Investigation of the growth potential and ecosystem impact of intensively farmed Atlantic salmon fed on experimental diets.

Thesis submitted for the degree of Doctor of Philosophy

By

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Dedicated To
My family

Declaration

I declare that the work contained within this thesis is my own and where the work of others has been used it has been properly cited.

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Abstract

There are increasing concerns regarding the environmental impacts and sustainability of intensive fish farming. In particular, criticism has centred on the use of fish meal and fish oil in the diets of farmed carnivorous fish species such as Atlantic salmon. If the industry is to continue to expand, reduction in the levels of fish meal and fish oil incorporated into diets and/or the use of alternative terrestrial sources of protein and oil must occur. The present study assesses several diet types containing different inclusion levels and /or sources of protein and oil in terms of growth and performance as well as assessing the diets in terms of sustainability and their potential to impact on the marine environment.

In two nutritional studies, Atlantic salmon fed a low protein (LP) diet achieved similar growth and performance compared to fish fed a normal commercial diet (control diet) in both studies. Growth rates of fish fed a diet containing partial replacement of fishmeal with corn gluten and fish oil with rapeseed oil (SUS) were better than those of fish fed the control and LP diet and were similar to those of fish fed a high energy; nutrient dense (ND) diet. The amount of wild fish required to produce 1 Kg farmed salmon based on fish meal and fish oil inclusion levels were lowest for fish fed the SUS diet (1.3 kg) whilst fish fed LP diets had a lower conversion value compared to both the ND and control diets based on fish meal inclusion levels only. These results suggest there is potential for aquaculture to be more environmentally sustainable by reducing the amounts of marine fishmeal and oil used in diets fed to intensively farmed Atlantic salmon.

In terms of dissolved wastes, fish fed a nutrient dense diet had higher feed-related concentration peaks of ammonia detected which occurred earlier compared to fish fed other diet types. In contrast, fish introduced to low protein diets at different sizes throughout the marine phase of production had consistently lower concentration peaks of ammonia detected compared to fish fed a standard commercial ration. All groups fed a low protein diet had between 17 and 28 % less ammonia detected as a feed related concentration peak compared to the control group. The area under each concentration peak of ammonia ranged from 43.6 to 88.8 % ± 60 min of total ammonia detected over time for all diets. These results show that feeding fish diets containing lower inclusion levels of fish meal resulted in lower feed related concentration peaks of ammonia being detected. However most of the ammonia was excreted over a short time period and its potential to impact on the marine environment was assessed using mesocosm studies.

In the first of two studies, mesocosms were fertilised with NH_4Cl based on ammonia concentration peaks from either 500 or 1000 T rainbow trout production (LN and HN enclosures respectively). These enclosures had consistently more phytoplankton present than the control enclosures. There was evidence of rapid uptake of excess nutrients with the HN enclosures having more cells present than the LN enclosures. In the second experiment, enclosures were fertilised based on ammonia concentration peaks detected from Atlantic salmon fed a low protein (LP enclosures) or a nutrient dense diet (ND enclosures). Both had consistently more cells present than the control enclosures.. These results suggest that increases in phytoplankton

communities may occur as a result of a single pulsed release of ammonia simulating discharge from intensively farmed fish.

The predicted rate of dissolved nitrogen production was calculated from fish introduced to low protein diets throughout a complete marine production phase using a mass balance model. Fish introduced to low protein diets at 330, 800 and 1600 g had lower dissolved N discharge rates (22.51, 22.02 and 21.07 Kg^{-1} NT^{-1} Production respectively) compared to fish fed a standard commercial ration (23.32 Kg^{-1} NT^{-1} Production). These results show that there is potential to maximise use of low protein diets, which would result in less ammonia excretion and reduce the potential risk to impact on the marine environment.

In an attempt to accurately quantify waste outputs from intensively farmed Atlantic salmon a custom-made tarpaulin was designed. Initial studies have shown that there is potential to collect data on waste outputs from fish reared in the marine environment whilst taking in to account seasonal and daily fluctuations in water temperature and salinity. The system can be used to directly compare different feed types and feeding strategies.

It has been shown that changing the macronutrient inclusion level and sources results in differences in the physical characteristics of extruded feeds. High energy diets and substitution diets have similar settling velocities compared to a standard commercial diet but produce lower environmental impacts when modelled for solid waste impacts. Low protein pellets have slower sinking rates but generate more waste due to a higher FCR. The data from these results should be used in conjunction with other data for a range of feeds and environmental conditions to employ a "look-up table" approach to differentiate between diets when modelling waste dispersion.

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Chapter 1 – General Introduction

1.1 - Cage Aquaculture

There is an increased demand for fish protein as populations grow and fish supplies from traditional marine and inland fisheries are unlikely to increase substantially to meet the demand due to near full exploitation. Global fish production was 129 million tonnes in 2001 (FAO, 2002). Of this, 91.3 million tonnes (70.8%) was attributed to capture fisheries production, a small decline from a maximum production of 94.8 million tonnes in 2000. Whereas there has been no significant increases in production in capture fisheries, aquaculture production accounted for 37.5 million tonnes in 2001, a 12.3% increase in production from 33.4 million tonnes in 1999 (FAO, 2000). The slow down in growth of capture fisheries production is likely to continue and the projected shortfall in fish supply is likely to be met mainly from expansion of the aquaculture industry which is the only fisheries sector to be increasing production.

Aquaculture is highly diverse and consists of a broad spectrum of systems, practices and operations ranging from small household pond systems to large-scale, intensive, commercially orientated practices such as cage farming (NACA/FAO, 2000). The numerous advantages offered by cage aquaculture such as low labour costs per unit production, higher stocking densities per unit area compared to land based systems and ease of management and access to space has led to production of fish from cages increasing globally with operations well developed in Europe, Chile, China and South East Asia (NACA/FAO. 2000). The aquaculture industry in the coastal waters of northern Europe has experienced an increase in volume of salmonids farmed which dominates other fish culture at present. In Scotland, for example, production of

Atlantic salmon (*Salmo salar* L.) has increased from 244 tonnes in 1970 to 145, 609 tonnes in 2002, when they accounted for 13.6 % of the world total for farmed Atlantic salmon by weight (FAO Yearbook, 2004). Norway is the largest producer of Atlantic salmon in the world with production reaching 465, 609 tonnes in 2002 (69 % of all production in Europe) (FAO Yearbook, 2004).

Since the trend of increased production of fish in cages is likely to continue, there are several negative environmental impacts derived from the intensive production of fish that must be considered if the industry is to continue to expand and develop. These impacts mainly derived from feeding fish in cages are discussed in section 1.2.

1.2 - Environmental Impacts of Aquaculture

Intensive cage aquaculture systems such as those used to rear salmonids are reliant upon exogenous feed supplies that have the potential to impact on the environment (Chen, 2000). Initially these systems were viewed as "black-boxes" into which food was added and the fish harvested with little or no consideration given to the potential negative effect that this was having on the environment (Kadri *et a*l. 2001). However, over the past decade there has been increasing concern regarding the negative effects that these systems are having on the environment (Hall *et al.* 1990; Cho and Bureau. 2001).

Caged fish are dependent on the environment to provide a wide range of goods and services such as a steady supply of dissolved oxygen, water, feed and

removal and assimilation of wastes (Beveridge *et al.* 1997). Cages are ecologically open systems with a continuous exchange of water between the cage and the receiving water body (Troell and Norberg, 1998). Wastes derived from feeding can pass directly through the cages into the environment with very limited control. This is in direct contrast with land-based farms where there are more opportunities for the treatment and reduction of wastes (Seymour and Bergheim, 1991).

Although there is an advantage in that water quality requirements can usually be maintained by natural water movement, the disadvantage is that wastes pass directly into the surrounding water body and may accumulate (Kadri *et al.* 2001).

The following sections will focus on wastes derived from waste feed and feeding and the potential negative impacts that occur as a consequence of this.

1.2.1 - Particulate wastes from fish cages

The major particulate effluent from a cage farm consists of uneaten food and faecal material, which is produced by the fish. The amounts produced are dependant on factors such as the digestibility of the food supplied, the size and stocking density of the fish and on husbandry factors such as temperature and disease status of the fish (Harvey and Philips, 1996). Particulate wastes emanating from a cage farm will eventually settle onto the seabed. The eventual site of deposition will depend on the prevailing local hydrographic and

climatic conditions at each site (Harvey and Philips, 1996). Most of this waste by weight or volume is faecal, though much of the waste directly under the cages may be feed. (Gowen and Bradbury, 1987). Once waste food and faecal material have been incorporated into the sediments they can cause profound well documented effects on the receiving sediments.

Waste food and faecal material are a rich source of organic carbon (Harvey and Philips, 1996) and the primary effect of settled waste food and faeces from fish cages is the increase in organic carbon content of the sediments. High waste deposition rates can lead to an accumulation of organic detritus in the sediments that overwhelm the assimilative capacity of the benthic community and result in the formation of anaerobic bacterial mats and anoxic conditions (Krost *et al.* 1994). This leads to the generation and out-gassing of hydrogen sulphide H_2S) and methane (CH_4) (Black *et al.* 1996). As anaerobic processes predominate, benthic communities can become subjected to severe environmental perturbation (Tsutsumi *et al.* 1990). This can lead to decrease in species number and diversity of infaunal organisms (Brown *et al.* 1987; Hargrave *et al.* 1993).

The processes discussed above are well understood and have been used as the primary method of assessing impacts derived from feeding fish in cages. However, there is growing concern of the potential impacts derived from dissolved wastes primarily due to a lack of understanding as to their environmental fate and effects on receiving water bodies (Gowen and Bradbury, 1987; Aure and Stigebrandt, 1990).

1.2.2 - Dissolved nutrient wastes from fish cages

Dissolved nutrient wastes largely refer to the excretion of ammonia and urea from farmed fish. Ammonia and urea are the main nitrogen-containing compounds excreted by marine teleosts including Atlantic salmon (Fivelstad *et al.* 1990). The gills are the major site of ammonia excretion in fishes (Randall and Wright, 1987; Kaushik and Cowey, 1991). Ammonia is the main waste product from protein catabolism as is mainly excreted across the gills and urea, formed through purine catabolism is also mainly excreted across the gills (Rychly and Marina, 1977; Handy and Poxton, 1993).

Several studies have suggested that excretion of ammonia and urea represents 70-90% and 5-15% of the total nitrogenous wastes from fish respectively (Randall and Wright, 1987; Fivelstad *et al.* 1990; Dosdat *et al.* 1996). A study by (Forsberg, 1996) on post-smolt Atlantic salmon showed that about 35% of the estimated nitrogen intake was excreted as ammonia and in rainbow trout, 40-45% of nitrogen intake was excreted as ammonia (Kaushik and Gomez, 1988).

The release of nitrogenous compounds from intensively farmed fish has led to considerable concern and speculation regarding the effects that these nutrients may have on receiving water bodies (Aure and Stigebrandt, 1990). The following sections will discuss current issues relating to the effects of the release of dissolved nutrients on marine ecosystems.

1.2.3 - Marine eutrophication

Assessment of the environmental effects of intensive marine monoculture of salmonids is primarily based on the measurement of change on the benthos in the immediate vicinity of the cage site. However, the diffusive nature of dissolved wastes means that in order to try and determine their effect on marine systems, the whole embayment/inlet has to be considered.

There is perceived to be a problem of marine eutrophication in many oceans of the world (Gowen *et al.* 1992; Meyer-Reil and Köster, 2000; Nordvarg and Hakanson, 2002). The intensive nature of fish farming represents a point source of dissolve inorganic nutrients and there is little doubt that fish farms do contribute to the pool of plant nutrients in marine environments (Gowen, 1994; The Scottish Association of Marine Science and Napier University, 2002).

The primary effect of increased nutrient load in the water column is an increase in phytoplankton and macroalgae primary production (Larsson *et al.* 1985). Subsequent decay of high plant biomass results in an increase in oxygen consumption due to additional biochemical oxygen demand (BOD) (Larsson *et al.* 1985; Meyer-Reil and Köster, 2000). The increased sedimentation of organic matter will initially support an increase in benthic macrofaunal biomass. But when the demand for oxygen exceeds the supply due to extra BOD, bottom waters and sediments may become anoxic in enclosed waters (Tett *et al.* 1986; Aure and Stigebrandt, 1990).

Other undesirable consequences of marine eutrophication include discolouration of the sea due to increased abundance of micro-algae species such as the colonial flagellate *Phaeocystis pouchetii* which caused waters in

the southern North Sea to become red in colour and resulted in the foaming of seawater (Lancelot *et al.* 1987; Gowen, 1994). There may be occurrences of intense toxic algal blooms which may cause illness and death among seabirds and mammals and to fish such as those being grown. For example, a bloom of the dinoflagellate *Gyrodinium aereolus* caused fish kills in 1980 in Loch Fyne (Jones *et al.* 1982).

1.2.4 - Potential effects of aquaculture on phytoplankton ecology

In many countries as well as in Scotland, there are concerns that the discharges of nutrients from intensively farmed fish are disturbing the natural ecology of phytoplankton. One concern relating to fish farm wastes is that the food fed to fish does not contain silicon (Si) thus it is likely that the natural ratio of N to Si will be altered by the nutrient input from fish farms. This may result in a shift from diatom to flagellate dominated phytoplankton (Tett and Edwards, 2002). Increased N:Si ratios have been associated with a greater increase in annual mean flagellate abundance than in diatom abundance in the inner German Bight of the North Sea (Radach *et al.* 1990). Gilpin and Davidson (L.Gilpin Napier Univ., *pers comm.* 2001) found that flagellates began to dominate over diatoms in mesocosms once the ratio in the supplied nutrients N:Si exceeded 2. Tett and Edwards (2002) suggest that N:Si molar ratios less than 2.5:1 should be considered acceptable in Scottish coastal waters. There may be heavily enriched lochs where the "safe" N:Si limit of 2.5:1 is exceeded. Although N: Si limits may be exceeded in some of the most nutrient-enriched

sea lochs, especially in the waters of the Northern Isles where nitrate is naturally abundant (Tett and Edwards, 2002),other factors contribute to maintaining the balance of species. These include physical conditions, grazing and the success and survival of individual species (Black *et al.* 1997; Tett and Edwards, 2002).

There may be a perturbation effect on the nutrient element ratios of N and P resulting from the discharge of wastes from fish farms. Changes in the N:P ratio have been mentioned as likely causes for the development of a number of different types of toxic algal blooms (Aure and Stigebrandt, 1990; Folke *et al.* 1994).

Phytoplankton require approximately 16 Mol of N for every Mol of P they take in. This N:P ratio is known as the Redfield ratio (Redfield *et al.* 1963). Thus a N:P ratio of less than 16:1 suggests that N would be the limiting nutrient whilst higher ratios would indicate that P would be the most likely to limit growth. It is generally accepted that blooms of nitrogen–fixing blue-green algae are stimulated by lower N:P ratios since these blooms tend to occur during periods of N deficiency (Graneli *et al.* 1989). Similarly, high N:P ratios where blamed on the increase in production of the prymnesiophycean *Chrysochromulina polyepsis* that bloomed in the Kattegatt/Skagerak area in Scandinavia in 1988. This bloom caused massive economic loss to the salmon industry due to fish kills (Folke *et al.* 1994).

Another concern relating to the aquaculture industry is that severe depletion of one nutrient relative to another may increase cellular toxin content of individual algal cells. There have been laboratory evidence that physiological stress in the

form of nutrient depletion does increase cellular toxin content but the N:P ratios of fish farm wastes is typically close to the optimal (11:1) in Scottish waters and this would be unlikely to cause severe physiological stress to algal populations (Tett and Edwards, 2002).

The concerns discussed above and the desire to maximise profitability and performance in intensive fish farming have led to extensive research to minimise waste outputs. The following section will discuss ways to reduce waste outputs.

