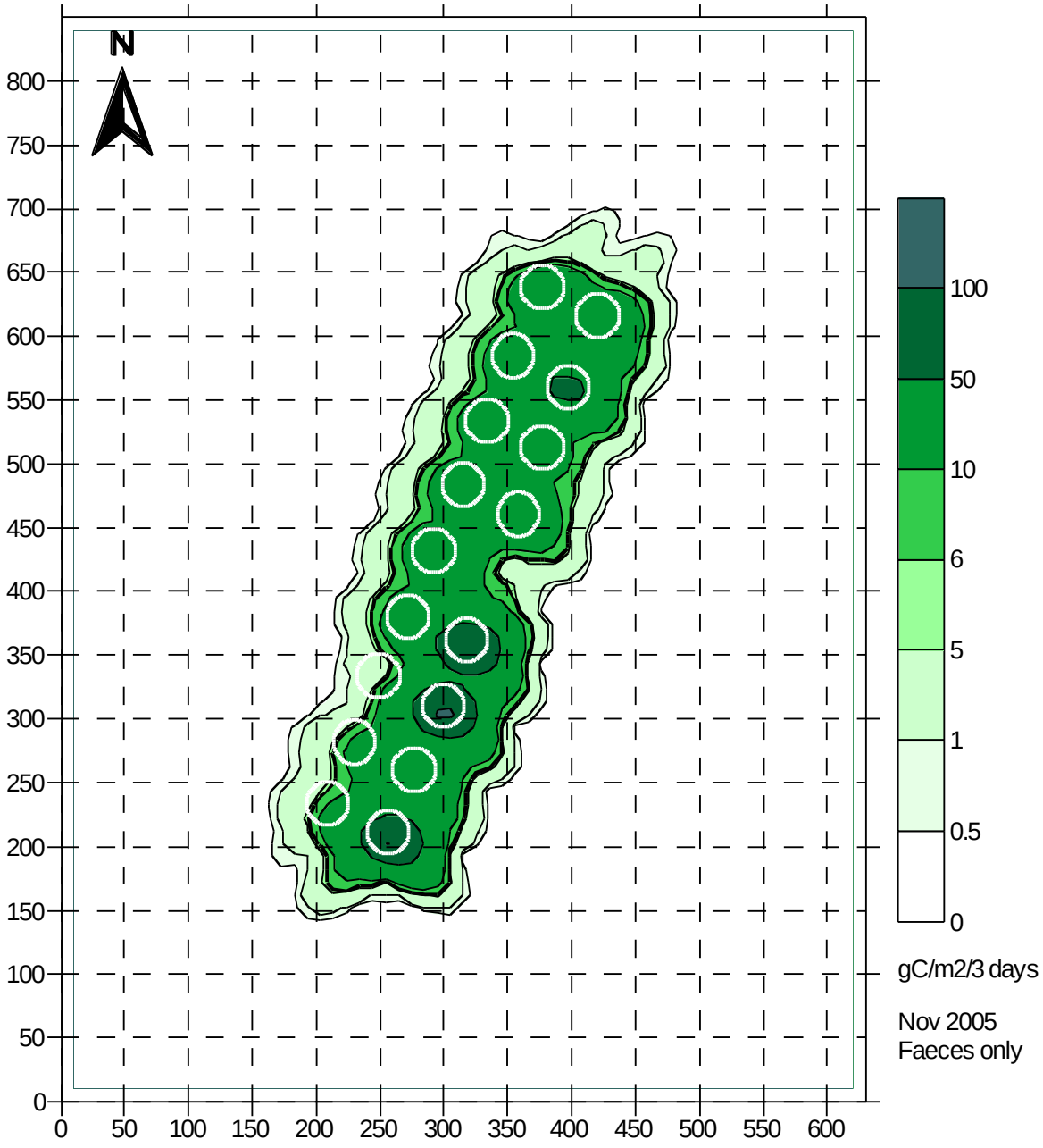



Figure 7.14 Model of Suspended Solid production for September 2005.
The values have been extrapolated to $m^2$ per year.



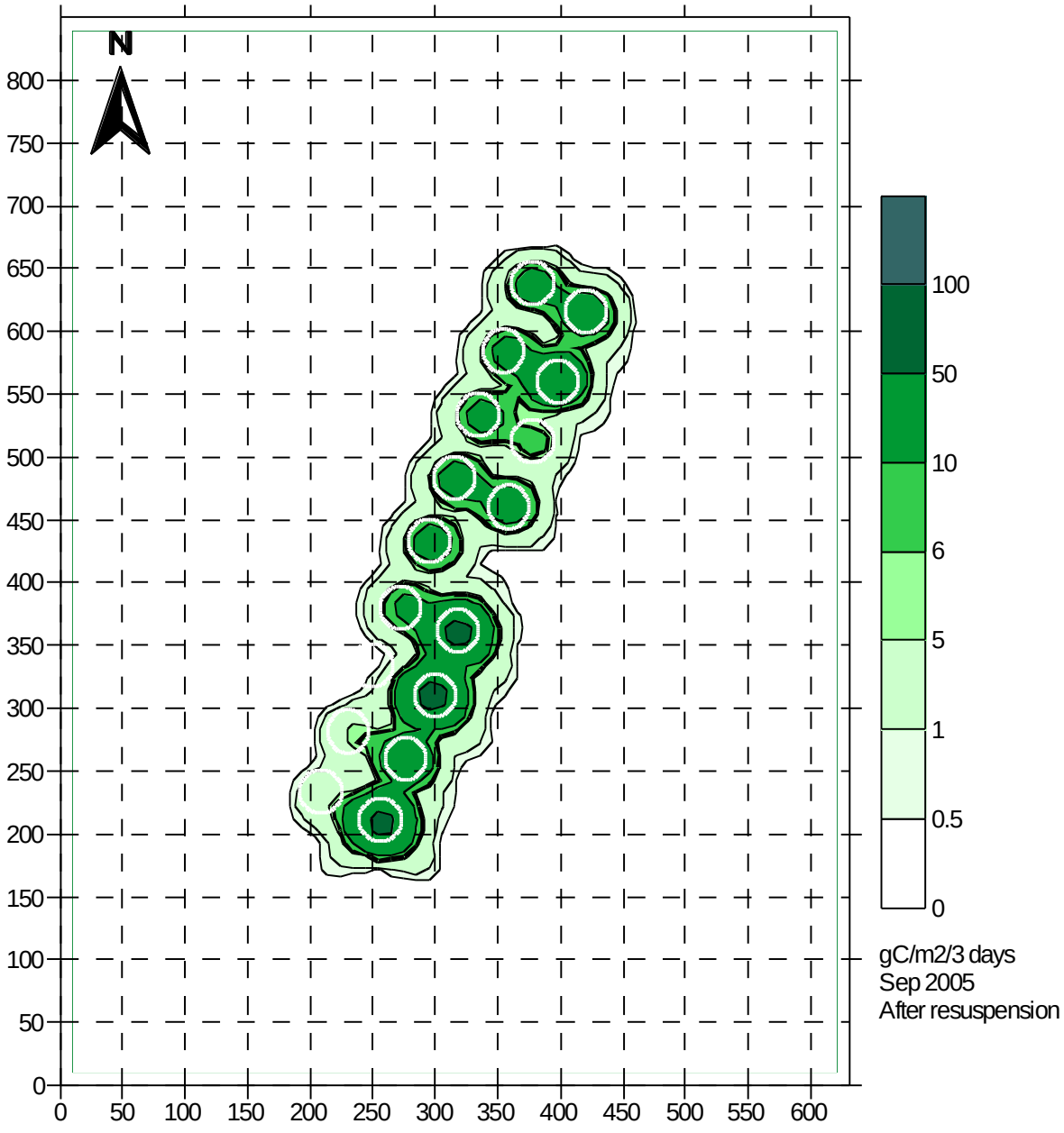

N.B. Scale is in metres. The black line around the site represents the limit of 6g/$m^2$/3 day (2g/$m^2$/day).