1.3 - Reduction of solid and dissolved wastes through diet formulation

The advent of technology, which has allowed the production of extruded pellets, has undoubtedly played a major role in the reduction of wastes from feed. The material in extruded pellets is expanded and fused together, this reduces the amount of surface area of the pellet which comes into contact with water, the result is that extruded feeds are far more stable in water than standard dry pellets (Seymour and Bergheim, 1991). A further advantage of extruded pellets is that they can contain a higher fat content and these high energy diets reduce the feed requirement per unit of production and spare protein which is the major source of nitrogen pollution from fish farms (Kaushik and Cowey, 1991). Where the protein content in feed was reduced from 45.6% to 41.5% and the fat content was increased from 22% to 30% respectively, this resulted in 35-37%

reduction in nitrogen load from post-smolt Atlantic salmon (Johnsen and Wandsvik, 1991).

Nutrient composition and digestibility of the ingredients of the diet determine the level of discharge of wastes derived from fish farming (Ackefors and Enell, 1994; Cho and Bureau. 2001). Solid waste outputs of fish fed practical diets consists mainly of undigested starch and fibre (cellulose, hemicellulose, oligosaccharides, pectins, lignin, polyphenols etc) from grain and various plant products, and minerals from the various ingredients (Cho and Bureau. 2001). Reduction of solid wastes can easily be achieved by using highly digestible ingredients with high lipid and/or protein content and excluding ingredients such as grain by-products rich in starch and fibre that are poorly digested (Cho *et al.* 1994). For example, reductions in carbohydrate in diets have resulted in the reduction of the faecal component of solid wastes due to improved digestibility (Kadri *et al.* 2001).

The use of high energy (HE) diets has resulted in the reduction in faecal production in the order of 10-25% (Cho *et al.* 1994; Talbot and Hole, 1994). These high nutrient-dense diets have been shown to reduce solid waste and phosphorus outputs (with 190kg and 3kg output per tonne of fish respectively) compared to 240kg and 4kg respectively from a regular grower diet. Another study by (Chen *et al.* 2003) compared a HE diet to a standard diet fed to Atlantic salmon and found that the use of HE diets resulted in a 12% reduction in faecal carbon content and an 8% reduction in faecal nitrogen when compared to the standard diet.

The amount of ammonia excreted is dependant on the quality and quantity of dietary protein. Feeding excess amino acids results in catabolism of the amino acid with subsequent excretion of ammonia and loss of energy (Kaushik and Cowey, 1991). Protein sources with poor amino acid profiles results in fish retaining digestible nitrogen less efficiently with subsequent increases in dissolved nitrogenous wastes. Increased nitrogen retention efficiency and a decrease in ammonia production have been achieved by decreasing the dietary digestible protein (DP) to digestible energy (DE) ratio by increasing the dietary non-protein energy content of pellets (Einen and Roem, 1997; Hillestad *et al.* 1998; Steffens *et al.* 1999). Commonly known as protein sparing, it is the non-protein energy source, which is used to meet energy requirements. Experimental data suggest that DP/DE ratio of about 18g/MJ reduces amino acid catabolism and dissolved nitrogenous wastes without affecting growth rate and feed efficiency of salmonid fish species. Higher DP/DE is generally required by smaller fish compared to larger ones (Einen and Roem, 1997).

Although the mitigation measures discussed above do achieve reductions in solid and dissolved wastes, other factors need to be considered. The use of nutrient dense/high energy diets and inclusion of highly digestible ingredients in the diets fed to cultivated carnivorous fish species such as salmonids usually rely on the use of fish meal and fish oil from capture fisheries. However, the use of these products has lead to growing criticism that the industry is unsustainable due to its use of these products from declining wild fish stocks (Tidwell and Allan, 2001). The following section (section 1.4) will discuss the concern that aquaculture is a contributing factor to the collapse of wild fishery stocks.

1.4 - Sustainability and aquaculture

Projections of worldwide aquaculture production show that for species such as salmon, carp, tilapia, milkfish, bass and other marine species that there is expected to be a steady annual percent rate of growth in production up to 2010 (Pike and Barlow, 2002). This increase in production will result in a subsequent increase in the demand for the use of dry feed to meet the dietary requirements of the cultured organisms. The main species used in the manufacture of fish meal and fish oil include anchovies, sardines, pilchards, capelin and sandeel (The Scottish Association of Marine Science and Napier University, 2002).

In 2000, 35% of the fishmeal produced was used in aquaculture diets with the remainder mainly used for terrestrial livestock production. It has been estimated that with the current growth of aquaculture, by the year 2005, aquaculture will utilise 45% of fish meal and by the year 2010 this will have increased to 56% (Pike and Barlow, 2002). Conversely, fish oil usage was estimated to be 54% in 2000 with a projected increase to be over 90% by the year 2010 (Zaldivar and Pike, 2002).

A proportion of this projected increase in the availability of fishmeal is accounted for through the decline in the use of this product in poultry, pig and ruminant diets (Pike and Barlow, 2002). Indeed, this has led some authors to suggest that a shift in fishmeal use toward aquaculture may represent a more environmentally beneficial use of this resource as fish are regarded as more efficient feed converters than the primary users, terrestrial livestock such as pigs and poultry (Tidwell and Allan, 2001; Zaldivar and Pike, 2002). Even

though aquaculture will utilise over half of fishmeal production by the year 2010, this still leaves a substantial remainder to meet future growth of the industry and this will possibly be reflected by priority of use (Zaldivar and Pike, 2002). The demand for fish meal could also be potentially met by the use of by-catch from wild capture fisheries (Howgate, 1995). The amount of by-catch killed and discarded each year is estimated to be between 18 and 40 million tonnes (FAO, 2000). This is comparable to the total amount of fish currently being harvested for fish meal production (30 million tonnes) (Tidwell and Allan, 2001). Norway, Canada and Iceland have all introduced a ban on the "at-sea" discarding of certain commercial species and a proportion of the by-catch is used by fish feed manufacturers (The Scottish Association of Marine Science and Napier University, 2002).

The use of fish oil in the aquaculture industry has potentially more serious repercussions. Aquaculture is the current major user for this product (54% in the year 2000) and the predicted amount required by the industry is expected to be 77% by the year 2005 and increase to over 90% by the year 2010. This will result in increasing competition with other users of fish oil. Interest in fish oil has also been increasing in the pharmaceutical, health and technical industries, which currently utilise the remainder not used by aquaculture. These sectors currently purchase fish oil at prices that are significantly higher than the prices paid by fish feed manufactures for premium oils (The Scottish Association of Marine Science and Napier University, 2002).

As an industry, intensive cage aquaculture is still relatively young and the knowledge of the nutritional requirements for many cultivated species is rather

limited. Livestock feeds typically contain 2 –3 % fish meal (Naylor *et al.* 2000) yet 20 years ago, fish meal was the preferred source of protein for poultry feeds just as is the case for several aquaculture species today (Tidwell and Allan, 2001). Reduced reliance on fish meal came as a result of nutritional research particularly in the understanding and quantification of the requirement for essential amino acids and energy needs as well as the evaluation of alternative ingredients (Tidwell and Allan, 2001). The search for alternative ingredients is already a research priority for aquaculture for exactly the same reasons.

1.5- Aims and Objectives

The overall aim of the present thesis has been to determine the growth potential and ecosystem impact of intensively farmed Atlantic salmon fed a range of experimental diets. Particular emphasis focussed on fish being fed a range of diets containing lower levels of fishmeal and fish oil and/or partial replacement of fish meal and fish oil with suitable terrestrial sources. Each diet was assessed in terms of growth performance and its sustainability in terms of how much fish meal and fish oil would be required to produce 1 kg farmed salmon. In addition, each diet was assessed in terms of the amounts of dissolved and particulate wastes being produced and the potential for dissolved wastes to impact on the marine environment was also assessed. Specific objectives were:

1. To assess the growth performance and efficiency of feed utilisation of four different diets formulated to contain different inclusion levels and sources of

dietary protein and oil fed to large Atlantic salmon and to determine the effects on growth and dietary performance of triplicate groups of fish introduced to low protein diets at different sizes throughout the marine phase of the production cycle. The first low protein feed was introduced when fish had grown to approximately 330 g in size with subsequent introductions of low protein feeds as the fish grew.

2. To determine the amounts of marine fish meal and fish oil required to produce 1 Kg intensively farmed Atlantic salmon fed different diet types based on inclusion levels of fish meal and fish oil in each of the diets and growth performance.

3. To determine the ammonia excretion rates and patterns from intensively farmed Atlantic salmon fed different diet types. There has been little or no research undertaken on the patterns of ammonia excretion from intensively farmed salmonids. The ultimate aim was to determine whether there was food related concentration peaks of ammonia present and whether there were differences in ammonia excretion rates from Atlantic salmon fed different diet types.

4. To determine whether an ecological risk existed from discharges of dissolved ammonia from intensively farmed rainbow trout and Atlantic salmon and uptake of these excess nutrients by phytoplankton communities. The study attempted to determine whether phytoplankton community composition and densities would be affected by addition of excess nutrients within enclosed mesocosms.

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5. To determine the rate of nitrogen released from (a) Atlantic salmon fed different diet types and (b) Atlantic salmon introduced to low protein diets throughout a complete marine phase of production. The experiment integrates information on the protein and nitrogen content of each diet used along with feeding practices with a mass balance model to provide estimates of waste production.

6. To determine the amounts of dissolved and solid wastes discharged from farmed fish using enclosed net pens. The aim of the experiment was to collect data on feed input and output and to measure the amount of ammonia produced from farmed Atlantic salmon under semi-commercial conditions. Thus the data collected will allow for calculating an accurate mass balance for any diet. This information can then be used to assess the potential environmental impacts of any diet fed to intensively farmed Atlantic salmon.

7. To determine relevant physical characteristics and settling velocities of a range of different feeds under defined laboratory conditions and to determine the environmental implications based on waste dispersion modelling. The modelling of wastes from farmed Atlantic salmon relies on feed settling velocity values provided by (Gowen and Bradbury, 1987). Clearly, advances in feed manufacturing technology and the use of alternative ingredients may affect physical characteristics such as settling velocities of pellets fed to Atlantic salmon. Settling velocities may also change with environmental conditions (temperature and salinity).

Chapter 2 – Assessment of different feed types on growth performance, efficiency of feed utilisation and flesh quality in large Atlantic salmon (*Salmo salar* L.).

2.1- Introduction

The continuing growth of the aquaculture industry has led to increasing concerns regarding the environmental impacts and sustainability of intensive fish farming (Gillibrand, 2001). In particular, criticism has centred on the use of fish meal and fish oil in the diets of farmed carnivorous fish species such as Atlantic salmon, Atlantic cod and halibut (Tidwell and Allan, 2001).

The search for alternative sources of protein and oil for use in the diets fed to carnivorous fish species have centred on terrestrial products such as soy meal, gluten meal and rapeseed oil to provide suitable protein and oil substitutes. Corn gluten has been used in the diets fed to Atlantic salmon (Anderson *et al.* 1992) and it has been shown that rapeseed oil is a potential substitute for fish oil in diets for Atlantic salmon culture with inclusion levels < 50% (Bell *et al.* 2001).

However, if the industry is to become more sustainable by using alternative terrestrial sources to reduce reliance on marine proteins and oils, it also has to ensure that fish farmers can produce fish with the same as if not better quality than is currently achievable at present. Fish farmers will not utilise feeds that are more expensive and result in poorer growth than feed in use at present.

Marine fish oils in "oily fish", such as Atlantic salmon, contain considerable amounts of polyunsaturated fatty acids (PUFAs), which have been shown to have considerable human health benefits (De Deckere *et al.* 1998). In farmed fish these are provided in the diet by fish oils from capture fisheries. Any substitute for this fish oil in the diets of farmed salmon must ensure that the fish

produced continue to give the same health benefits. It has been shown that rapeseed oil is a potential substitute for fish oil in diets for Atlantic salmon culture. However, if rapeseed oil is above 50% of added oil, substantial reductions occur in muscle 20:5n-3 and 22:6n-3 and the n-3/n-6 PUFA ratio which results in reduced availability of the n-3 highly unsaturated fatty acid (HUFA), that are beneficial for human health (Bell *et al.* 2001). However, it has been shown that if fish are given feed containing marine fish oils as the only source of fatty acids for a period of time before harvest, this will potentially counteract any potential loss in flesh quality caused by feeding fish diets containing high inclusion levels of vegetable oils (Bell *et al.* 2003).

Fishmeal supplies the largest part of dietary protein for salmonid culture and as the industry continues to expand so does the need for high quality protein sources (Hardy, 1996). Fishmeal is an increasingly expensive component of commercial salmon feeds and as demand for the world fisheries catch increases, higher global demand for fish landings may increase the price of fishmeal. This was recently demonstrated during the decline in catches due to El Niño in 1998 (Sargent and Tacon, 1999; Vielma *et al.* 2000). There have been numerous studies undertaken to investigate alternative protein sources. Use of vegetable protein such as soybean and gluten are of particular interest. Soybean is now widely used to partially replace some fishmeal in salmonid feeds (Carter, 2000). Other plant proteins offer potential or replacement but the resulting lower feed costs may not reduce production costs if growth is reduced and feed conversion ratio impaired due to anti-nutritional factors, unfavourable amino acid profiles and low protein content (Opstveldt *et al.* 2003)

The study described here was undertaken to assess four different diets formulated to contain different inclusion levels and sources of dietary protein and oil fed to Atlantic salmon. Each diet was assessed in terms of growth performance and efficiency of feed utilisation. The four different diets formulated used in the trial were:

1. A high energy, nutrient dense (ND) feed rich in marine fishmeal and fish oil. High-energy diets that contain high lipid levels and optimal Digestible protein: digestible energy ratios have been used extensively in farming of salmonids. They have been shown to improve growth and feed utilisation as well as reducing the production of solid and nitrogenous wastes (Cho *et al.* 1991; Kaushik and Cowey, 1991).

2. The second feed used was a low protein diet (LP). Potentially, using lower protein diets, will result in less ammonia being excreted by fish and as a result will reduce the potential ecological risk associated with dissolved wastes as well as reducing the cost associated with the use of fishmeal.

3. The third feed was formulated to contain partial replacement of fishmeal with corn gluten and fish oil with rapeseed oil (SUS). Such feeds containing partial substitution of fish oil and fishmeal with vegetable oils and terrestrial proteins have the potential to make fisheries more sustainable by reducing the amount of marine fish derived products used in feed formulation.

4. The fourth feed used in the trial as a control, has similar dietary levels of fishmeal and fish oil as normal commercial grower diet used in the industry at present.

The main hypothesis that was tested was:

Ho: Feeding diets to groups of Atlantic salmon with different inclusion levels and sources of dietary protein and oil does not result in differences in growth and flesh quality

2.2 - Material and methods

2.2.1 – Stocking and husbandry

The field trial described here was conducted at Gifas Research Station, Inndyr, Norway. A total of 1800 Atlantic salmon with a mean weight of 1.78 kg ± 0.09 kg were randomly distributed into 12 trial-cages (5 x 5 x 5 m) with 150 fish allocated to each cage. Fish with wounds or deviations from normal with respect to shape or appearance were not used in the trial. All fish were sorted from the same cages and shared the same genetic, nutritional and environmental background. To prevent a skewed distribution of treatments with respect to water-current and quality, lots were drawn among four predetermined replicate distributions. The trial was started on the 20 June 2003 after the fish had a period of acclimatisation to the rearing environment, and lasted for a period of 139 days. Growth was measured after 69 and 139 days.

Sea lice infestations through the summer period were controlled by a bath treatment with Betamax (0.3 mg/L) (active ingredient deltamethrin) for 30 min on 18th September 2003. The fish were starved for one day prior to medication and the net pens were enclosed with a tarpaulin during treatment. The fish were continually monitored during the period and oxygen injection was available if required.

2.2.2 - Feeding

The fish were fed by hand to satiation twice a day, with a minimum of four hours between meals. In order to facilitate accurate calculations of feed intake and food conversion ratio (FCR), each cage was fitted with an air uplift system to allow accurate determination of food wastage. During each meal, each population of fish was fed randomly to avoid any systematic differences among treatments.

2.2.3 - Diets

Four experimental diets were formulated and manufactured by BioMar Ltd to have different inclusion levels of protein and oil. The four diets were a nutrient dense diet (ND), a low protein diet, a partial vegetable oil substitution diet and a control diet. Table 2.1 shows the dietary composition, chemical analysis and calculated energy content of the experimental diets.

Each diet was fed to fish in triplicate groups for a period of 139 days, to evaluate the performance and commercial potential of each diet. For each diet,

dietary protein content was measured by the Kjeldahl method (ISO 5983, 1997) and dietary oil content by the Soxhlet method (AOAC, 2000). Moisture content was measured by drying to a consistent weight and ash content by combustion at 500 ^{0}C for 16 h. Percent carbohydrate was calculated indirectly (100 - ∑protein, oil, ash and moisture). The gross energy content was calculated from the energetic values of the protein (23.6 MJ/kg), fat (39.5 MJ (kg) and carbohydrate (17.5 MJ/kg) components of the feed. The digestible energy content was calculated from the digestibility study (see below), using yttrium oxide as an inert marker and the DP:DE ratio of each diet was calculated from the protein content and energy content of each diet

2.2.4 - Sampling

The first 30 fish selected for each cage were anaesthetised with Benzocain (10 % solution) at a concentration of between 0.5 to 1 ml/l of seawater. Once anaesthetised, individual fork length and weights were recorded using a Marel M 2000 series, type M60 animal weighing scale. The remaining 120 fish were bulk weighed before being assigned to each cage.

Weight and length of 30 individual fish from each cage, were also recorded at day 69 and again at the end of the trial (day 139) with the remaining fish bulk weighed during these times.

Table 2.1 Dietary ingredient, chemical analysis and calculated values of the experimental diets

	Diet type			
	LP	SUS	ND	Control
Ingredient (%)				
Fish meal	20.0	20.0	42.8	28.1
Corn gluten	-	17.5	12.5	-
Oil/Legume seed meals	20.0	12.0	0	27.0
Starch source	20.0	13.0	6.0	12.0
Fish oil	36.0	10.0	39.2	33.1
Rapeseed oil	-	23.4	-	-
Mineral and vitamin premix	0.75	0.75	0.75	0.75
Methionine	0.16	0.11	-	0.08
Lysine	0.58	1.07	0.15	0.41
Threonine	0.10	0.07	-	0.05
MSP[1]	1.75	1.03	-	0.37
Carophyll pink[2]	0.05	0.05	0.05	0.05
Chemical composition (%)				
Protein	31.5	35.3	35.6	35.6
Oil	40.59	34.58	45.58	37.68
Ash	4.8	5.1	5.5	6.8
Moisture	6.0	5.8	4.4	3.1
Carbohydrate	17.11	19.22	8.92	16.82
Calculations				
Gross energy (MJ kg^{-1})	26.45	25.35	27.97	26.22
Digestible energy (MJ kg^{-1})	21.22	22.44	23.97	22.56
DP:E ratio	11.90	13.90	12.70	13.60

[1]Mono sodium phosphate (phosphate source); [2] DSM Astaxanthin product (based on 8% Astaxanthin).

2.2.5 - Carcass analysis

At the start of the trial, eight fish were selected at random from the stock cage for analysis. In addition, at the end of the trial, a further four fish from each cage (N = 48) were selected at random. All sampled fish were humanely killed, tagged and placed on ice. Fork length and body weight was recorded before gutting and washing. Dress-out loss was then recorded along with any instances of deformation, cataract or vaccine damage.

The visual colour of salmon fillets was assessed by comparison with the Roche *Salmor*Fan^{TM} (Hoffmann-La Roche, Basel, Switzerland), under standard conditions using a light cabinet fitted with a fluorescent light source. The light source had a colour rendering index (Ra)>90 and a colour temperature > 6000 K to allow accurate colour matching.

Two scorers independently measured the colour of sample fillets at the front, mid and tail part of the fillet, and from the front cross-section of the Norwegian quality cut (NQC) which was taken at a position behind the first dorsal fin ray and pectoral fin ray and finishes anterior of the anal fin ray (Figure 2.1). The muscle tissue was then dissected from the NQC-region of the two sides of the fillet and homogenized. The samples were then frozen at -20 ^{0}C in duplicate containers for analysis of protein, fat, astaxanthin, water and ash content.

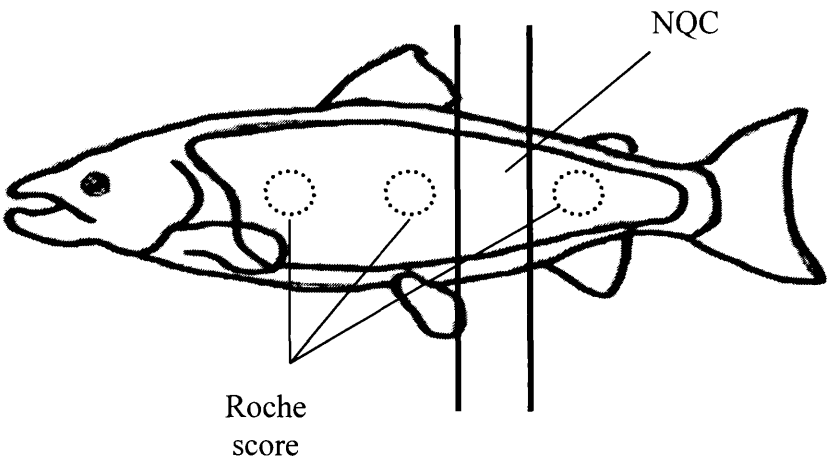


Figure 2.1 Sample sites on the fillet from the Roche score and the NQC sample taken at the start and end of the trial.

2.2.6 - Fatty acid analysis of the diets

The fatty acid composition of each diet was detected by gas liquid chromatography. Samples were homogenized in trichlormethane by means of Ultraturrax^{TM} and the total lipid extracted according to the method proposed by (Folch *et al.* 1957).

The lipid classes were separated by thin layer chromatography on silicagel G 60 using isohexane/diethyl ether/acetic acid (90:10:1, v:v:v) as the developing solvent. The plates were then sprayed with 1% (w/v) iodine in $CHCl_3$ to visualise the methylated fatty acids (FAME). The FAME bands were then marked and scraped from the TLC plates. The FAMEs were then eluted from the silica with 10ml isohexane: diethyl ether (1:1, v:v), mixed on vortex mixer, and centrifuged as described above, to precipitate the silica. The solvent was

the evaporated under oxygen-free nitrogen (OFN) and the samples transferred to 2 ml sample vials in 1 ml isohexane, evaporated to dryness and re-dissolved in isohexane to a concentration of 2 mg ml. The FAMEs were separated by gas liquid chromatography at 190 ^{0}C using a flame ionisation detector. The component peaks were identified by reference to the retention time of authentic standards. The relative proportion of each fatty acid in the fatty acid pattern was expressed as a percentage of the sum of the fatty acids resolved.

2.2.7 - Digestibility and nutrient utilisation

For the last 5 days of the trial the fish were fed diets containing 100 mg yttrium oxide (Y_2O_3) kg^{-1} feed with no other change in feeding regime. The feeding regime continued to be based on satiation feeding by hand, using distinctive meals. On each day of feeding a 100 g sample of each yttrium feed type fed to each cage was retained and placed in a bag ready for analysis. The feed samples from each cage were then mixed and pooled for diet type leaving four composite yttrium labelled feed samples (1500 g per diet). The four composite feed samples were then stored at – 20 ^{0}C prior to analysis.

On day 5, post feeding, the fish were sampled. Between 10 and 20 fish from each cage were stripped to collect approximately 80 ml of faeces. The pooled faecal samples were immediately stored at – 20 ^{0}C prior to analysis. The protein, oil, starch, dry matter and yttrium content of both feed and faeces were analysed.

2.2.8 - Water quality

Temperature and salinity were logged daily at 1, 3 and 5 m water depth. Temperature was logged using a WTX – OXI 197 probe and salinity with a WTW LI -196 microprocessor conductivity meter.

2.2.9 - Calculations and statistics

The specific growth rate (SGR) was calculated as:

$$SGR = 100 \ln(W_2 - W_1)/ (t_2 - t_1) \times 100$$

where:

W_1 is the weight (g) at time t_1 (beginning of period),

W_2 is the weight in g at time t_2 (end of period),

t is time in days.

Thermal growth coefficient (TGC) (Cho, 1992) was calculated according to the formula:

$$TGC = (W_2^{1/3} - W_1^{1/3})*1000 / \sum(t * \text{feeding days})$$

where:

W_1 and W_2 were the initial and final body weights respectively,

$\sum(t *$ feeding days) is the sum of water temperatures (^{0}C) for every feeding days in the experiment.

Feed conversion ratio (FCR) was calculated as follows:

$$FCR = FI\ (B_2 + B_{dead} - B_1)^{-1}$$

Where:

FI is feed offered on a dry weight basis,

B_1 and B_2 are the biomass at the start and end respectively,

B_{dead} is the biomass of the dead fish.

Condition factor (K) was calculated as:

$$K = [(W \times 100)\ (L^3)$$

where:

W is weight (g),

L is fork length (cm).

The dress out percentage (DOP) was calculated as:

$$DOP = [1 - (BW_{gutted}BW^{-1}_{ungutted})]\ 100$$

where:

BW_{gutted} and $BW_{ungutted}$ were the weights of gutted and ungutted fish respectively.

Apparent Digestibility (AD) for protein, oil, starch and dry matter was calculated as follows:

$$AD = 100 - [100\ (\%I_{feed}\ \%N_{faeces})(I_{faeces}\ N_{feed})$$

where:

I_{feed} and I_{faeces} are the concentration of marker in the feed and faeces,

N_{feed} and N_{faeces} are the nutrient concentration in the feed and faeces.

Statistical significance of differences among diet types was computed from one-way or two-way analysis of variance (ANOVA) using Minitab^{TM} version 13 statistical software (Ryan & Joiner, 1994). The normality and homogeneity of the variance of all data sets was tested prior to parametric statistical analysis. Normality was tested by graphic examination of probability plots and the Anderson-Darling test. Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$) and results are presented as mean values ± standard error of the mean (SE).

2.3 – Results

Mean fish weights calculated at day 1, day 69 and day 139 can be seen in Figure 2.2. There was no significant differences in mean fish weight between treatments at the start of the trail ($F_{3.356}$ 0.52; $p > 0.05$) nor were there significant differences in mean fish weight at day 69 ($F_{3.356}$ 0.29; $p > 0.05$) and at the end of the trail at day 139 ($F_{3.356}$ 2.32; $p > 0.05$).

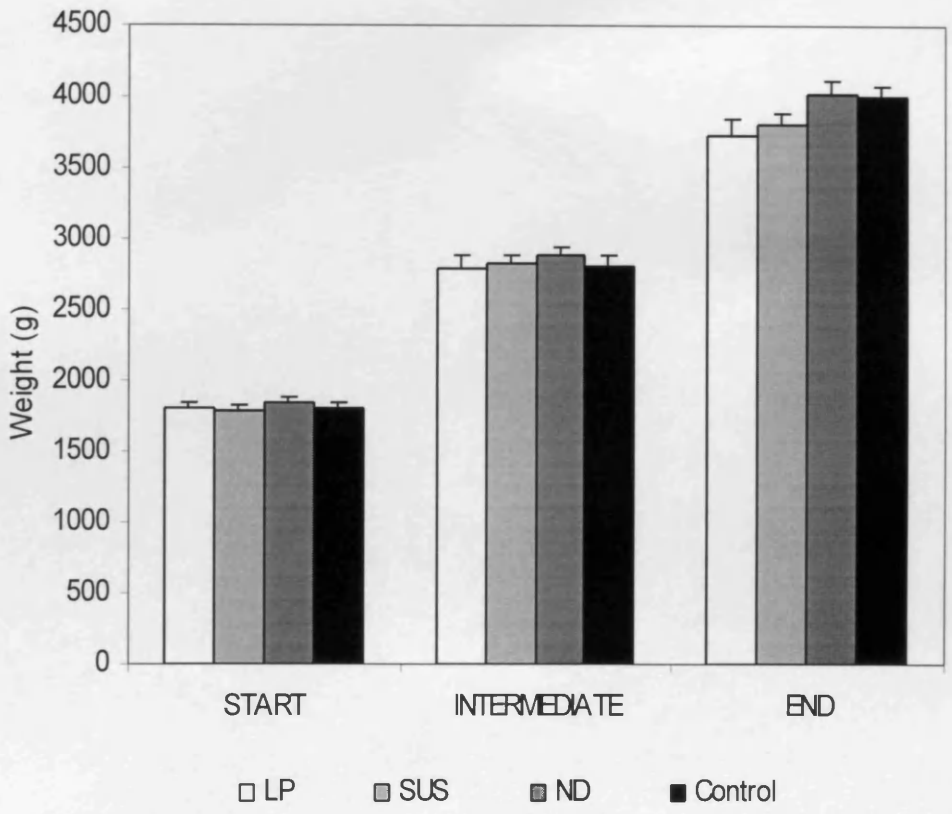


Figure 2.2 Mean fish weights calculated for each group of fish fed the LP, SUS, ND and control diets at day 1(START), day 69 (INTERMEDIATE) and day 139 (END). (n = 150; N = 450). Values represent means ± S.E.

There was no significant difference in FCR for individual fish in replicate cages within treatments so the data was combined. At the intermediate sampling (day 69), FCR values obtained can be seen in Figure 2.3. There were no significant differences between fish fed the LP, SUS and control diets with fish fed the control diet having the highest FCR of 1.19. Fish fed the ND diet had the lowest FCR (0.97) which was significantly lower than fish fed the SUS diet ($F_{3.8}$ 6.06; $p < 0.05$). There were no significant differences in FCR for all groups from intermediate sampling to the end of the trail ($F_{3.8}$ 1.15; $p > 0.05$) (see Figure 2.3). Overall (from day 0 – 139), fish fed the ND and SUS diets had the lowest FCR at the end of the trial (0.97 AND 1.00 respectively) with no significant differences between these diets. Fish fed the LP and the control diets had similar but significantly higher FCR values (1.09) compared to the ND and SUS diets ($F_{3.8}$ 10.02; $p < 0.05$). SGR values calculated at day 69 can be seen in Figure 2.4. Fish fed the ND diet had the highest SGR (0.75) compared to the other groups. This was significantly higher than fish fed the LP diet ($F_{3.8}$ 5.40; $p < 0.05$). There were no significant differences between the other treatments (Figure 2.4). From intermediate sampling to the end of the trial, the control group had the highest SGR (0.53) which was significantly higher than the SUS group ($F_{3.8}$ 5.19; $p < 0.05$) which had the lowest SGR of all groups (0.42 compared to 0.44 and 0.49 for the LP and ND groups respectively). At the end of the trial, fish fed the ND diet had the highest SGR. This was significantly higher compared to fish fed the LP diet ($F_{3.8}$ 9.76; $p < 0.05$). SGRs for all other diets did not differ significantly from the LP diet.