Figure 7.15 Carbon settlement in September 2005, resulting from faecal inputs (g/$m^2$/3days). The values are extrapolated to 3 days, which represents the period of time the sediment traps were deployed.



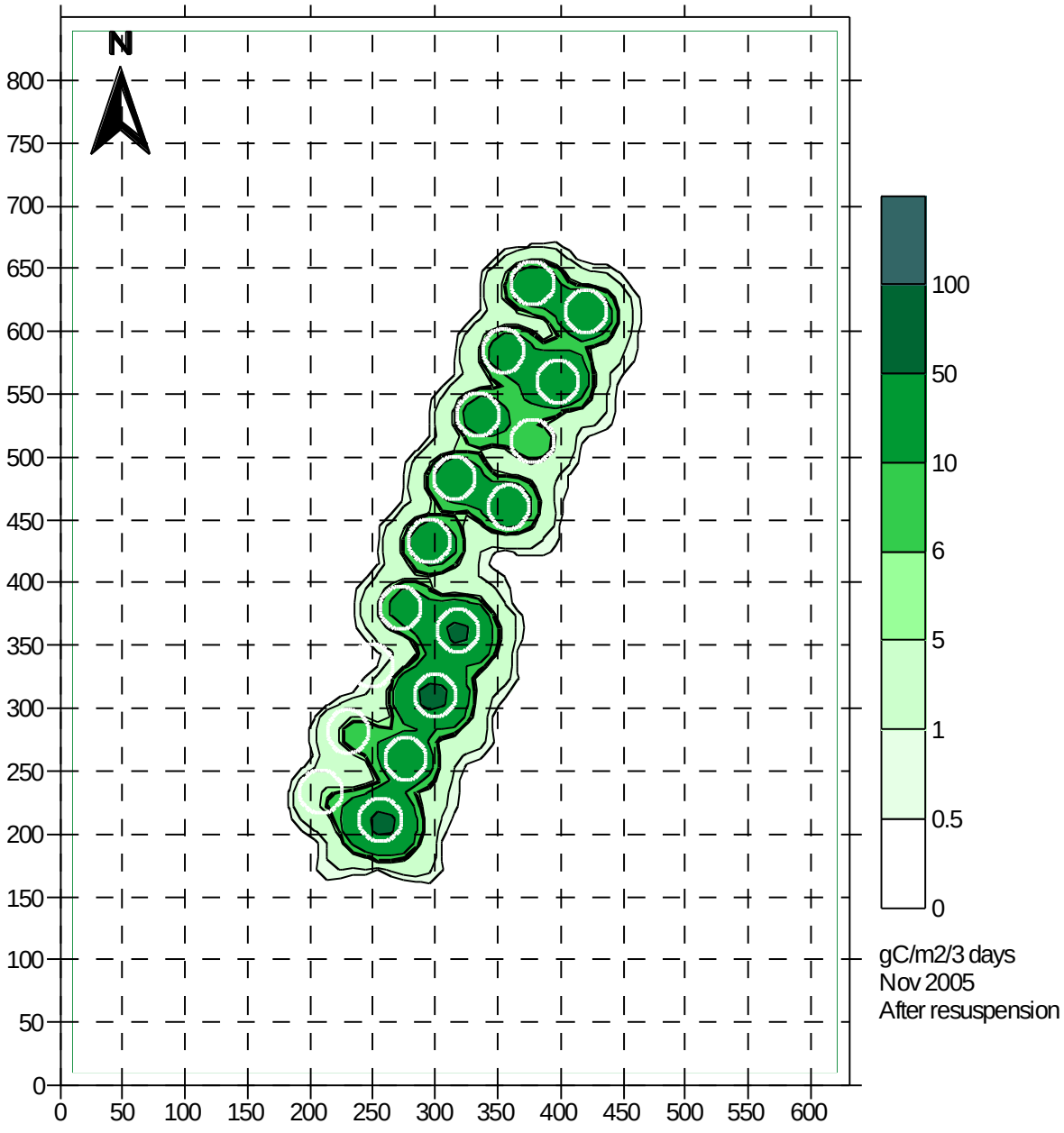

N.B. Scale is in metres. The black line around the site represents the limit of 6g/m$^2$/3 day (2g/m$^2$/day).

Figure 7.16 Carbon settlement in November 2005, resulting from faecal inputs (g/m$^2$/3days). The values are extrapolated to 3 days, which represents the period of time the sediment traps were deployed.




N.B. Scale is in metres. The black line around the site represents the limit of 6g/$m^2$/3 day (2g/$m^2$/day).

Figure 7.17 Carbon settlement in September 2005, resulting from faecal inputs (g/$m^2$/3days) after resuspension. The values are extrapolated to 3 days, which represents the period of time the sediment traps were deployed.




N.B. Scale is in metres. The black line around the site represents the limit of 6g/$m^2$/3 day (2g/$m^2$/day).

Figure 7.18 Carbon settlement in November 2005, resulting from faecal inputs (g/$m^2$/3 days), after resuspension. The values are extrapolated to 3 days, which represents the period of time the sediment traps were deployed.

Figure 7.14 shows a model of suspended solid production using data collected in September 2005, which has been extrapolated to give g SS/$m^2$/yr. It shows the greatest sedimentation following resuspension around the cages containing the greatest biomass, (Cages 14 - 17) which are the bottom right cages (Figure 7.13). The greatest deposition in relation to cage biomass is also observed in Figures 7.14 to 7.18.

Figures 7.15 and 7.16 show carbon production from faecal inputs alone in September 2005 and November 2005. The models include resuspension and the black line present in all models represents a limit of 6 gC/$m^2$/3 day, which assimilates to 2 gC/$m^2$/day seen as the acceptable limit for organic carbon deposition in Scottish waters.

It can be seen that a greater sedimentation of faecal material occurs in September 2005 compared to that observed in November 2005. This maybe related to feeding; as the fish were fed 0.5% bw/day in September 2005 compared to 0.4% bw/day in November 2005. Another reason may be due to calmer weather conditions observed in September 2005 to that in November 2005, resulting in less spatial dispersal of faecal material. It can also be observed in Figures 7.17 and 7.18 that faecal deposition in both models is further extended to the east of the cages than to the west.

Figures 7.17 and 7.18 show organic carbon deposition (g/$m^2$/3 days) in September 2005 and November 2005, after resuspension and show a defined localisation of faecal deposition, in particular the black line showing the 6 gC/$m^2$/3 day limit to the cages. Both Figures show a similar trend although a greater intensity of deposition can be seen in September 2005. This may be again due to higher feeding rates as discussed earlier.