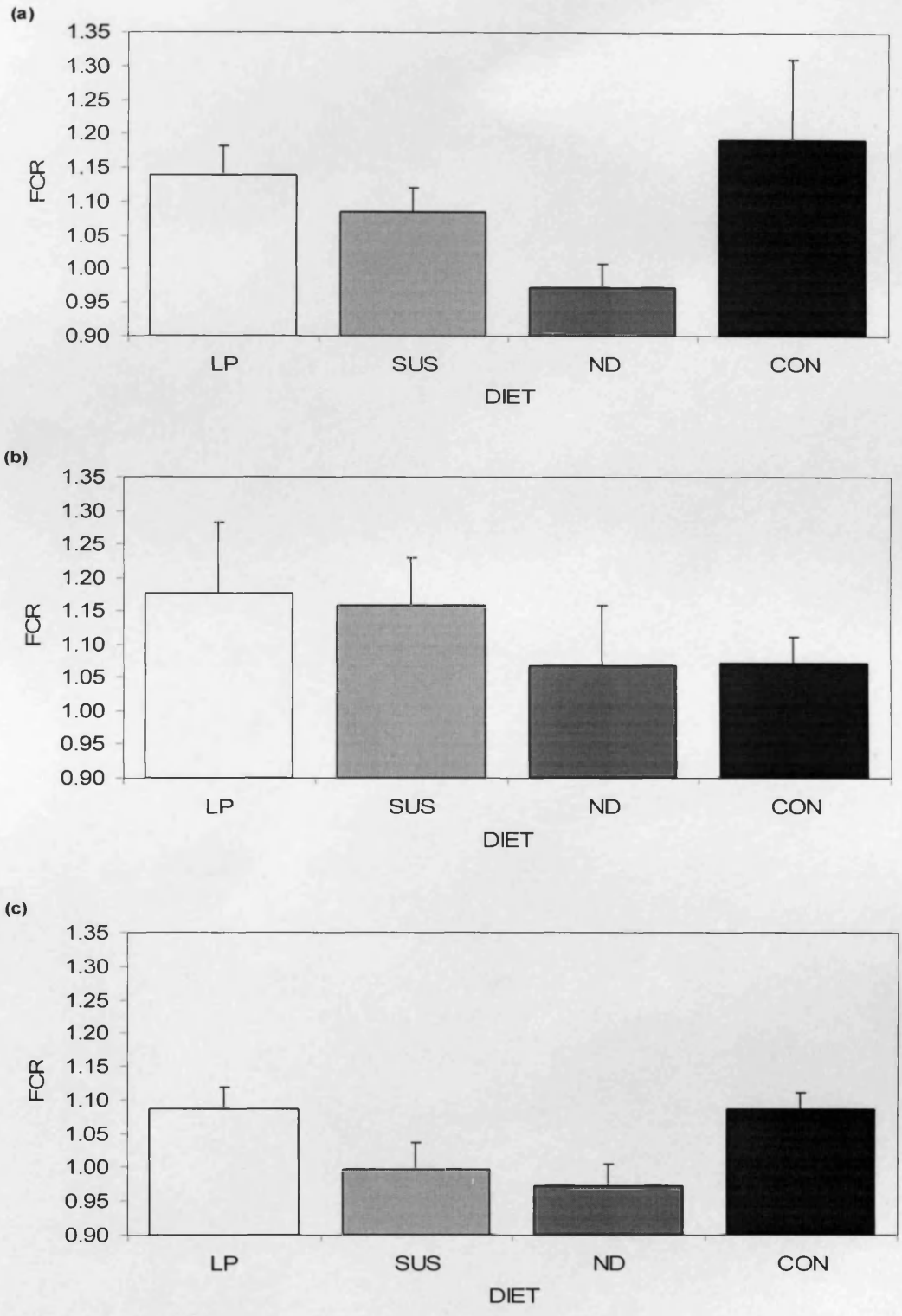


Figure 2.3 Mean FCR calculated for fish fed the LP, SUS, ND and control diets (CON) at (a) intermediate sampling (day 0 -69), (b) end sampling (day 70-139) and (c) overall FCR (day 0 – 139). Values represent means ± S.E.

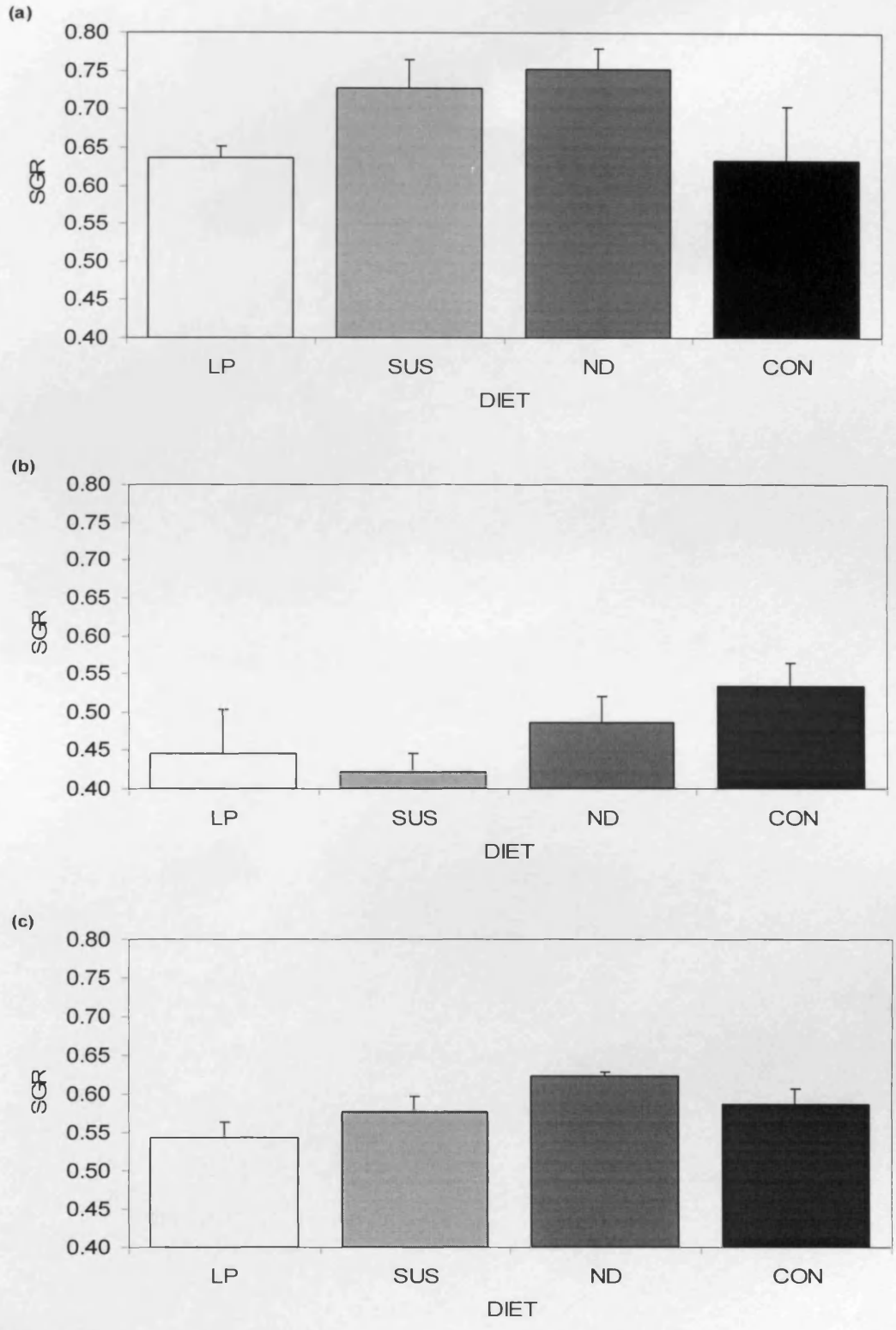


Figure 2.4 Mean SGR calculated for fish fed the LP, SUS, ND and control diets (CON) at (a) intermediate sampling (day 0 -69), (b) end sampling (day 70-139) and (c) overall FCR (day 0 – 139). Values represent means ± S.E.

TGC values calculated at day 69 can be seen in Figure 2.5. Fish fed the ND diet attained the highest TGC (2.85) of all the groups. This was significantly higher than the TGC of fish fed the control diet (2.36) ($F_{3.8}$ 5.10: $p < 0.05$). There were no significant differences in TGC between the other groups. The TGC values calculated from intermediate sampling to the end of the trial showed that fish fed the control diet has the highest TGC (2.94) which was significantly higher than the TGC of fish fed the SUS diet (2.27) ($F_{3,8}$ 6.98: $p < 0.05$). There were no significant differences in TGC between the other groups with fish fed the LP and ND diets having a TGC of 2.50 and 2.71 respectively. There were significant differences in TGC between diet types. Fish fed the ND diet had a significantly higher TGC compared to fish fed the LP and SUS diets at the end of the trial ($p < 0.01$; one-way ANOVA) but was not significantly different to the fish fed the control diet.

At intermediate sampling, there were no significant differences in condition factor (K) between diet types ($F_{3.356}$ 0.70; $p > 0.05$)(Figure 2.6). This trend continued from days 69 to 139 ($F_{3.356}$ 2.02: $p > 0.05$) and overall (from day 0-139) with no significant systematic effects due to diet type on condition factor ($F_{3.356}$ 1.53: $p > 0.05$).

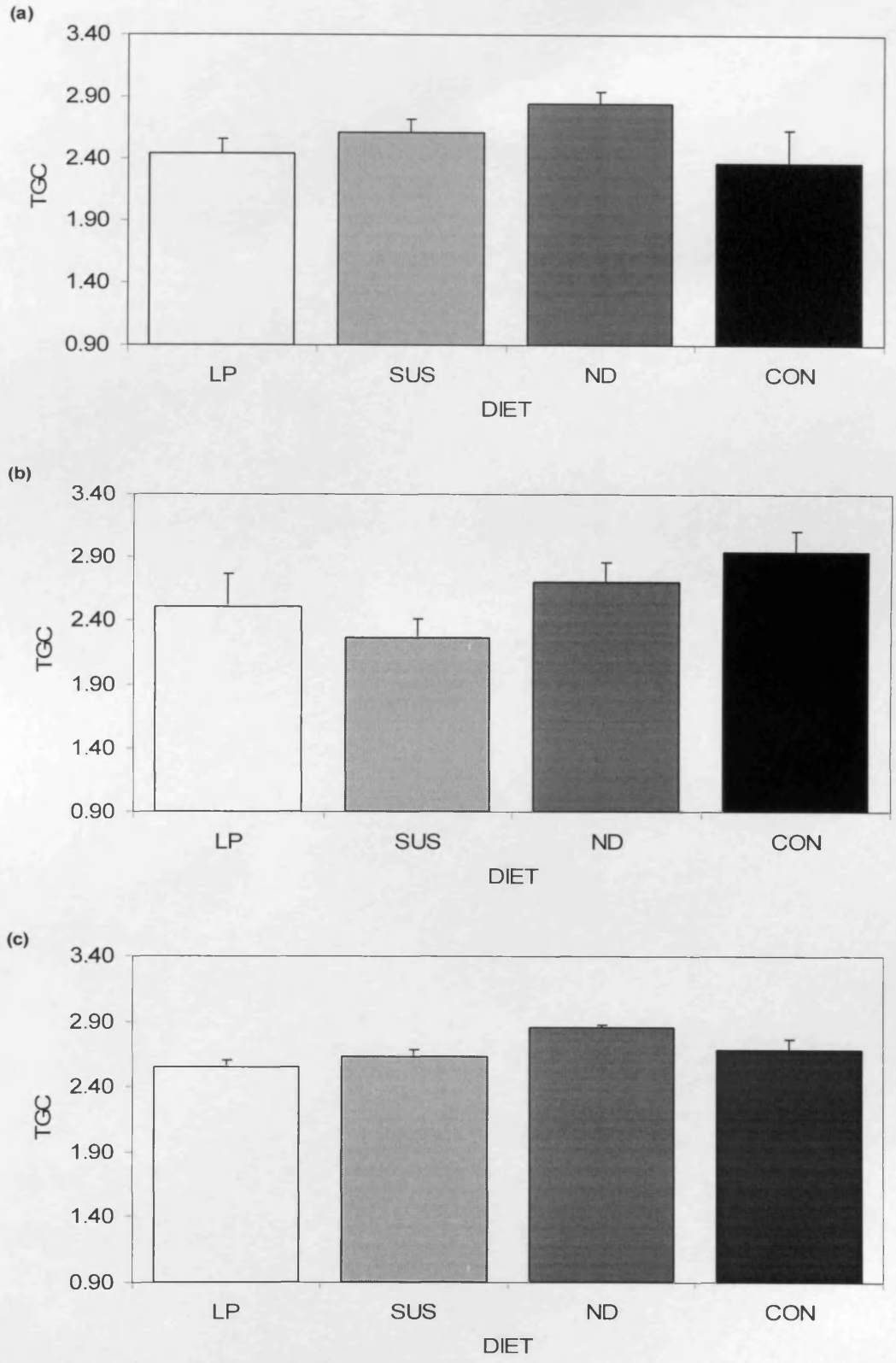


Figure 2.5 Mean TGC calculated for fish fed the LP, SUS, ND and control diets (CON) at (a) intermediate sampling (day 0 -69), (b) end sampling (day 70-139) and (c) overall FCR (day 0 – 139). Values represent means ± S.E.

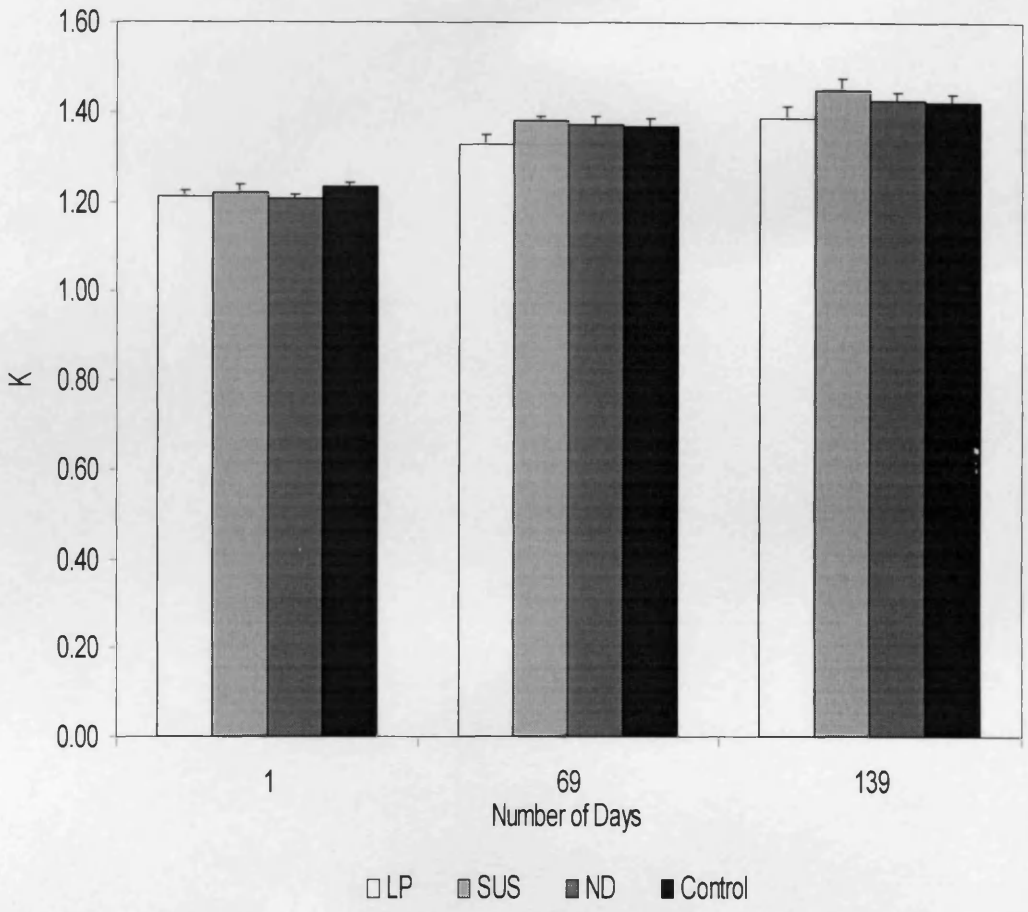


Figure 2.6 Condition Factor (K) calculated for each group of fish fed the LP, SUS, ND and control diets at day 1, day 69 and day 139. (n = 150; N = 450). Values represent means ± S.E.

The mean values for the flesh quality characteristics assessed are shown in Table 2.2. There were no significant differences in body weight ($F_{3.44}$ 1.15; $p > 0.05$) and gutted weight ($F_{3.44}$ 0.94) between diets at the end of the trial. The DOP was found to be significantly higher in the fish fed the ND diet (15.29 %) compared to the fish fed the SUS and the Control diets (13.48 and 13.25 respectively) ($F_{3.44}$ 4.09; $p < 0.05$). There were no significant differences between the LP and ND diets. There was no significant difference in body protein content between groups ($F_{3.44}$ 1.08; $p > 0.05$). Flesh oil averaged 11.64 – 13.61% wet mass at the end of the trial with no significant differences between diets ($F_{3.44}$ 1.93; $p > 0.05$). Astaxanthin concentration was significantly higher in fish fed the LP and control diet compared to fish fed the ND and SUS diets ($F_{3.44}$ 11.35; $p < 0.05$). There were no significant differences in percentage water content between the groups ($F_{3.44}$ 1.81; $p > 0.05$) with values ranging from 65.74 % for fish fed the ND diet to 67.48 % for fish fed the SUS diet. There was a significant decrease on body water content in all the test groups compared to fish sampled at the start of the trial ($F_{4.53}$ 8.22; $p < 0.05$). Analysis of ash content showed that all groups had similar ash content with no significant differences between them ($F_{3.44}$ 1.96; $p > 0.05$).

Average values for Roche *Salmo*Fan™ scores (see Table 2.2) were significantly lower in the NQC ($F_{3.44}$ 4.68; $p < 0.05$) and front fillets ($F_{3.44}$ 4.02; $p < 0.05$) from fish fed the SUS diet compared to fish fed the LP and C diets at the end of the trail). The scores from the middle fillet indicate a significant difference between the SUS and all other three diets ($F_{3.44}$ 6.44; $p < 0.05$) and scores obtained from the tail also indicate significant difference between the SUS and control group ($F_{3.44}$ 4.23; $p < 0.05$).

Table 2.2 Flesh quality parameters in Atlantic salmon (*Salmo salar* L.) fed low protein (LP), sustainable (SUS), nutrient dense (ND) and control diets for 139 days. Values represent means ± S.E.

Parameter	Day 0	Day 139				ANOVA[1]	
	(*n* = 10)	LP (*n* = 12)	SUS (*n* = 12)	ND (*n* = 12)	Control (*n* = 12)	*F*	*P*
Body weight (g)	2088 ± 106	3939 ± 237	3890 ± 213	4383 ± 272	4279 ± 181	1.15	NS
Gutted weight (g)	1835 ± 97.3	3391 ± 204	3369 ± 190	3714 ± 233	3712 ± 159	0.94	NS
Dress loss (percent waste)	12.01 ± 0.45	13.83 ± 0.53^{ab}	13.48 ± 0.46^{a}	15.29 ± 0.33^{b}	13.25 ± 0.48^{a}	4.09	*
Protein (%)	19.38 ± 0.10	19.66 ± 0.16	19.68 ± 0.06	19.49 ± 0.12	19.65 ± 0.14	1.08	NS
Flesh oil (%)	9.80 ± 0.37	12.78 ± 0.59	11.64 ± 0.57	13.61 ± 0.76	12.97 ± 0.35	1.93	NS
Pigment (mg kg^{-1})	6.30 ± 0.26	7.97 ± 0.34^{b}	6.25 0.26^{a}	6.00 ± 0.31^{a}	7.35 ± 0.17^{b}	11.35	***
Water (%)	69.64 ± 0.29	66.42 ± 0.53	67.48 ± 0.56	65.74 ± 0.73	66.21 ± 0.30	1.81	NS
Ash (%)	1.21 ± 0.01	1.13 ± 0.02	1.19 ± 0.02	1.17 ± 0.03	1.17 ± 0.01	1.96	NS
Roche *Salmo*Fan™ scores:							
NQC	24.7 ± 0.37	26.3 ± 0.38^{a}	25.3 ± 0.18^{b}	26.0 ± 0.17^{ab}	26.6 ± 0.29^{a}	4.68	**
Front	24.9 ± 0.23	25.9 ± 0.34^{a}	24.9 ± 0.15^{b}	25.7 ± 0.14^{ab}	25.9 ± 0.26^{a}	4.02	*
Middle	25.1 ± 0.23	26.2 ± 0.34^{a}	24.8 ± 0.21^{b}	25.8 ± 0.11^{a}	26.3 ± 0.27^{a}	6.44	***
Tail	26.0 ± 0.30	26.4 ± 0.43^{ab}	25.3 ± 0.26^{b}	26.3 ± 0.14^{ab}	26.8 ± 0.28^{a}	4.23	*

[1]Results from analysis of variance (ANOVA) where *P* is the significance level (d.f. =3, 44). Mean values marked with different superscripts on the same line, between diets, where found significantly different by Tukeys *post hoc* tests (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

The data for the digestibility study is summarised in Table 2.3. There were no significant differences in protein digestibility between diet types with all diets having similar AD for protein ($F_{3.8}$ 0.16; $p > 0.05$). The digestibility of oil in the SUS diet was 94.51 %, which was significantly higher compared to the LP, ND and control diets (85.43, 86.38 and 89.76 % respectively) ($F_{3.8}$ 15.54; $p < 0.05$). The digestibility of starch in the LP diet (67.98) was significantly lower compared to the ND diet (76.92) ($F_{3.8}$ 129.69; $p < 0.01$. There were no significant differences in starch digestibility between the other diet types. Digestibility of dry matter was significantly higher in the ND diet (76.20 %) compared to values of 63.66 %, 71.24 % and 66.34 % for the LP, SUS and control diets respectively ($F_{3.8}$ 36.35; $p < 0.05$).

Fatty acid composition (see Table 2.4) of the diets was distinctly different and consistent with different inclusion levels of fish oil and plant oil (Table 2.1). The ND diet, which contained fish oil as the only lipid source, contained the highest and lowest concentration of n-3 and n-6 PUFA. In contrast, the SUS diet which contained the majority of oil as rapeseed oil had the lowest and highest concentration of n-3 and n-6 PUFA. The LP and control diets had similar concentrations of n-3 and n-6 PUFA. The n-3/n-6 ratios were highest in the ND diet and lowest in the SUS diet. The control and LP diet ratios were 4.85 and 6.08 respectively. The ND, LP and control diets contained similar concentration of total saturates (28.5 to 30%), whilst the SUS diet contained the lowest concentration (18.7%). The SUS diet contained the highest concentration of monosaturates, in particular 18:1n-9 compared to the other diets that had similar amounts present.

Table 2.3 Apparent digestibility (AD) of protein, oil, starch and dry matter. Values represent means ± S.E

	Diet type				[1] ANOVA	
Parameter	**LP**	**SUS**	**ND**	**Control**	***F***	***P***
Digestibility						
Protein	85.57 ± 0.72	85.45 ± 0.11	85.99 ± 0.38	85.42 ± 0.31	0.16	NS
Oil	85.43 ± 0.72^{a}	94.51 ± 0.06^{b}	86.38 ± 0.75^{a}	89.76 ± 0.08^{a}	15.54	***
Starch	67.98 ± 1.24^{b}	71.72 ± 3.01^{ab}	76.92 ± 0.52^{a}	68.83 ±1.45^{ab}	129.69	*
Dry matter	63.66 ± 0.55^{a}	71.24 ± 0.29^{a}	76.20 ± 0.61^{b}	66.34 ±0.27^{a}	36.35	***

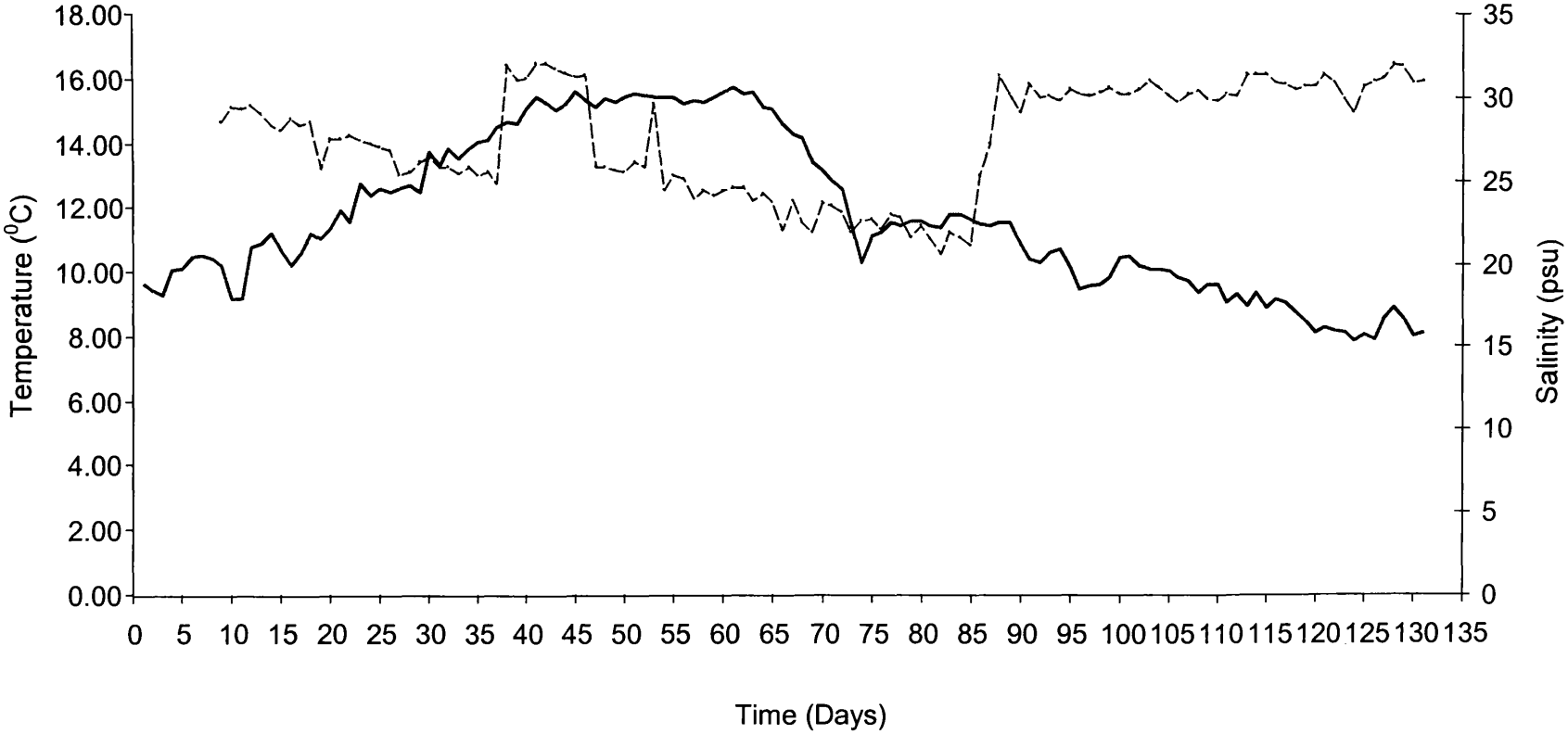
[1]Results from analysis of variance (ANOVA) where *P* is the significance level (d.f. = 3, 44). Mean values marked with different subscripts on the same line, between diets, where found significantly different by Turkeys *post hoc* tests (NS = not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2.4 Percentage fatty acid compositions of the four experimental diets used in the trial.

	Diet type			
Fatty acid	LP	SUS	ND	Control
14:0	4.8	1.7	5.4	5.4
15:0	0.5	0.2	0.6	0.6
16:0	17.0	10.0	17.3	17.3
18:0	4.3	4.1	4.1	4.5
20:0	0.6	0.8	0.5	0.6
22:0	1.3	1.9	0.8	1.6
16:1n-9	0.2	0.1	0.2	0.3
16:1n-7	6.8	2.1	7.1	6.7
18:1n-9	13.2	34.0	12.2	14.6
18:1n-7	2.7	2.5	2.8	2.8
20:1n-9*	4.1	2.5	3.6	5.4
20:1n-7	0.3	0.1	0.3	0.4
22:1n-11**	5.2	2.9	4.3	7.0
24:1n-9	0.8	0.3	0.7	0.8
18:2n-6	3.1	22.1	3.2	3.5
18:3n-6	0.2	0.1	0.2	0.2
20:2n-6	0.3	0.1	0.2	0.3
20:3n-6	0.1	0.0	0.1	0.1
20:4n-6	0.8	0.2	0.8	0.7
22:4n-6	0.1	0.0	0.1	0.1
22:5n-6	0.4	0.1	0.4	0.3
18:3n-3	1.5	5.7	1.3	1.5
18:4n-3	2.7	0.8	2.6	2.3
20:3n-3	0.1	0.0	0.1	0.1
20:4n-3	0.8	0.2	0.8	0.7
20:5n-3	11.0	2.7	12.1	8.4
22:5n-3	1.5	0.4	1.7	1.3
22:6n-3	13.3	3.6	13.8	10.9
16:2	0.6	0.2	0.7	0.5
16:3	0.6	0.1	0.7	0.5
16:4	0.7	0.2	0.9	0.4
Σ saturates	28.5	18.7	28.8	30.0
Σ monosaturates	33.4	44.7	31.3	38.1
Σ PUFA	38.1	36.6	39.9	31.9
Σ (n – 3)	31.0	13.5	32.5	25.2
Σ(n – 6)	5.1	22.6	5.1	5.2
(n – 3)/(n – 6)	6.08	0.60	6.37	4.85

The average monthly temperature (see Figure2.7) ranged from 10.6 ^{0}C in June at the start of the trial to 15.1 ^{0}C during August. This was followed by a decrease in temperature to an average of 9.0 ^{0}C during October at the end of the trial.

Figure2.7 Daily mean temperature (^{0}C) and salinity (psu) taken at 1, 3 and 5 m depth throughout the duration of the experiment. (—) denotes temperature and (---) denotes salinity.



2.4 - Discussion

Assessment of the suitability of each feed types in terms of growth performance showed that the overall growth rates obtained at the end of the experiment were higher for all diets used than growth rate estimates given by Austreng *et al.* (1987), but were similar to growth rates obtained by Einen and Roem, (1997) with similar numbers of fish and experimental conditions. Growth rates over period 1 (days 0 – 69) were lower compared to growth rates in period 2 (days 70 – 139). This was possibly due to higher daily mean water temperatures from day 30 to 69 (14.9 ^{0} C) (Figure 2.7) in period 1, which resulted in higher growth rates for all groups. It has been estimated that growth rates for fish over 2000 g is higher (0.7 % wt/day) at 14 ^{0} C compared to growth rates achieved at lower temperatures (Austreng *et al.* 1987).

At the end of the experiment, the ND diet was shown to give a small but positive effect in terms of growth compared to the other test diets but in terms of inclusion levels of fish meal and fish oil and the associated cost of producing such a diet, the small positive effects obtained in terms of growth would result in diets being unsustainable in both provision of fishmeal and cost. Growth rates of fish fed the SUS diet were comparable to fish fed the ND diet. Such a diet with partial replacement of fishmeal and fish oil with corn gluten and rapeseed oil would be more environmentally sustainable than high energy/nutrient dense diets. Growth rates obtained from fish fed the LP diet, though showing lower growth rates than in fish fed ND and SUS diets, were the same as those obtained from fish fed the control diet. Such a diet would be

more economical to produce and certainly more environmentally sustainable in terms of the amount of fish meal required compared to diets used in the industry at present. However, the poorer performance of such a diet would potentially limit its use as a commercial grower diet.