### 7.4.1 Validation of model

The sediment trap data can be used to validate the models shown in Figures 7.14 through to 7.18 inclusive. No feed pellets were collected in any traps deployed during any of the three sampling trips. Consequently, the models concentrate exclusively on faecal deposition. However, it can be seen that the comparison of the collected (observed) sedimentation data and the predicted data from the model show little correlation for September 2005 (Figure 7.19) and November 2005 (Figure 7.20). The large differences between the collected (observed) data and the values predicted by the model will be influenced by several factors. There may be high variability in the collected data due to influences of weather and variable current speed / direction. On the following page, Figure 7.19 and 7.20 show carbon deposition around the production site in September 2005 and November 2005 respectively. Both Figures chart observed (sediment trap data) against predicted (CAPOT model). All raw data is shown in Appendix 34.

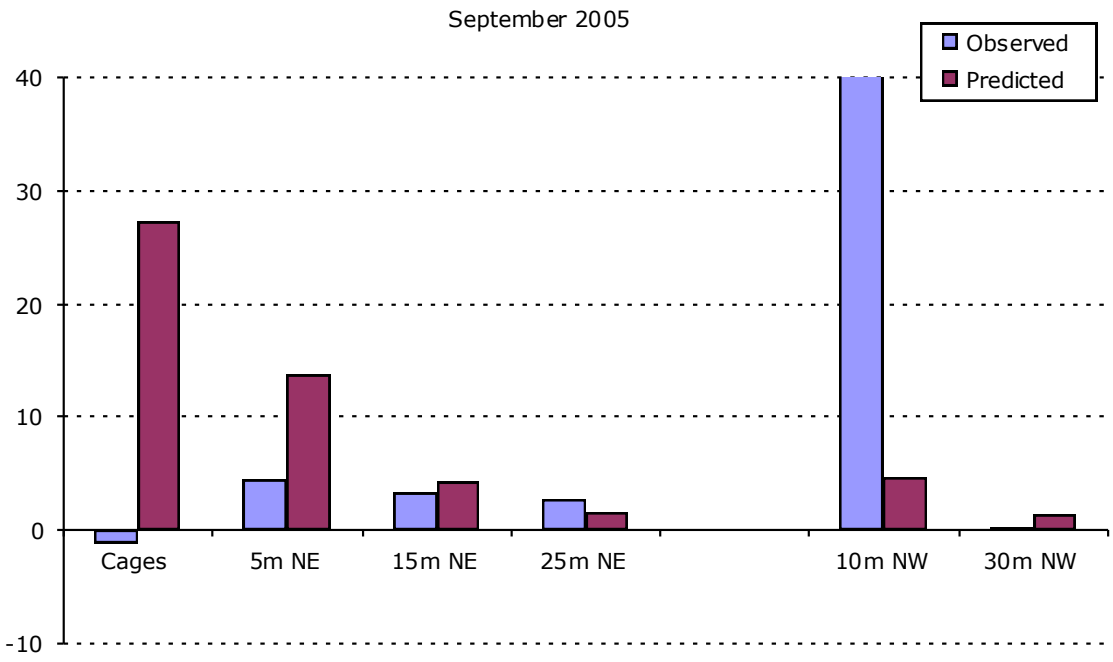



Figure 7.19 Carbon deposition around production site in September 2005 (observed .v. predicted).

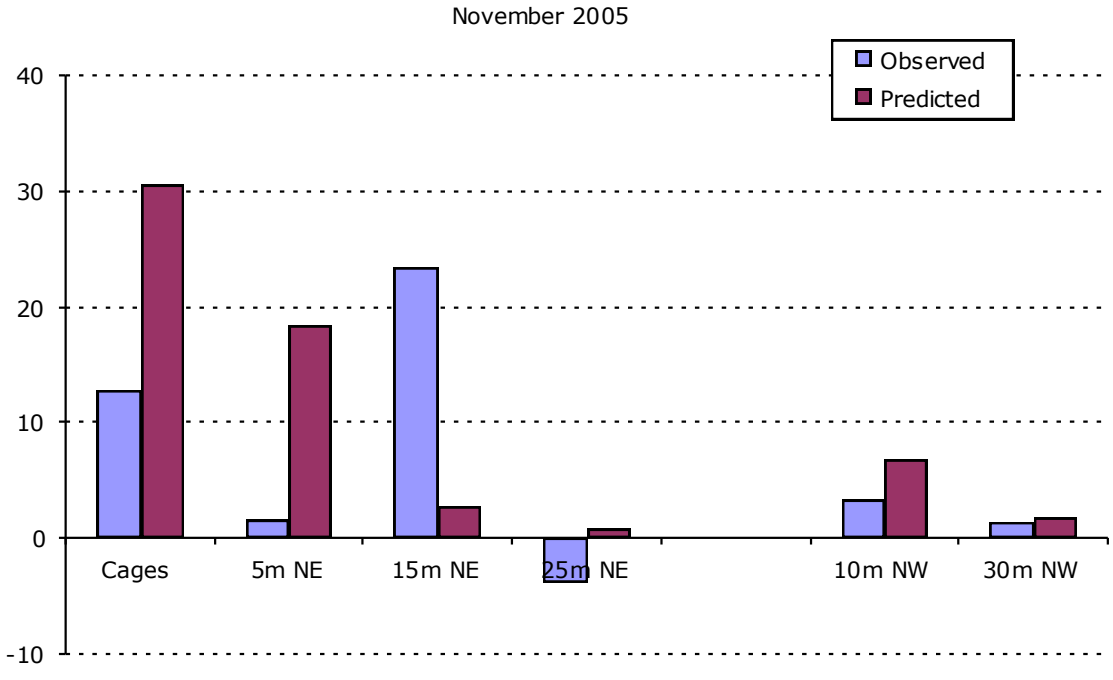



Figure 7.20 Carbon deposition around production site in November 2005 (observed .v. predicted).

When comparing the CAPOT model to DEPOMOD (Figure 7.21), a strong correlation can be seen suggesting that both models are comparable, but are not accurate in terms of the predicted value versus the observed sediment data (Figures 7.19 and 7.20). The spreadsheet model has many advantages as it can be used on any computer and does not require any specific software to generate models.

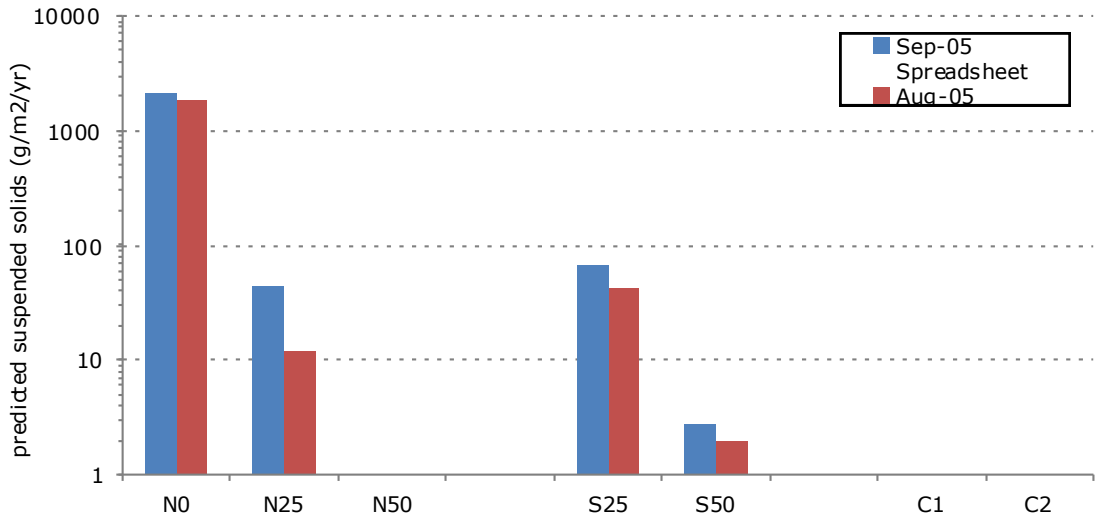



Figure 7.21 Carbon deposition rates calculated by CAPOT (blue) compared to that calculated by DEPOMOD (red).

The parameterisation of the CAPOT model was validated as it was run against the trap data and also the DEPOMOD data from a previous study at the same site (Cromey *et al*, 2007). The correlation with the trap data (Figure 7.20) is poor as to be expected due to the high variability of the aquaculture system. However, the correlation with the DEPOMOD results (Figure 7.21) is much stronger making the models comparable when assessing the impact of an aquaculture system on the local aquatic environment. All associated raw data is shown in Appendix 35.

## 7.5 Discussion

### 7.5.1 Settling velocities

According to Stokes' Law, the settling velocity of a particle is dependent upon its dimensions, shape, density and the viscosity of the medium. Viscosity in turn is dependent on temperature, solute concentration and pressure. In order that the law may be applied, the Reynolds number (Re) should be less than 0.5 and hence the maximum settling velocity is required to be less than 1 cm/s (Smith, 1975). As all settling velocities measured are greater than 1 cm/s Stokes law is not directly applicable but aspects such as pellet size, density and water temperature should be considered.

Settling velocities of aquafeeds have been studied and it has been found that settling velocities increase with pellet size (Chen, 2000). Such an observation was made in the current study at MERL with the 7 mm EWOS® pellet settling at a faster rate (approximately 9 cm/s), than the 4 mm EWOS® pellet (approximately 6 cm/s). A similar trend was observed within this study at No Catch® Ltd. with the 12 mm Biomar® diets settling at 12.4 cm/s ± 1.8, 11.64 cm/s ± 1.9 and 11.4 cm/s ± 1.9 for 8 ºC, 10 ºC and 13 ºC respectively. Settling velocity of the 3 mm Biomar® diet was 8.38 cm/s. These results are comparable with data recorded by Findlay & Watling (1994), who provide data on seven pellet types and quote settling rates ranging from 5.5 cm/s for a 3 mm pellet to 15.5 cm/s for 10 mm pellets. Elberizon & Kelly (1998) recorded settling rates of 5 cm/s - 12 cm/s for 2 mm and 8 mm salmonid pellets respectively. In a more recent study, Chen, (2000) states an increase in settling velocity from 6 cm/s for a 2 mm EWOS® pellet, to 13 cm/s for a 10 mm EWOS® pellet. This is compared to 10 cm/s for a 4 mm Trouw® pellet through to 12 cm/s for an 11 mm Trouw® pellet. This difference in settling velocities for similar sized pellets produced by different manufacturers (EWOS®, Biomar® and Trouw®) may be due to differing raw materials and manufacturing criteria. Any differences between

species specific diets produced by different manufacturers will be negligible and may be related to raw materials, formulation and manufacturing criteria,

As density of the medium effects settling velocity, it could be deduced that a higher temperature (resulting in a less dense medium) would promote increased settling velocities. However, in the current study and similar work undertaken by Chen (2000), the opposite is observed. Chen (2000) states that the density of sea-water decreases by 0.2 % between 10 ºC and 20 ºC but found that diets settled faster at 10 ºC than at 20 ºC. Due to the relatively small range of temperatures investigated (8 ºC - 13 ºC) in the current study, the differences between the settling velocities as a result of temperature is not significant.

In the present study, the use of large sample sizes (25 repetitions per diet) increases the statistical validity of the settling velocity data. As the settling velocities were only measured over 1 m in depth, the difference in settling rates between the formulations for each pellet size is not significant for tank systems. The depth of 1 m is not ideal as it is not deep enough but the 1 m column was the only facility available to measure settling velocities. Temperature is a factor affecting settling velocity as it can affect viscosity of the medium (warmer water is less dense). However, settling velocities of the diets investigated in the tank studies were studied at a mean temperature of 11 ºC with a small outlying range. A large range in temperature would affect settling velocities due to changes in water density. However, the narrow range investigated in the tank and cage studies do not promote significantly different settling velocities. It was considered that the impact of temperature on a settling depth of 1 m would not be significant.

### 7.5.2 Leaching of ammoniacal nitrogen

Leaching of ammoniacal nitrogen from the studied diet formulations was related to protein content and pellet size. Both the 4 mm and 7 mm EWOS® pellets followed the same trend with the greatest protein composition promoting the greatest ammonia concentration in the water following 30 min. of immersion. It should be also noted that leaching from the 4 mm EWOS® pellet was greater than the respective formulations as a 7 mm EWOS® pellet. As 1 g of each formulation for each pellet size was analysed, the greater leaching rate exhibited by the 4 mm EWOS® pellet was due to a greater surface area of the smaller pellet in contact with the water.

Leaching of ammoniacal nitrogen from the 12mm diet was studied over a range of temperatures (8 ºC, 10 ºC and 13 ºC). Following 5 min. of immersion in artificial seawater, leaching was similar at each temperature. However, at 10 min. onwards, and ammonia concentration in the artificial seawater (Section 2.4.3) could be correlated to temperature with the highest temperature promoting greatest leaching and the coolest temperature promoting the least. However, the temperature range was small, the immersion period short and the actual differences between leaching, as a result of temperature was not significant. It should be also noted that leaching from the 3 mm pellet was more than double (µg/L) than that of the 12 mm pellet. This is related to pellet size as 1 g of each diet was analysed. The greater leaching exhibited by the 3 mm pellet was due to a greater surface area of the pellets in contact with the water. When comparing leaching of the EWOS® diets and Biomar® diets, it could be seen that the Biomar® diets generally exhibited far greater leaching over 30 min. Composition and pellet size of the 3 mm Biomar® pellet was similar to Diet F (60% protein and 4 mm) as investigated in the juvenile tank study. The difference in leaching of the diets could be attributed to differences in raw materials and / or production standards and methods. It is published that extruded pellets are less polluting as they have a smaller surface area, are more stable in water and generally promote higher digestibility. Seymour & Bergheim (1991) developed an extruded pellet that remained 84 % intact following 24 hours of immersion. Although extruded pellets are

stable for longer periods of time, it became apparent in the tank studies that cod only readily accepted food as long as it was in the water column. Therefore, optimal feeding regimes exploiting cod feeding behaviour need to be investigated in order to maximise ingestion rates and maximize growth and minimise leaching of ammoniacal nitrogen from uneaten pellets and consequently reduce environmental impacts.

### 7.5.3 Carbon and nitrogen loading into the environment

Uneaten food, faecal and excretory losses to the environment can be estimated using data on FCR, feed quality and quantities, and retention levels in the fish. The following equation can be used to calculate a very approximate value for nitrogen loading into the environment:

$$(FCR \times N_2 \text{ in diet}) - (1000 \times N_2 \text{ in fish})$$

In order to quantify and compare the effect of waste discharges from large marine farms in Nordic countries, the Norwegian Institute of Water Research (NIVA) surveyed waste discharges from salmonid cage farms in Norway and found that from the culture of 290,000 tonnes of salmonids in commercial culture cages has led to feed-based pollution of 4,255 tonnes of phosphorus and 20,286 tonnes of nitrogen annually (Bergheim, 2000). This load is not particularly high in comparison to the total load of these elements in sea-water, and also when considering that the total annual load of nitrogen derived from agriculture, industry and waste water treatment in Denmark alone was estimated to be 460,000 tonnes.