In the present study, the ND, SUS and control diets had similar but higher DP:DE ratios than the LP diet. The FCRs for all diets were in the same range as those found in other studies (Juell *et al.* 1994; Aksnes, 1995; Einen and Roem, 1997). The ND and SUS diet had a higher energy contents than the LP diet and it has been shown that high-energy diets containing a substantial quantity of readily digestible lipid give increased growth and reduced FCR in salmonids (Alsted *et al.* 1995). Even though fish fed both ND and SUS diets had lower FCRs, fish fed the Control and LP diets had similar FCRs to those achieved in the industry at present.

Both the ND and SUS diets contained corn gluten as an additional protein source, which has been shown to be a good alternative to fishmeal in Atlantic salmon diets (Mundheim *et al.* 2004). Even at high inclusion levels, fish fed diets containing corn gluten show good growth and feed efficiency (Anderson *et al.* 1992; Mente *et al.* 2003). In this study condition factor (K) was higher than recorded previously in Atlantic salmon (Juell *et al.* 1994; Sveier *et al.* 1999), reflecting that the fish were fed to satiation and may have increased deposition of visceral and intermuscular fat (Sorebakken and Austreng, 1987).

Dress out percentage is an important factor directly affecting salmon yield (Hillestad *et al.* 1998). Fish fed the ND diet had higher dress loss than fish fed the other diets. The ND diet had the highest lipid level and flesh oil content of

all diets used, and so probably had higher lipid deposition (Grisdale-Helland and Helland, 1997; Einen and Skrede, 1998). This high dress loss from fish fed the ND diet is likely to result in economic loss to farmers, as the deposited visceral fat will be discarded during product processing.

The reduced flesh astaxanthin concentration obtained from fish fed the ND and SUS diets may be partially explained by the reduction of feed required for a given weight gain in fish fed ND and SUS diets compared to the control and LP diets. The different flesh colour (indicated by Roche *Salmo*Fan^{TM} scores) in fish fed the SUS and ND diets may also be attributed to use of corn gluten meal. Use of this in other feeds for food-fish has been limited because of fear that its high concentration of xanthophylls and lutein may produce undesirable pigmentation or decrease pigmentation efficiency due to competition with the synthetic pigments added (Skonberg *et al.* 1998; Mente *et al.* 2003). It has been shown that variation in the type and quality of dietary PUFA can influence uptake and deposition of carotenoid pigments in salmon flesh (Bjerkeng *et al.* 1999). Thus the SUS diet, containing high levels of n (6) PUFA, may also inhibit pigment uptake.

The experimental feeds used in this study contained a wide range of fatty acid compositions. The control and LP feed contained a fatty acid profile considered appropriate for the intensive production of salmon, whilst the ND feed had a fatty acid profile similar to that of a predominantly pisciverous diet, as reflected in its high concentration of HUFAs. However, the SUS feed (23.4 % rapeseed oil, 10 % fish oil) has a fatty acid composition low in HUFA but high in PUFA products such as 18:2 (6) and gave similar fish growth and food conversion as

fish fed the ND feed (39.2 % fish oil as sole lipid source). These results are consistent with previous studies that have shown that rapeseed oil can be a successful substitute for fish oil in diets for Atlantic salmon with no adverse effects at levels below 50 % inclusion. (Bell *et al.* 2001). It has been shown that salmon are able to convert the essential fatty acids (EFA) 18:3n (3) and 18:2 (6) to the longer chain, more unsaturated HUFA 20:5 (3), 22:6 (3) and 20:4 (6) via a series of fatty acid desaturase and elongase enzymes (Tocher *et al.* 2000). However, it is possible that the capacity for the production of HUFA products may not meet optimal requirements. For optimal growth and to maintain health in the fish as well as to obtain acceptable levels in fish flesh at harvest, it may be that an optimal inclusion level of dietary 20:5 (3) and 22:6 (3) will be required (Bell *et al.*, 2003). It may be that the SUS diet reflects the lowest level of fish oil that can be included in the diets of Atlantic salmon when partially substituting it with terrestrial oil products rich in PUFA products.

Intensively produced salmon using marine fish oil is rich in n-3 HUFA and with a high n-3/n-6 PUFA ratio (Bell *et al.* 1998; Schmidt *et al.* 2001) and thus highly nutritious as food for humans (De Deckere *et al.* 1998). Any alternatives to fish oil used in fish feeds should therefore ensure that these nutritional qualities are maintained (Bell *et al.,* 2003). Studies have shown that by feeding salmon, previously fed on high vegetable oil feeds, feeding with diets containing fish oil as the sole lipid source for 12 weeks restored HUFA 20:5 (3) and 22:6 (3) levels to those comparable to fish fed standard commercial diets (Bell *et al.*, 2003).

Protein digestibility was similar for all diets. This is in accordance with data from Anderson *et al.* (1992) and Opstveldt *et al.* (2003) who reported values for protein digestibility of 86.6% and 89.3%, respectively, in salmon fed high quality fishmeal diets, and of 83.3% and 87.0%, respectively, for those fed corn gluten meal diets. The results of the present study showed increased levels of dietary vegetable oil or dietary protein did not affect digestibility of protein. This is in agreement with the study by Mundheim *et al.* (2004).

The AD of fat was significantly higher in the SUS feed than in the other feeds used. This may be due to the low water temperature during the study (mean = 8°C) as the digestibility of saturated fatty acids is reduced at low water temperature (Olsen and Ringo, 1998), and the SUS diet contained lower concentrations of these than the other feeds. The improved lipid digestibility in fish fed the SUS feed significantly improved the energy availability of this feed (88.5% available energy) and could be the reason for the low FCR experienced by fish fed the SUS diet.

The digestibility of starch was similar for fish fed the LP, SUS and control diets which had similar inclusion levels of carbohydrate. These inclusion levels were higher compared to inclusion levels in the ND diet which had the highest digestibility of starch. It has been shown that starch digestibility in salmonids is affected by carbohydrate source (Arnesen *et al.* 1995) and inclusion levels (Aksnes, 1995; Grisdale-Helland and Helland, 1997).

Apparent digestibility of dry matter was lowest in the LP feed, which contained the highest starch level. The low AD of dry matter in the LP feed may be explained by the low digestibility of starch for this feed shown in the results,

which is similar to digestibility values obtained by (Thodesen and Sturebakken, 1998) for pre-cooked rye or wheat in Atlantic salmon. The ND diet, which contained the lowest starch content of all the feed used, had the highest AD of dry matter. These results are in agreement with Aksnes (1995) who showed that AD of dry matter decreased with increasing levels of carbohydrate in diets fed to Atlantic salmon.

2.5 - Conclusion

In conclusion, the diets used in this study demonstrated the range of different diets that could be provided by feed manufactures to salmon farmers. Each experimental diet used here was manufactured to reflect the potential to change the level or source of feed components based on current industry knowledge.

Feeding fish a nutrient dense diet resulted in a small but positive increase in growth rate. However, the higher dress loss, reduced flesh pigment uptake and high inclusion levels of fishmeal and fish oil would ensure that such a diet is both environmentally unsustainable and uneconomical.

Use of low protein diets does not necessarily result in significantly poorer growth compared to commercial grower diets used in the industry at present. There may be potential to maximise use of low protein diets, which are clearly more sustainable in terms of the amounts of marine fishmeal that would be required to achieve reasonable performance. This would represent a significant economic saving as well as reducing reliance on fishmeal. Indeed there is a

need to determine whether low protein diets can be used under commercial conditions.

Growth rates of fish fed the SUS diet were comparable to those of fish fed the control diet and the overall FCR was similar to that of fish fed the nutrient dense diet. The reduced cost of producing the SUS diet through reduced reliance on fishmeal and fish oil would make such a diet more environmentally sustainable compared to diets used in the industry at present when considering the reduction in wild pelagic fish that would be required to produce this type of diet.

Based on the results the hypothesis "H_o: Feeding diets to groups of Atlantic salmon with different inclusion levels and sources of dietary protein and oil does not result in differences in growth and flesh quality" may be rejected as fish fed the ND and SUS diets had better growth rates compared to fish fed the LP and control diets which achieved similar growth and flesh quality. There were significant differences in flesh quality between the groups. With fish fed the ND diet having higher dress loss and reduced pigment uptake compared to all other groups.

Chapter 3 - The use of low protein diets - optimal introduction time and effects on performance and harvest quality of Atlantic salmon (*Salmo salar* L.).

3.1- Introduction

Feed cost is the largest single expense in commercial salmon production. In Norway, approximately 50-60% of the production costs are related to the purchase of fish feed (Fiskeridirektoratet, 2003). Protein is the most expensive component in modern fish feeds and accounts for 45 – 50% of the total raw material cost (Sveier, 2004). This puts considerable strain on feed producers to deliver the most cost efficient feed and feeding solutions to fish farmers. The quality of the dietary protein is usually high with an apparent digestibility coefficient of about 90% (Sveier, 2004). However, only 35- 40% of the protein eaten is incorporated into fish growth (Einen and Roem, 1997) whilst the remainder is excreted as particulate and dissolved waste.

The requirement of specific amino acids depend on both biotic and abiotic factors such as age, size and life stages of the fish, rearing conditions and dietary energy density (Cowey, 1994). Present day feeds are generally well balanced in regard to essential amino acid concentrations and contain high energy levels, which offer the fish farmer the potential for excellent performance in regard to both growth rate and FCR. As understanding of the essential amino acid and energy requirement for different sizes of fish has evolved, commercial rations have in turn shown a reduction in dietary protein content and associated increase in dietary oil and energy content (Bell *et al.* 1998; Hillestad *et al.* 1998; Biomar historical data). The use of alternative protein sources coupled with increasing knowledge on the use of synthetic amino acids has led to the potential to further reduce feed cost and production costs.

Added to this, environmental sustainability in aquaculture is the key parameter that both feed manufacturer and salmon producer will have to consider in order to successfully farm salmon and other species in the future. In Scotland the amount of biomass a particular farm can produce is already controlled by the amount of waste discharged in the form of dissolved nitrogen and particulate matter. Fish meal and fish oil are also finite resources that are becoming increasingly limited as both aquaculture and agriculture develops.

Low protein diets have substantial inclusion levels of vegetable-based raw materials. As a result they are more cost effective to produce, more sustainable in regard to fishmeal and fish oil inclusion and lead to decreased environmental discharge, especially dissolved nitrogen discharge (Einen and Roem, 1997; Hillestad *et al.* 1998). In chapter 2, it was shown that the use of low protein diets does not necessarily result in significantly poorer growth compared to commercial grower diets used in the industry at present. Low protein feeds manufactured and tested by Biomar Ltd have shown the potential to decrease N discharge by 30% when compared to today's commercial feeds, giving the same performance in terms of growth and feed conversion (Biomar historical data). In a situation with the use of feed quotas as used in Norway, attention is inevitably drawn to feed utilization i.e. the amount of feed needed per unit fish produced. The lowest feed conversion ratio (FCR; feed:gain) and the lowest waste output from the farming site may be achieved by providing diets with an optimal energy and protein-to-energy ratio, adequate amounts of essential components, at a rate adapted to the fish size. However, any imbalances and inadequacies of feed composition will affect fish performance and be

manifested as reduced appetite, sub-optimal growth, increased feed conversion or increased fat accretion.

As mentioned earlier, in 2002, the total amount of Atlantic salmon produced in Scotland was 145,609 tonnes. The amount of feed required to be purchased by the Scottish aquaculture industry, based on an FCR of 1.3, would be 182, 292 tonnes. A reduction of even 2% in the amount of protein incorporated into fish feeds given protein inclusion levels of 30 – 45% would result in a reduction of 3645 tonnes of protein that would otherwise be used in the manufacture of fish feeds.

There is a need to determine whether low protein diets can be used under commercial conditions. Past trials carried out by (Hillestad and Johnsen, 1994) and Biomar (Biomar historical data) have clearly shown the huge potential for use of low-protein diets described above. However, these results are from tightly controlled small-scale trials with well-graded fish, feed waste collection systems and careful feeding regimes. This can offer the potential for results to be quite different to what is achieved under commercial conditions. It is therefore essential to test the concept under large-scale conditions.

In the study described here, it was intended to introduce low protein feeds to triplicate groups of fish at different sizes throughout the production cycle. The first low protein feed was introduced when fish had grown to approximately 330 g in size with subsequent introductions of low protein feeds as the fish grow. This allowed identification of the optimal size of fish in relation to the variation in size within the populations to ensure the most cost effective production costs.

Fish were assessed in terms of growth and dietary performance throughout the production cycle.

The main hypothesis that was tested was:

H_o: Introducing groups of Atlantic salmon to low protein feeds at different times throughout the marine phase of production will not result in differences in growth or dietary performance

3.2 - Materials and methods

3.2.1 - Stocking and husbandry

The field trial described here was conducted at Gifas Research Station, Inndyr, Norway. Approximately 290 000 Atlantic salmon from the NLA strain where used in the trial. After completing smoltification, as assessed by regular seawater challenge tests, the fish were held in seawater tanks prior to transport by well boat to GIFAS trial site at Røssøy. The estimated average body weight upon transfer was 75g.

Twelve groups of smolts were established in 60 m circular cages with equal amounts of fish in each cage (23 000 – 24 000). The sizes of the groups were kept unchanged throughout the experiment. Smolts were delivered to GIFAS by means of a well-boat in three trips. During each trip, three counts of smolt numbers was undertaken using a macro counter; (a) counting in the freshwater facility, (b) a control count during loading onto the well-boat, and (c) a final count from the well-boat to the cage. Each shipment was evenly distributed between all the 12 cages, to avoid systematic differences amongst the fish-

groups. Thus, all cages received three batches of fish. All handling of fish was done at a relatively slow pace, in order to minimize handling stress and to maximize counting accuracy. The smolt-nets were 12 m deep, giving a volume of ca. 3000 m^3, whereas the nets used during on-growth were 20 m deep and contained ca. 5000 m^3 volume. The trial commenced in July 2003 and continued until August 2004.

Fish were treated for sea lice infestation with SLICE Premix 50µg (kg biomass)$^{-1}$ (day)$^{-1}$ (active ingredient, emamectin benzoate) for seven consecutive days between 01/09/2003 and 07/09/2003.

3.2.2 – Feeding

Daily feed delivery was based on distinct meals; one daily meal at water temperatures below 5°C and two-three meals above 5°C. When the smolts had arrived at GIFAS, they were fed by hand for 2 months, after which feeding was by means of automatic feeders. A "moderate restrictive" feeding regime was used in all groups to obtain a good FCR and minimize the risk of feed waste. Each cage was equipped with a Betten automatic feeder, modified to minimise feed breakage (ca 0.2 % on 12 mm pellet, less on smaller pellet sizes). Thus, feed wastage due to breakage and dust can be considered negligible.

3.2.3 – Diets

The feeds were produced by BioMar AS, Norway. Table 3 1 shows the dietary composition, chemical analysis and calculated energy content of the experimental diets.

Table 3 1 Dietary ingredient, chemical analysis and calculated values of the experimental diets.