Coastal areas are nutrient rich from both terrestrial and oceanic inputs. Nutrient upwelling in coastal areas combined with terrestrial inputs, results in greater production in coastal areas compared to that observed in the open ocean. Microbial processes help to create the balance between nitrogen input and outflow to an ecosystem and microbial activity is integral to the removal of nitrogen loading through denitrfication. (Christiansen *et al*, 2000) found that

denitrifcation processes observed in a Norwegian fjord can remove up to 50 % of nitrogen loading from terrestrial sources, compared to 25 % removal of terrestrial input into Chesapeake Bay (Boynton *et al,* 1995). Following work undertaken in the North Sea, Livingstone *et al* (2000). states that denitrification in the sediment occurs mostly in the uppermost 5 cm of the sediment, decreasing with depth. Eutrophication may result from enrichment and can cause hypoxia and anoxia due to the increased oxygen uptake of microbes involved in the breakdown of organic material. This can ultimately result is a decrease in species diversity.

As discussed by De Silva and Anderson (1995), based on work undertaken by Hakanson (1986) and Kryvi (1989) a typical flow of nitrogen in salmonid pens shows that approximately only 25 % of the nitrogen ingested is retained within the fish. The remaining 75 % is released into the environment either as ammoniotelic nitrogen or nitrogen contained within solid wastes. This calculation was based on an FCR of 1.5. By reducing protein content of the diet through partial replacement with another constituent such as carbohydrate may be possible but could lead to increased carbon pollution. Further work would be required to investigate commercial cod diet formulations that would maximise growth while keeping carbon and nitrogen pollution to a minimal or sustainable level.

The development of commercial aquafeeds used in the culture of Atlantic salmon has reduced protein (and associated nitrogen content) through protein sparing using lipid (up to 30 % of formulation). Such sparing is not possible in cod culture due to the ready deposition of excess lipid almost exclusively in the liver. The amount of waste food is dependent upon feeding efficiency, which is influenced by feed composition, feeding methodology and water currents at the cage location. Uneaten food wastes have been suggested at less than 5 % (Weston, 1986), between 1 % and 5 % (Gowen & Bradbury, 1987) and an average value of less than 5 % (Findlay & Watling, 1994).

Based upon work carried out by Weston (1986) and given the accepted digestibility of commercial aquafeeds to be around 90 %, Brooks *et al* (2002) suggest that approximately 12.5

% of the ingested food will be ejected as faeces. Based upon the given 5 % uneaten feed, Brooks *et al* (2002) concluded that approximately 8.8 % of organic carbon in the feed is discharged in the particulate form. Ellis (1996) suggests that waste feed and faecal material produced by all salmon farms in British Columbia, Canada is equivalent to human sewage produced by a city the size of Edinburgh, while Folke *et al* (1994) compared the waste produced through the production of 100 tonnes of Atlantic salmon to that produced by a human settlement of between 880 and 3200 individuals. Such comparisons have been criticised based on many assumptions on which they were made.

Ackefors & Enell (1994) estimated that total organic output from a salmon farm to be 2.5 tonnes wet weight per tonne of fish produced. Gowen *et al* (1988) estimated that 21 % - 23 % of carbon in the feed was retained in the fish and 75 % - 80 % was lost to the environment, predominantly as $CO_2$. Findlay & Watling (1994) measured sedimentation rates between 1 g C/$m^2$/day and 1.6 g C/$m^2$/day while Hargrave (1994), when summarising sedimentation associated with salmon cage operations, stated a range from less than 1 g C/$m^2$/day to over 100 g C/$m^2$/day. This conclusion was made through the comparison of work described by a number of authors. Johnsen *et al* (1993b) estimated a range of 20.5 g/N to 30 g/N and 6.7 g/P were released per kg of salmon produced when fed high lipid (30%) diets. Based upon these calculations, Leving (1997) concluded that 880 tonnes of nitrogen and 118.6 tonnes of phosphorus are released into the marine environment of British Columbia annually by salmon farms alone. Uneaten food was not considered in these calculations.

### 7.5.4 Dispersion modelling

Modelling is an important tool in assessing the direction and severity of deposition of an investigated compound or element (in this case the loading of organic carbon and nitrogen) into the marine environment. These models are useful for regulation when assessing the direction and severity of effluent deposition from a marine cage site into its environment. As yet, there are no models used specifically for commercial cod culture and the data concerning discharge consents granted by SEPA to the industry use the increased protein level of cod diets and retro-fit the associated nitrogen data into DEPOMOD carbon models, created for Atlantic salmon culture.

The model developed here through the spreadsheet of the salmon model CAPOT has several advantages of the DEPOMOD modelling system. CAPOT utilises a spreadsheet in which data can be entered and the model run with great simplicity. However, the parameterisation of DEPOMOD is already in the code of the model. It is therefore necessary for the model to be re-coded specifically for each data set. This effectively means that data needs to be added to a new DEPOMOD database rendering the DEPOMOD inflexible and clumsy as a research tool, as coding for each new data set is time consuming and requires circulation of each individual data set. CAPOT is much better as a flexible research tool as it is simple to change additional data and tweak existing data to incorporate into the model allowing quicker development of the model.

As well as being a flexible research tool, modelling allows the comparison of environmental impacts from different production scenarios (i.e. regulation based on husbandry). Such modeling would be useful in assessing the different environmental impacts using different feeding regimes such as 40% protein diet fed daily (similar regime to Atlantic salmon) compared to a 50% protein diet fed 2 times per week. However, the use of the spreadsheet model allows rapid modelling of any production scenario, which is useful to the regulatory body (SEPA), when granting accurate discharge consents specific to cod aquaculture.

Given the confirmed accuracy of the CAPOT model, Figures 7.14, through to and including 7.18 show a local deposition of particulate carbon and nitrogen to the cage site, supported by R.O.V. work undertaken by No Catch® Ltd. these results are positive and provide further data to the direction and degree of organic sedimentation associated with a commercial cod cage system.

As there is little other work to compare the current models to, there is much scope for future work into the topic. These studies provide baseline models to which future work can be compared. Although the models (DEPOMOD and CAPOT) provide comparable predicted results to each other, there is very little correlation between the models (predicted results) and the sediment traps (observed results). This is to be expected due to the dynamic marine environment and the number of variables influencing the settlement rates. The models are beneficial in that they are indicative of the degree of settlement of particulate matter in relation to a particular site given site-specific hydrodynamic data. However, at best they are indicative and should not be read as definite values given the highly dynamic nature of the marine environment. There is scope for much future work, investigating different sites, different diets and different feeding regimes. As all sites will have different hydrodynamic data, such models are very site specific. Future work would build upon the current study, increasing the understanding of particulate matter settlement associated with the commercial culture of adult and juvenile Atlantic cod in cage systems, the influences of weather and currents and how formulations and regimes can effect the settlement of organic carbon and nitrogen.

# Chapter 8

## GENERAL DISCUSSION

## GENERAL DISCUSSION

With the current low levels of Atlantic cod populations, one readily realises the desire for the development of a successful cod farming industry. Considering individually the various elements of study carried out within this thesis, results indicate the aims of the study (as discussed in Chapter 1), were achieved through a series of four separate studies. With discharge consents granted to cod farming by SEPA set at 66 % of those granted to salmon farming, the work included in this study offers the data to create more accurate nitrogen models for cod farming, thereby supporting expansion of the industry in part by the granting of greater discharge consents. The models created in the current study offer a starting point into the modeling of wastes associated with the commercial culture of Atlantic cod. The dissolved nitrogenous waste profiles show a direct relationship between feeding and ammonia excretion and show that excretion rates of cod are comparable with those of salmon, despite the increased protein levels in commercial cod diets.