	C50	C100	C250		C800		C1500		C2000a		C2000b		C2000c	
			Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test
Recipe														
Fishmeal, %	55.9	59.7	48.1	34.5	32.6	36.4	33.8	32.6	30.2	27.4	36.4	28.6	33.9	30.8
Fish oil, %	16.6	17.4	22.7	25.5	27.7	31.1	31.9	32.6	33.3	34.5	21.2	21.0	32.3	33.3
*Astaxanthin (mg Kg^{-1})	*40*	*40*	50	54	58	55	58	65	34	39	31	32	32	31
Analysed composition														
Crude protein, %	46.1	46.3	45.7	40.6	40.5	35.3	36.4	32.3	35.2	32.3	36.3	33.2	35.2	33.2
**Nitrogen content (%)	7.38	7.41	7.31	6.50	6.49	5.65	5.82	5.17	5.63	5.17	5.81	5.31	5.63	5.31
Crude fat, %	24.8	25.1	26.7	29.8	32.0	34.7	37.1	37.6	35.9	37.2	35.9	37.7	36.0	38.6
Ash, %	9.6	9.8	7.8	7.6	6.5	7.0	6.3	6.5	6.9	7.2	7.0	6.5	7.1	7.0
Water, %	7.7	7.4	8.1	6.9	6.4	6.6	5.3	5.1	4.6	4.4	5.2	4.5	4.7	4.4
Residue, %	11.8	11.4	11.7	15.1	14.6	16.4	14.9	18.5	17.3	18.9	15.6	18.2	17.0	16.9
***Gross energy (GE), MJ kg^{-1}	22.7	22.8	23.3	23.9	24.7	24.9	25.8	25.6	25.5	25.6	25.4	25.8	25.4	26.0
****Protein to energy ratio	20.3	20.3	19.6	17.0	16.4	14.2	14.1	12.6	13.8	12.6	14.3	12.8	13.8	12.8

* Deciared values underlined otherwise determined by laboratory analysis.
**Caiculated from the assumption that 16 % of the protein content is Nitrogen
*** Estimated from caloric values of 39.5, 23.6 and 17.2 kJ g-1 for fat, protein and carbohydrate, respectively.
**** Calculated g protein kJ^{-1}

From smolt input until approximately 330 g weight, all groups were fed the BioOptimal Classic products C50 and C100 (Control feed). From this size a low-protein diet (Test diet C250) was introduced to three cages of fish while the remaining nine cages continued on the standard product (C250 control). At approximately 1000 g live weight another three cages of fish were transferred to the Test diet, while fish in the remaining six cages continue on the control diet (C800). The last three cages were given low-protein feed from 1600 g onwards, while fish in the control group continued on the standard product (C1500-C2000) until harvest. By this procedure, nine cages of fish were transferred to the Test diet at 330g, 1000g or 1600g, respectively; while three cages were kept on a standard feed control from smolt input to harvest. The experimental set-up is illustrated in Table 3.2 below.

Table 3.2. Illustration of the experimental set-up where the use of control feed is shown by white colour and the use of a low-protein product are shown by grey colour. The experimental groups were fed a low-protein feed from 330, 1000 and 1600g fish size, respectively, while the control group was fed the standard feed from smolt input to harvest. Each group consisted of three net pens.

	DIETS					
	C100	**C250**	**C800**	**C1500**	**C2000a**	**C2000b**
Fish size (g)	100	330	1000	1600	2000	3-6000
Control						
Group 1(T250)		03/10/2003 ONWARDS				
Group2 (T800)			17/12/2003 ONWARDS			
Group3 (T1500)				18/04/2004 ONWARDS		

3.2.4 – Sampling

In November, 2003, nine fish from the control and T250 groups were selected from random cages and anaesthetised with Benzocain (10 % solution) at a concentration of between 0.5 to 1 ml/l of seawater. Once anaesthetised, individual fork length and weights were recorded using a Marel M 2000 series, type M60 animal weighing scale. This process was repeated in April and June 2004 with 18 fish being selected from all four groups and again in August 2004 when thirty-six fish were sampled from each of the four groups. All sampled fish were humanly killed, tagged and placed on ice. Fork length and body weight was recorded before gutting and washing. Dress-out loss was then recorded along with any instances of deformation, cataract or vaccine damage.

Mean fish weights for each triplicate group were determined at 1-2 month intervals using a Storvik Biomass Estimator (Storvik AS, Sunndalsøra). During registration the open rectangular frame (17.5cm deep, 85cm high and 67cm wide) was submerged at 3-6m, and was used to measure a minimum of 300 fish in each cage over 1-4 days. Sample weighing commenced on 30th August 2003 with an average 437 fish sampled from each cage on each occasion.

The visual colour of salmon fillets was assessed by comparison with the Roche *Salmor*Fan^{TM} (Hoffmann-La Roche, Basel, Switzerland), under standard conditions using a light cabinet fitted with a fluorescent light source. The light source had a colour rendering index (Ra)>90 and a colour temperature > 6000 K to allow accurate colour matching.

Two scorers independently measured the colour of sample fillets. The fillets were then homogenized and then frozen at -20 ^{0}C in duplicate containers for analysis of astaxanthin

3.2.5 – Water quality

Temperature was logged daily at 1, 3, 5 and 7 m water depth using a WTX – OXI 197.

3.2.6 – Calculations and statistics

The specific growth rate (SGR), thermal growth coefficient (TGC), feed conversion ratio (FCR) and dress out percentage (DOP) were calculated as described in chapter 2. Viscerosomatic index (VSI) was calculated as: VSI = 100 (visceral weight / body weight) and hepatosomatic index (HSI) was calculated as: HSI = 100 (liver weight / body weight).

Statistical significance of differences among diet types was computed from one-way or two-way analysis of variance (ANOVA). The normality and homogeneity of the variance of all data sets was tested prior to parametric statistical analysis. Normality was tested by graphic examination of probability plots and the Anderson-Darling test. Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$) and results are presented as mean values ± standard error of the mean (SE). Regression analysis was used to determine the relationship between flesh pigment level (mg kg^{-1}) and flesh colour using Minitab^{TM} version 13 statistical software (Ryan and Joiner, 1994).

3.3 – Results

The data obtained for mean fish weights during the trial period can be seen in Figure 3.1. At no time were significant differences found in mean body weight between treatments ($F_{3.8} < 2.39$; $p > 0.05$; $n = 437$). Fish fed the control diet had a final weight of 3676 g, whilst fish fed the T250, T800 and T1500 diets had final weights of 3475, 3479 and 3630 g respectively.

The data obtained for the FCR of all the salmon during the trial (Figure 3.2) showed that there were no significant differences in FCR ($F_{3.8} < 2.89$; $p > 0.05$; $n = 437$) throughout the trial period. Fish fed the control diet had an overall FCR of 1.04 whilst fish fed the T250, T800 and T1500 diets had overall FCRs of 1.04, 1.06 and 1.04 respectively.

Similarly, there were no significant differences in SGR between groups ($F_{3.8} < 3.43$; $p > 0.05$) between the start of the trail until April, 2004 (Figure 3.3). There were significant differences in SGR during the months of May 2004 and June 2004. In May the T800 diet group had a significantly lower SGR (0.64) compared with both the control diet (0.77) and T 1500 diet (0.76) groups ($F_{3.8}$ 9.58; $p < 0.05$). And in June, both the T250 and T800 diet groups (0.62 and 0.63 respectively) had significantly lower SGR ($F_{3.8}$ 15.83; $p < 0.05$) compared to both the control and T1500 diet groups (0.69 and 0.71 respectively). Fish fed the four different diets had similar SGRs at the end of the trial (0.57 for both the control and T250 diet group and 0.60 and 0.52 for the T800 and T1500 diet groups respectively).

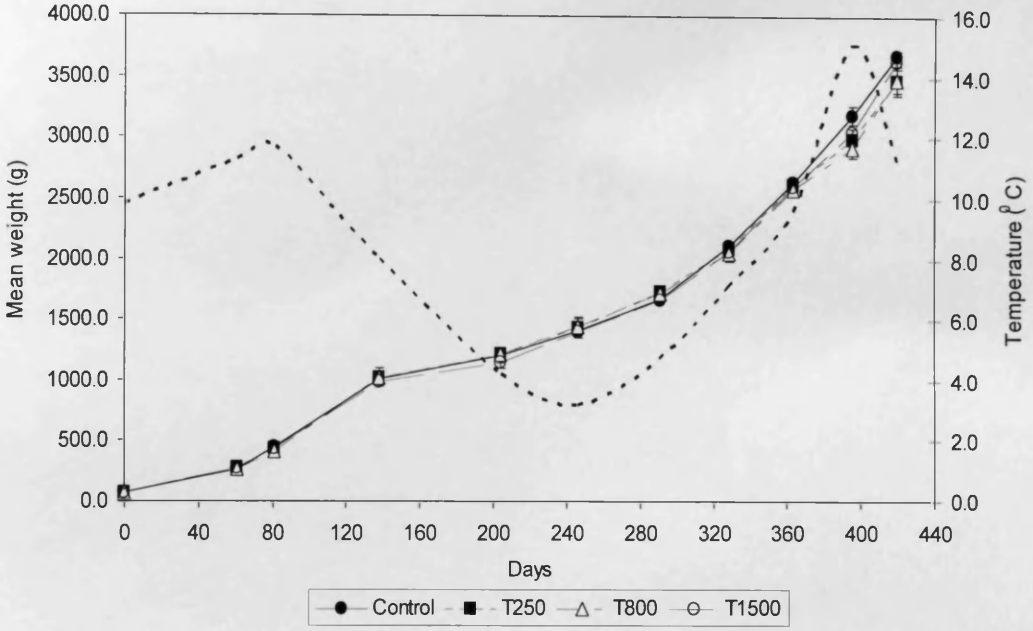


Figure 3.1Temperature (dotted line) and mean fish weights determined for each group of fish throughout the trial period. Values represent means ± S.E. (n = 437).

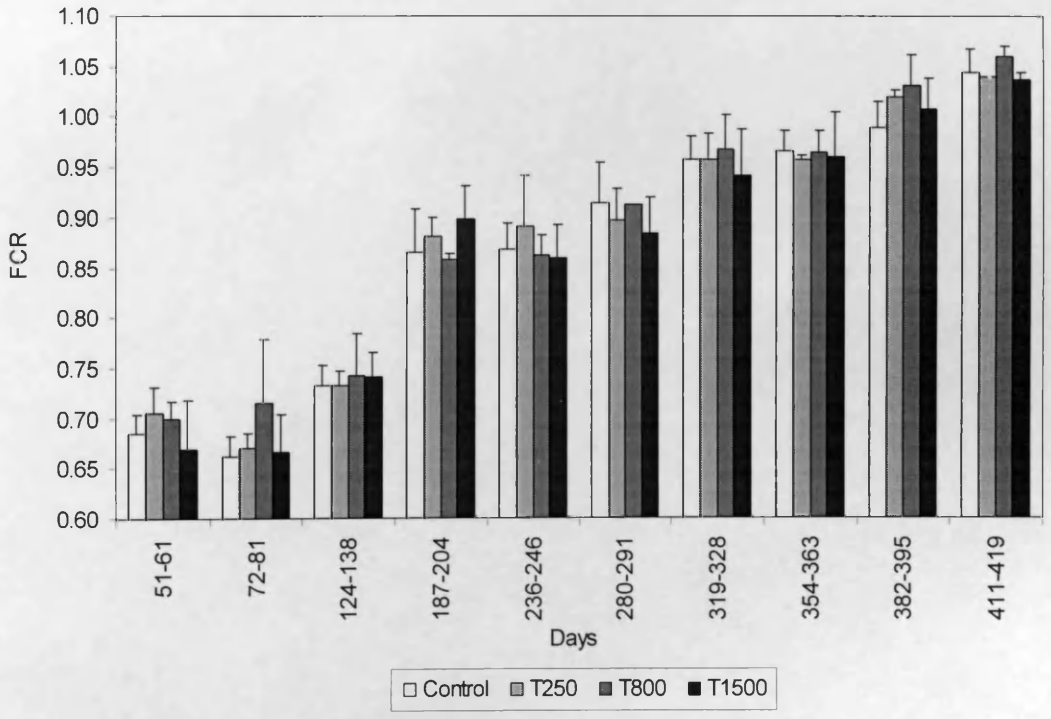


Figure 3.2 Mean feed conversion ratios of groups of Atlantic salmon introduced to low protein diets at different times throughout the marine phase of production. Values represent means ± S.E. (n = 437).

Calculated TGC for each month can be seen in Figure 3.4. There were no significant differences in TGC between all groups from July, 2003 to April, 2004 (F $F_{3.8}$ < 1.34; p > 0.05) and between July, 2004 until the end of the trail period (F $F_{3.8}$ < 2.64; p > 0.05). However, there were significant differences in TGC in May and June 2004. At the end of May, both the control and T1500 diet groups had a significantly higher TGC (4.20 and 4.16 respectively) compared to the T800 diet group (3.57) ($F_{3.8}$ 6.44; p < 0.05). In June both the T250 and T800 diet groups (3.02 and 3.06 respectively) had significantly lower TGC ($F_{3.8}$ 19.82; p < 0.05) compared to both the control and T1500 groups. Fish fed the control diet had an overall TGC of 3.24 whilst fish fed the T250, T800 and T1500 diets had an overall TGC of 3.20, 3.20 and 3.18 respectively.

The mean values for the flesh quality characteristics (body weight, gutted weight, and condition factor and dress loss) are shown in Table 3.3. There were no significant differences in body weight and gutted weight at days 296 and 357 ($F_{3.68}$ < 2.44; p > 0.05). At day 419 (at the end of the trial period), fish fed the control diet had significantly higher body weight compared to fish fed the T250 diet ($F_{3.140}$ 2.67; p < 0.05;n = 36). There were no significant differences between the other groups. In addition, fish fed the control diet also had significantly higher gutted weight ($F_{3.140}$ 2.91; p < 0.05;n = 36) compared to the T250 diet group and again there were no significant differences between the other groups. There were no significant differences in condition factor (K) between groups at days 296 and 357 ($F_{3.68}$ < 2.06; p > 0.05;n = 18). At day 419, fish fed the control diet had a significantly higher K compared to the control group ($F_{3.140}$ 3.54; p < 0.05;n = 36). There were no significant differences between the other groups.

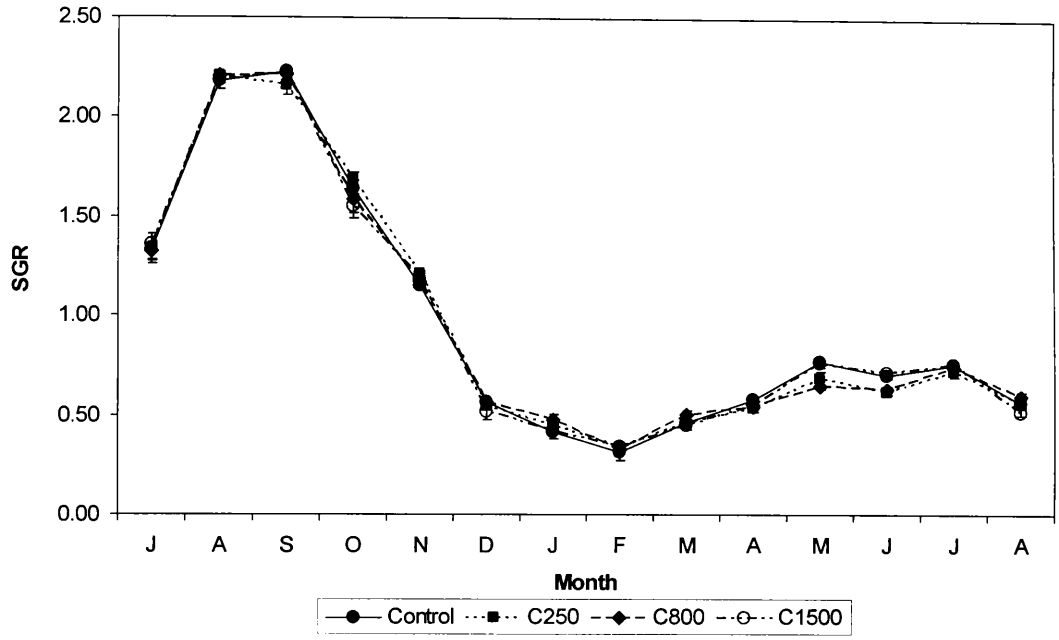


Figure 3.3 Mean specific growth rates of groups of Atlantic salmon introduced to low protein diets at different times throughout the marine phase of production. (July2003 to August 2004). Values represent means ± S.E.