The 66 % discharge consent value granted by SEPA is probably valid due to the increased level of protein in the cod diet. The calculation is based upon cod being fed on a similar regime to that of Atlantic salmon. The current work found that feeding on this regime was extremely wasteful and studies at MERL showed growth of the adult fish is similar over the 40 %, 50 % and 60% range of protein content of those diets. As it stands, the 66 % discharge consent value may be inaccurate as the current study found that the excretion of dissolved nitrogenous wastes associated with the commercial culture of cod are comparable with that of commercial salmon culture despite the increased protein levels in the cod diet. However, further work would be required to substantiate the findings of the current study and encourage SEPA to increase the discharge consent granted to cod farmers. Such findings give SEPA fundamental cod - relevant data when considering the discharge consent for the commercial culture of the species. Further detailed work investigating different diet formulations and feeding regimes is necessary, such as

considering the effect on growth, condition and nitrogen production when feeding cod a 40 % protein diet daily, compared to feeding the cod a 50 % diet on alternate days. Such work would provide SEPA with highly detailed data related to a range of diet formulations and feeding regimes, which should be taken into account when calculating an accurate discharge content directly related to cod farming.

In a regulated environment, the tank study showed clearly that juvenile fish (up to 380 g in this instance) required a higher protein diet than adult fish. However, it also showed that there was no significant difference in the growth promoted by the 50 % and 60 % protein diets suggesting that a 50 % protein diet may be used to successfully culture juvenile cod in tank systems. It was also observed that the 50 % and 60 % protein diets promote a similar FCR and a similar increase in K - Factor over the full range of the 154 - day study. Publications (Pillay & Kutty, 2005; Morais *et al*, 2001 and Foster *et al,* 1993) acknowledge that Atlantic cod (juveniles in particular) require high protein diets. Currently, commercial juvenile cod diets contain approximately 57 % protein (the juvenile Biomar® diet as used at No Catch® Ltd.). However, the use of a lower protein content diet (closer to 50 % protein) would present considerable economic benefit to the industry and also potentially reducing the nitrogen impact upon the environment.

The adult tank study also shows that there was no significant difference in growth promoted by each diet, suggesting that a low protein diet (40 % protein) promoted similar growth as a high protein diet (60 % protein), indicating that a lower protein diet may be used for the culture of adult cod. The associated condition of the adult fish was similar under each treatment and improved over the course of the study under all three formulations. However, as the fish completed spawning immediately prior to the commencement of the study, an increase in condition was not observed until approximately day 90 of the 210 - day investigation and consequently, no significant growth was noted up to approximately 90 days. It was also noted

that the energy was directed to the liver to regain condition before growth commenced. Therefore, the use of the 40 % protein diet (low protein, high lipid) would be economically and environmentally beneficial (in terms of nitrogen excretion). However, exclusive use of a 40 % protein diet throughout the production cycle is not recommended as it would promote enlarged livers and potentially unhealthy fish.

Other work undertaken by EWOS® found a 54 % protein content of a commercial diet for cod to be optimal. Commercial diets used at No Catch® Ltd. as supplied by Biomar® were analysed as part of this study and were found to contain, 52 % protein for adult fish. However, the current results suggest that a lower protein diet may be used. However, as a narrow optimal range of protein has been defined in the present study and other works, further work concentrating on this narrowed range of protein content would further refine the relationship between an optimal protein content of the diet, growth and reduced nitrogen excretion, potentially reducing production costs and environmental impacts.

Unfortunately, apparent digestibility was not measured accurately due to the preparation of the dry weight diet and faecal samples. Acid digestion of the samples through heating in a microwave oven as opposed to an aged hotplate (as recommended to me) would have allowed greater control of temperature, time and quality of the prepared samples. In turn, this would have allowed the accurate calculation of apparent digestibility promoted by each of the EWOS® diets studied.

In the juvenile tank study, nitrogen digestion was directly related to the protein (and associated nitrogen) content of the diet and expressed as a percentage of the content of the diet as 59.19 % (40 % Protein), 56.90 % (50 % protein) and 52.23 % (60 % protein) suggesting that nitrogen digestion in juvenile cod is more efficient at lower protein content in the diet. Such a finding gives rise to future work investigating the relationships between protein content of the diet, digestion rates and growth / condition of the juvenile cod.

In the adult tank study, nitrogen digestion was similar regardless of the protein (and associated nitrogen) content of the diet and when expressed as a percentage of the nitrogen content of the diet, nitrogen digestion observed in the adult study was 60.55 %, (40 % protein) 60.92 % (50 % protein) and 60.60 % (60 % protein) respectively, suggesting protein digestion is similar regardless of protein content and that feeding a high protein diet is inefficient.

A higher carbon and nitrogen content is observed in the faecal material collected under an automated regime, suggesting that when the cod are fed continuously, digestion is less efficient. It is also evident that the carbon and nitrogen in juvenile faecal material is greater than that observed in adult faecal material suggesting that digestion of carbon and nitrogen in juvenile fish is less efficient than in adult fish. Such findings can have very important implications on feeding regimes, which should be considered in future work.

Tissue samples from both adult and juvenile fish were collected throughout the tank and cage studies, and the dry weight samples analysed for proximate composition. In the tank studies, dry weight adult muscle was found to contain between 93 % and 94 % protein and between 14 % and 15 % nitrogen, compared to approximately 80.5 % protein and between 13 % and 14 % nitrogen of the juvenile carcass. Similar values were obtained from the cage studies with protein values between 92.5 % and 93 %, and nitrogen between 14.5 % and 15 % of the adult muscle. Values obtained for juvenile carcass sampled in the cage study reflected those obtained in the juvenile tank study (c. 80 % protein and c. 13.5 % nitrogen). Lower values were observed in the juvenile carcass as the whole clean fish (including skeleton) was analysed, which increased the proportion of ash in the sample. Muscle alone was analysed for the adult fish, reducing proportionate ash content and increasing proportionate protein content.

From the studies it is clearly evident that ammonia production is related to the feeding regime and to the protein content of the diet. When comparing the manual to the automated feeding

regimes this is particularly evident in the controlled environment of the tank studies. With manual feeding (twice daily) the ammonia production is higher than that produced by an automated regime (small rations throughout the day). Ammonia production under the automated system is at a lower level throughout the course of the day and the peaks and troughs observed under a manual system are not so pronounced.

In the cage studies, it is again noticeable that ammonia production is related to feeding. An increase in ammonia levels were recorded at the onset of feeding and decreasing levels recorded upon cessation of feeding. Unlike salmon, which evacuates its gut on an exponential fashion, a more linear response is observed with cod. Having the experience in the tank study of being unable to retrieve any faecal material, because of its high moisture content (c. 90 %) it may be deduced that the liberation of nitrogenous wastes from the faecal material will be rapid. Gut evacuation in response to feeding could explain the correlation between feeding and the rise at that time of dissolved nitrogenous wastes.

As suggested in Section 4.4.3 with gut evacuation, appetite returns. Jobling (1995) states that larger particles of food remain in the gut for a longer time for extraction of nearly all nutrients from the food. Research carried out with Atlantic cod found that feeding small rations delayed emptying of undigested foods. It was also found the wild cod prefer to eat large prey swallowed whole. dos Santos *et al* (1993) experimented with natural food pieces of varying sizes and varying number of rations and his results supported the above findings. This work was done with pieces of herring but there is scope for further detailed work with larger pellet size (cod prefer large prey swallowed whole) and feeding regime. There could be several benefits of feeding large pellet size; longer gut retention and hence greater digestion and subsequently less nutrient evacuation, less foraging for a given uptake of ration and therefore less energy expenditure. Theoretically for a given ration of a given size of pellets, only 50 % in number of a pellet twice that size would be required for the same ration. Therefore it could be argued that with the commercially accepted 5 % uneaten food wastes, a ration of 15 larger pellets would

produce considerably less uneaten food waste than a ration of 30 smaller pellets. In turn, this would greatly reduce dissolved nitrogenous wastes from uneaten pellets. Again with less foraging required for a satiation meal, less energy will be expended. It was observed in the tank studies that cod would not accept the food once it had settled on the tank bottom. The development of pellets with an increased buoyancy would reduce settling velocities increasing the availability of the pellets in the water column. This consideration would contribute to reduce pellet sedimentation and there-by uneaten food waste. An optimal feeding regime is essential to keep uneaten feed wastes to a minimum.

When discussing the gut evacuation of cod compared to that of salmon one must also give consideration to the different physical nature of the faeces of these two species. The observed 90 % moisture content was calculated from the dry weight stripped faeces of the cod from the tank studies. This high moisture content could assist the dispersal of cod faeces in a sea-cage situation, and this dispersal would contribute to the low ammonia level (linear gut evacuation) using an automated feeding system. Sedimentation of cod faecal material will locally be immeasurable. However, with salmon the more solid nature of the faeces combined with an exponential gut evacuation will result in a considerably higher sedimentation in a closer proximity to the cages. Following a three-year period of cod culture at No Catch® Ltd., the examination of the seabed below these cages by a Remotely Operated Vehicle, No Catch® Ltd. report that the seabed below the cages shows little impact. The model shows that impact on the seabed occurs (settlement of particulate matter). However, as discussed in Chapter 7, the marine environment is highly dynamic and the present study found little correlation between the modeled data (predicted) and the sediment trap data (observed) suggesting that models are at best, only indicative of the character of waste dispersion and should not be used as a definitive representation.

The sedimentation rates of organic nutrients were found to decrease with an increasing distance from the cages. Sedimentation was also dependent on which transect the trap was deployed.

Traps on the prevailing current transect consistently recorded higher results than those recorded on the transect perpendicular to the prevailing current. Such a result suggests that the prevailing current plays and important role in the dispersal of organic nutrients. Lower organic nitrogen and carbon in the trap deployed between the adult cages was observed, highlighting the rapid dispersal of cod faecal material and the role of currents in the dispersal of organic waste. Times of inclement weather increased organic 'sedimentation' in all traps but resuspension (Chapter 7) would account for elevated levels. No feed pellets were recorded in any of the deployed sediment traps. Given the high quantities of feed input into the production cages, the fact that no pellets were collected, suggests in part that the feeding regime was extremely accurate. However, it could also be interpreted that more sediment traps would need to be deployed to ascertain a more accurate view of uneaten feed wastes associated with that particular feeding regime.

Tables 8.1 and 8.2 show a brief summary of results obtained in the tank studies, while Tables 8.3 and 8.4 offer a brief overview of results obtained in the cage investigations.

Table 8.1 Summary of results for adult cod under manual and automated feeding regimes in tank systems.

| Manual | | Manual | | | Automated | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | A | B | C |
| | | | | | | | |
| No. of fish | | 88 | 90 | 89 | 47 | 52 | 50 |
| Mean Fish Weight (g) | | 1380.4 | 1383.2 | 1397.5 | 2089.4 | 2126.3 | 2152.4 |
| Biomass (kg) | | 121.48 | 124.49 | 124.38 | 98.20 | 110.57 | 107.62 |
| Ration (g) | | 803.5 | 813 | 794.5 | 703 | 770 | 747 |
| | | | | | | | |
| Ingested (% feed offered) | | 79.7 | 79.4 | 79.7 | 92.75 | 92.87 | 93.23 |
| | | | | | | | |
| Diet (% dry weight) | C | 48.03 | 47.19 | 46.19 | 48.24 | 47.05 | 45.79 |
| | N | 7.62 | 9.03 | 10.55 | 7.70 | 9.26 | 10.64 |
| | | | | | | | |
| Tissue (% dry weight) | C | 46.32 | 46.79 | 47.20 | 44.70 | 45.45 | 45.32 |
| | N | 14.90 | 15.00 | 15.05 | 14.43 | 14.66 | 14.62 |
| | | | | | | | |
| Faeces (% dry weight) | C | 30.11 | 27.13 | 21.09 | 36.16 | 31.37 | 29.72 |
| | N | 2.39 | 2.95 | 3.04 | 3.20 | 3.46 | 4.65 |
| | | | | | | | |
| Ammonia | Base | 0.60 | 0.80 | 1.00 | 0.50 | 0.60 | 0.80 |
| (ug/l/kg biomass) | Peak | 1.00 | 1.10 | 1.20 | 0.60 | 0.80 | 0.90 |
| | Mean | 0.80 | 0.95 | 1.10 | 0.55 | 0.70 | 0.85 |

Table 8.2 Summary of results for juvenile cod under a manual feeding regime in tank systems.

| Manual | | Diet | | |
|---|---|---|---|---|
| | | D | E | F |
| | | | | |
| No. of fish | | 38 | 38 | 38 |
| Mean Fish Weight (g) | | 229 | 264.5 | 263.4 |
| Biomass (kg) | | 8.70 | 10.05 | 10.01 |
| Ration (g) | | 127 | 127 | 125 |
| | | | | |
| Ingested (% feed offered) | | 92.4 | 91.1 | 91 |
| | | | | |
| Diet (% dry weight) | C | 46.07 | 46.45 | 47.87 |
| | N | 7.57 | 9.09 | 10.57 |
| | | | | |
| Tissue (% dry weight) | C | 40.45 | 41.25 | 40.95 |
| | N | 14.06 | 14.04 | 14.56 |
| | | | | |
| Faeces (% dry weight) | C | 38.43 | 36.03 | 31.23 |
| | N | 3.05 | 3.95 | 5.01 |
| | | | | |
| Ammonia | Base | 4.00 | 3.00 | 4.00 |
| (ug/l/kg biomass) | Peak | 5.00 | 4.00 | 7.00 |
| | Mean | 4.50 | 3.50 | 5.50 |

Table 8.3 Summary of cage related results for adult cod under an automated feeding regime.

| Septmeber 2005 | | 1 | 10 | 11 |
|---|---|---|---|---|
| No. of fish | | 46963 | 45354 | 61175 |
| Mean Fish Weight (g) | | 1009 | 1074 | 1366 |
| Biomass (kg) | | 47385.67 | 48710.20 | 83565.05 |
| Ration (kg) | | 53.40 | 90.20 | 115.00 |
| Ingested* (% feed offered) | | 95 | 95 | 95 |
| Diet (% dry weight) | C | 47.9 | 47.9 | 47.9 |
| | N | 8.6 | 8.6 | 8.6 |
| Tissue (% dry weight) | C | 47.1 | 47.1 | 47.1 |
| | N | 14.8 | 14.8 | 14.8 |
| Ammonia | Base | 0.1 | 0.4 | 0.2 |
| (ug/l/tonne biomass) | Peak | 1.0 | 1.6 | 0.4 |
| | Mean | 0.55 | 1.0 | 0.3 |

| November 2005 | | 1 | 10 | 11 |
|---|---|---|---|---|
| No. of fish | | 46390 | 44761 | 60533 |
| Mean Fish Weight (g) | | 1319 | 1422 | 1703 |
| Biomass (kg) | | 61188.41 | 63650.14 | 103087.70 |
| Ration (kg) | | 317 | 279 | 315 |
| Ingested* (% feed offered) | | 95 | 95 | 95 |
| Diet (% dry weight) | C | 47.7 | 47.7 | 47.7 |
| | N | 8.5 | 8.5 | 8.5 |
| Tissue (% dry weight) | C | 47.2 | 47.2 | 47.2 |
| | N | 14.8 | 14.8 | 14.8 |
| Ammonia | Base | 0.1 | 0.4 | 0.0 |
| (ug/l/tonne biomass) | Peak | 1.0 | 1.2 | 0.2 |
| | Mean | 0.55 | 0.8 | 0.1 |

| February 2006 | | 1 | 10 | 11 |
|---|---|---|---|---|
| No. of fish | | 45697 | 44126 | 69769 |
| Mean Fish Weight (g) | | 1760 | 1880 | 2155 |
| Biomass (tonnes) | | | | |
| Ration (kg) | | 287 | 216 | 508 |
| Ingested* (% feed offered) | | 95 | 95 | 95 |
| Diet (% dry weight) | C | 47.8 | 47.8 | 47.8 |
| | N | 8.9 | 8.9 | 8.9 |
| Tissue (% dry weight) | C | 46.8 | 46.8 | 46.8 |
| | N | 14.9 | 14.9 | 14.9 |
| Ammonia | Base | 0.2 | 0.1 | 0.2 |
| (ug/l/tonne biomass) | Peak | 0.4 | 0.5 | 0.4 |
| | Mean | 0.3 | 0.35 | 0.3 |

Table 8.4 Summary cage related results for juvenile cod under an automated feeding regime.

| February 2006 | | 1 | 10 | 11 |
|---|---|---|---|---|
| No. of fish | | 45697 | 44126 | 69769 |
| Mean Fish Weight (g) | | 1760 | 1880 | 2155 |
| Biomass (tonnes) | | | | |
| Ration (kg) | | 287 | 216 | 508 |
| Ingested* (% feed offered) | | 95 | 95 | 95 |
| Diet (% dry weight) | C | 47.8 | 47.8 | 47.8 |
| | N | 8.9 | 8.9 | 8.9 |
| Tissue (% dry weight) | C | 46.8 | 46.8 | 46.8 |
| | N | 14.9 | 14.9 | 14.9 |
| Ammonia | Base | 0.2 | 0.1 | 0.2 |
| (ug/l/tonne biomass) | Peak | 0.4 | 0.5 | 0.4 |
| | Mean | 0.3 | 0.35 | 0.3 |

When comparing nutrient budgets associated with adult cod in tank systems (Table 8.1), it can be seen that increased ingestion is observed under an automated regime. It is also evident that carbon and nitrogen content of dry weight tissue is marginally lower under an automated regime and that carbon and nitrogen content of the dry weight faecal material is higher under an automated regime, although in both cases, the difference is not significant. Higher carbon and nitrogen content in the faecal material suggests that digestion under an automated regime is less efficient than under a manual feeding regime. Ammonia production is generally lower under an automated regime with a more linear excretion pattern. The use of an automated regime could be a recommendation or even a condition set by the regulator when granting consent to discharge.

By comparison with juvenile cod in tank systems (Table 8.2), it can be seen that carbon and nitrogen content of dry weight juvenile tissue is lower than the adult flesh. However, the carbon and nitrogen in juvenile faecal material is greater than that observed in adult faecal material suggesting that digestion of carbon and nitrogen in juvenile fish is less efficient than in adult fish.

Throughout the cage studies, faecal material was not collected but carbon and nitrogen content of the adult and juvenile tissue was found to be similar to those values recorded for the respective size of fish in the tank studies.

## 8.1 Critique

The aim of this study is stated as "Quantifying and modeling of the dissolved nitrogenous wastes associated with the commercial culture of Atlantic cod (*Gadus morhua* L.)" and the individual aims are discussed in Sections 1.5 and 1.6. Although this study provides an initial set of results, experience offers an insight into further possibilities of how the studies undertaken when compiling this thesis could be modified and expanded upon to provide more comprehensive results.

The sampling regimes used in the tank study were created to monitor daily fluctuation over the duration of the sampling period (9 am - 4.30 pm). However, given the necessary volume of sample to be taken over each sampling trip to MERL, combined with the facilities available for water filtration, the regime was not as expansive as initially hoped. An electric pump was provided later in the study, which phenomenally increased the rate of filtration compared to the water vacuum filtration method initially used. By this stage, the studies had been running for too long to amend the sampling regime. With hindsight, and if an electric pump had been available from the outset, the sampling regime would have been altered to: 0 min., 15 min., 75 min., 135 min., 195 min., 255 min., 315 min., 375 min., 435 min., 450 min., 510 min. and 570 min., collecting from the inflow, within the tank and from the outflow of each tank in both studies, at all of the sampling times. Such a regime would provide a much more detailed daily profile of dissolved nitrogenous waste production.

The sampling regime as discussed in Chapter 4 could have been altered earlier in the study if the collected water samples could have been analysed between each sampling trip to MERL. Due to the time constraints arising from the intense sampling regime at MERL and sporadic

opportunities of the necessary equipment to analyse the water samples, this process did not commence until toward the end of the tank studies.

Fish from the tank studies (18 adult fish and 27 juvenile fish) were sampled every 14 days to Produce an HSI and allow proximate analysis tissue composition to be analysed. The results show that HSI and proximate composition entities (moisture, ash, lipid, protein and nitrogen) vary little between each 14 - day sampling period. It would have been beneficial to link this sampling with the 28 - day sampling period, the regime employed when monitoring growth. Further work should consider such a sampling regime as this would have made more time available both at MERL and the Institute, to be more expansive in the investigation of dissolved nitrogenous waste production in tank systems. Future work should also perhaps consider proximate analysis of all tissue (flesh, skeleton, liver, viscera and gonads during sexual maturation), which would allow the partitioning of nitrogen assimilation throughout the fish to be studied.

The PIT tagging program used in the study at MERL was unsuccessful as the fish were rarely caught. However, monitoring the change in condition of individual fish may also be a useful and future work on a similar scale should consider the implementation of a large - scale PIT tagging program.

With such an expansive topic, the scope for future work is enormous. The current work provides comprehensive initial results and has opened doors for future work, which can expand upon the current findings and investigate other areas giving a greater insight into the nitrogen budget associated with the commercial culture of Atlantic cod. Further investigation into the interaction of fish size, state of sexual maturation, diet formulations, stocking densities and feeding regimes is required to increase the knowledge and understanding of such interactions and discover the optimal diet formulations and feeding regimes specific to the differing life stages of Atlantic cod. All future studies undertaken should be concerned with both economic and environmental sustainability and all results achieved should be considered in terms of both factors.

With any future studies, especially in northern climates such as Vidlin, the time of year when sampling should be carefully considered as with the experience gained from this study, the winter sampling period proved difficult and uncertain.

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