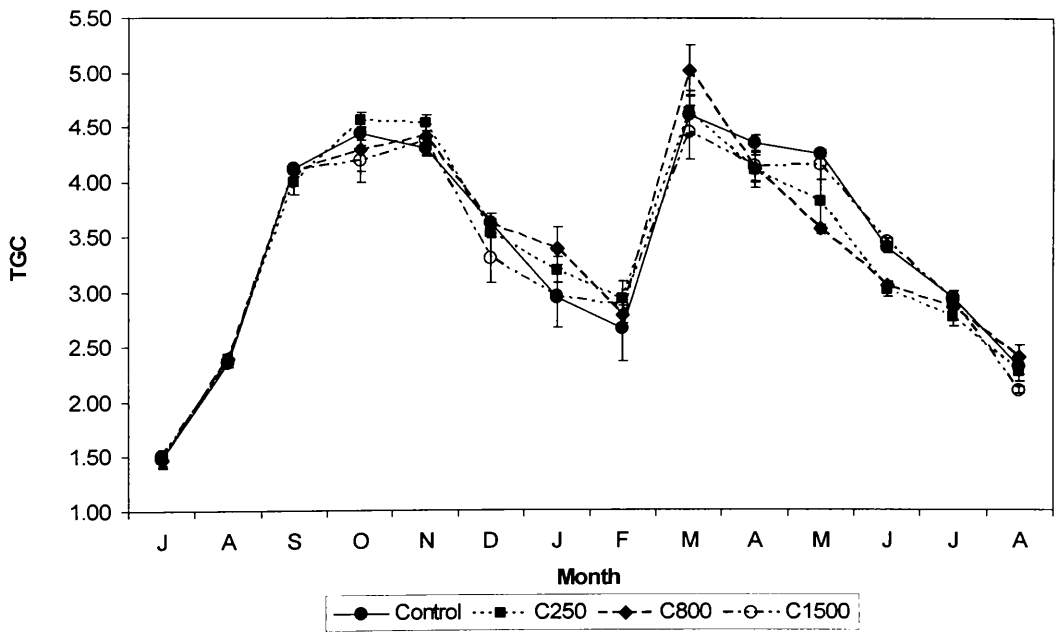


Figure 3.4 Mean thermal growth coefficient of groups of Atlantic salmon introduced to low protein diets at different times throughout the marine phase of production. (July2003 to August 2004). Values represent means ± S.E.

There were no significant differences in percentage dress loss between groups at days 296 and 357 ($F_{3.68}$, 0.73; $p > 0.05$;n = 18). Similarly, there were no significant differences in dress loss between groups at the end of the trial period ($F_{3.140}$ 0.28 ; $p > 0.05$;n = 36) with percentage loss ranging from 13.50 % for fish fed the control diet to 13.98 % for fish fed the T250 diet.

Mean values for flesh pigment, HSI, VSI and Roch colour scores are shown in Table 3.4. There were no significant differences in flesh pigment between groups at day 144 ($F_{3.8}$ 0.45; $p > 0.05$;n = 10). However, at day 296, fish fed the T250 diet had significantly higher ($F_{3.68}$ 2.96; $p < 0.05$) flesh pigment (6.42 mg Kg^{-1}) compared to fish fed the control diet (5.30 mg Kg^{-1}). There were no significant differences between the other groups with fish fed the T800 and T1500 diets having 5.30, 6.28 and 5.73 mg Kg^{-1} respectively. There were no significant differences between groups in the assessment of flesh colour score (Roch SalmoFan^{TM}) at day 144 ($F_{3.8}$ 0.05; $p > 0.05$;n = 10). At day 296, fish fed the T250 and T800 diets had significantly higher ($F_{3.68}$ 4.45; $p < 0.05$;n = 18) colour scores (26.61 and 26.5 respectively) compared to fish fed the control diet (25.39). There were no significant differences between the other diets with fish fed the T1500 diet having colour score of 25.78. There were no significant differences in colour between groups at day 357 ($F_{3.68}$ 0.87; $p > 0.05$;n = 18) and at day 419 ($F_{3.140}$ 1.52; $p > 0.05$;n = 36). Within the range of the flesh pigment concentrations observed in the study, there was a linear increase of Roche SalmoFan^{TM} score with increasing flesh total pigment concentration (Figure 3.5), where the r^2 value was significant at 0.89 ($p < 0.05$, $y = 0.82x + 21.09$, SE estimate = 0.62;n = 288).

In addition, there were no significant differences found in HSI and VSI between groups at days 296 and 357 ($F_{3.68} < 2.30$; $p > 0.05$;n = 18) and at day 419 ($F_{3.140} < 1.12$; $p > 0.05$;n = 36).

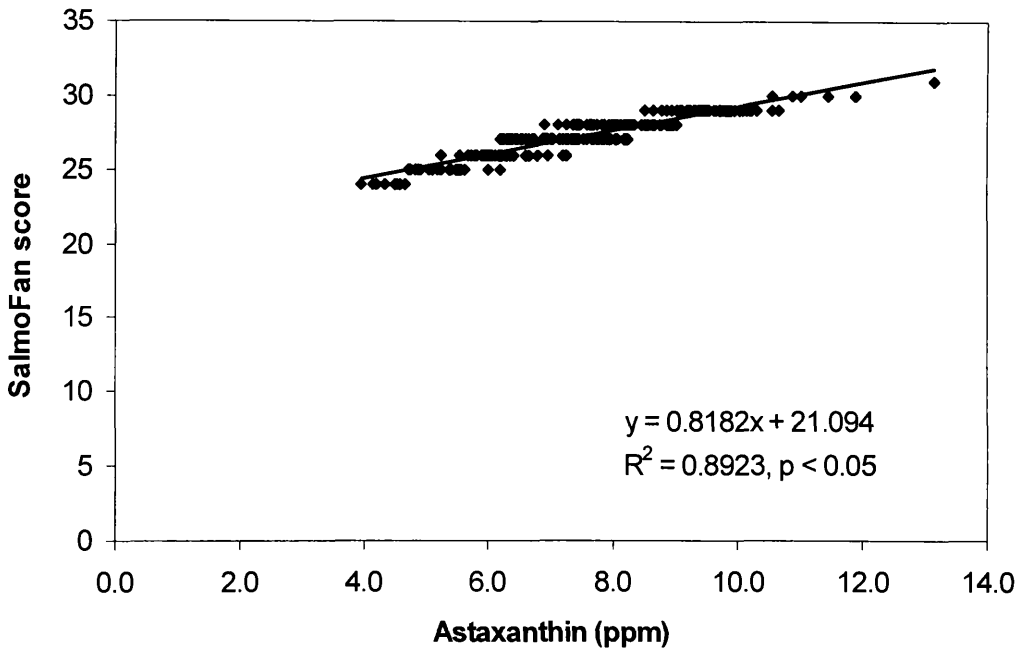


Figure 3.5 Regression analysis on the effect of dietary pigment level on Roche *Salmo*Fan™ scores in Atlantic salmon. Data are from fish analysed from a mean weight of 1620 g to a mean final weight of 4047 g. (n = 288).

Table 3.3 Mean body weight, gutted weight, condition factor and dress loss of groups of Atlantic salmon introduce\d to low protein diets at different times throughout the marine phase of production. Values represent means ± S.E. Number of fish sampled at days 296 and 357 (n = 18; N = 72). Number of fish sampled at day 419 (n = 36; N = 144).

	DIETS				ANOVA[1]	
Days	**Control**	**T250**	**T800**	**T1500**	***F***	***P***
Body weight (g)						
296	1600.56 ± 64.59	1647.17 ± 66.72	1640.83 ± 76.27	1594.11 ± 48.75	0.18	NS
357	2753.22 ± 120.77	2797.67 ± 114.38	2417.89 ± 122.83	2566.00 ± 100.53	2.33	NS
419	4203.78 ± 88.86^{a}	3859.78 ± 79.25^{b}	4079.17 ± 90.08^{ab}	4047.03 ± 92.01^{ab}	2.67	*
Gutted weight (g)						
296	1361.28 ± 55.98	1407.94 ± 59.13	1403.50 ± 64.45	1366.87 ± 41.88	0.19	NS
357	2389.83 ± 106.07	2429.22 ± 99.64	2089.56 ± 107.12	2226.28 ± 86.86	2.44	NS
419	3637.67 ± 78.71^{a}	3319.42 ± 67.46^{b}	3519.83 ± 80.74^{ab}	3477.00 ± 80.67^{ab}	2.91	*
Condition factor						
296	1.22 ± 0.02	1.27 ± 0.02	1.27 ± 0.02	1.21 ± 0.02	2.06	NS
357	1.37 ± 0.02	1.32 ± 0.03	1.30 ± 0.02	1.32 ± 0.01	1.80	NS
419	1.35 ± 0.02^{a}	1.29 ± 0.01^{b}	1.30 ± 0.02^{ab}	1.28 ± 0.02^{b}	3.54	*
Dress loss (percent waste)						
296	14.99 ± 0.33	14.57 ± 0.51	14.42 ± 0.22	14.26 ± 0.37	0.72	NS
357	13.22 ± 0.27	13.18 ± 0.19	13.60 ± 0.22	13.22 ± 0.24	0.73	NS
419	13.50 ± 0.17	13.98 ± 0.18	13.70 ± 0.65	13.55 ± 0.23	0.28	NS

[1]Results from analysis of variance (ANOVA) where P is the significance level (d.f. =3, 8; 3, 68 and 3, 140). Mean values marked with different superscripts on the same line, between diets, where found significantly different by Tukeys multiple range test (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

Table 3.4 Mean flesh pigment, HSI, VSI and colour scores of groups of Atlantic salmon introduce\d to low protein diets at different times throughout the marine phase of production. Values represent means ± S.E. Number of fish sampled at day 144 (n = 9; N = 18). Number of fish sampled at days 296 and 357 (n = 18; N = 72). Number of fish sampled at day 419 (n = 36; N = 144).

	DIETS				ANOVA[1]	
Days	**Control**	**T250**	**T800**	**T1500**	***F***	***P***
Astaxanthin (mg Kg^{-1})						
144	4.79 ± 0.12	4.56 ± 0.31	-	-	0.45	NS
296	5.30 ± 0.20^{a}	6.42 ± 0.28^{b}	6.28 ± 0.24^{b}	5.73 ± 0.19^{ab}	5.03	**
357	7.56 ± 0.28	8.16 ± 0.29	7.79 ± 0.31	7.41 ± 0.32	1.20	NS
419	8.48 ± 0.17	8.95 ± 0.21	8.58 ± 0.24	8.36 ± 0.14	1.75	NS
HSI						
296	1.23 ± 0.05	1.20 ± 0.03	1.23 ± 0.05	1.23 ± 0.04	0.12	NS
357	1.12 ± 0.03	1.02 ± 0.03	1.07 ± 0.02	1.03 ± 0.04	2.38	NS
419	1.06 ± 0.02	1.02 ± 0.01	1.03 ± 0.02	1.05 ± 0.02	1.10	NS
VSI						
296	10.11 ± 0.18	10.11 ± 0.25	9.86 ± 0.21	9.73 ± 0.21	0.74	NS
357	10.24 ± 0.20	10.03 ± 0.16	10.15 ± 0.13	10.22 ± 0.19	0.30	NS
419	10.63 ± 0.15	10.83 ± 0.15	11.03 ± 0.19	10.74 ± 0.14	1.12	NS
Roche *Salmo*Fan™ scores						
144	24.56 ± 0.18	24.44 ± 0.44	-	-	0.05	NS
296	25.39 ± 0.26^{a}	26.61 ± 0.32^{b}	26.50 ± 0.26^{b}	25.78 ± 0.26^{ab}	4.45	*
357	27.61 ± 0.26	28.06 ± 0.27	27.72 ± 0.29	27.44 ± 0.28	0.87	NS
419	27.81 ± 0.17	28.22 ± 0.19	27.94 ± 0.21	27.72 ± 0.13	1.52	NS

[1]Results from analysis of variance (ANOVA) where P is the significance level (d.f. =3, 8; 3, 68 and 3, 140). Mean values marked with different superscripts on the same line, between diets, where found significantly different by Tukeys multiple range test (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

3.4 Discussion

Assessment of the suitability of each feed type in terms of growth performance showed that growth rates were similar for each group of fish throughout the trial period. Growth rates were comparable to growth rates achieved under commercial conditions (Austreng *et al.* 1987). Fish fed the low protein diets at 330 g, 1000 g and 1600 g onwards had similar growth rates compared to fish fed a normal commercial ration at the same time. Dietary protein level did not significantly affect FCR. The FCRs for all diets were in the same range as those found in other studies (Juell *et al.* 1994). It may be argued that this is a dietary energy effect, with test and control diets having similar dietary energy levels throughout the trial period which were sufficient to support maximum somatic growth in all groups. It has been shown that increasing dietary energy levels results in reductions in FCR ((Hillestad and Johnsen, 1994; Aksnes, 1995). Increased FCR calculated from fish weighing 1 kg and upwards compared to those measured from the start of the experiment may be explained by a size-dependant energy requirement for growth (Hillestad *et al.* 1998). The overall SGR was slightly although not significantly lower in fish fed the T1500 diet compared to those fed the control and other test diets. (Sveier *et al.* 1999) found no such effect feeding fish high and low protein diets, but the trial period only lasted for 82 days. These results may indicate that reducing dietary protein to 40.6 % for salmon weighing 300 g or higher is sufficient to support maximum growth, which is in agreement with a study by (Sveier *et al.* 2000) which showed that 35 % dietary protein may be sufficient to support maximum growth in fish of similar size. The TGCs found under optimal farming conditions of Atlantic salmon fed high-energy diets are reported to be around 3.3 (Holmefjord

et al. 1994; Einen *et al.* 1999).This is similar to the overall TGCs found in this study which again suggests that dietary energy levels were sufficient to support maximum growth in all groups of fish. In the present study, all diets fed to fish had similar crude protein: gross energy ratios at each stage of the production cycle. Crude protein: gross energy ratios in the control diet ranged from 16.4 to 14.3 g MJ^{-1} and for the test diets ranged from 14.2 to 12.6 g MJ^{-1} for fish fed between 1 and 5 kg. This was lower than recommended by (Einen and Roem, 1997) who suggest that optimal levels are around 17 – 19 g MJ^{-1} for Atlantic salmon weighing between 1 and 5 kg, decreasing with increasing fish size.

Condition factor (K) was not influenced by dietary protein concentrations. Values recorded previously in Atlantic salmon by (Juell *et al.* 1994) but higher than values recorded by (Sveier *et al.* 1999). The lack of response in K is in agreement with a study by (Einen and Roem, 1997) which showed that there was no dietary influence on K after 5 months. Dress out percentage is an important factor directly affecting salmon yield (Hillestad *et al.* 1998). In the present study, percentage dress loss was not significantly influenced by the dietary protein concentration. Fish fed the lower protein diets had a similar dress loss percentage at the end of the experimental period compared to fish fed the control diet with values ranging from (13.5 to 13.9 %).

The findings of the present study show that astaxanthin deposition in Atlantic salmon does not appear to be influenced by feeding fish low protein diets. Flesh astaxanthin concentration increased in all groups during the course of the experiment with only a small but significant lower flesh pigment noted in the control group at day 296 compared to the other groups. Flesh pigment

concentrations observed were higher than those reported by Einen *et al.* (1999) but similar to those reported by Aksnes (1995). Results from the Roche *Salmo*Fan™ scores indicated that there was an increase in colour perception throughout the trail period. This is in agreement with a study by Torrissen, (1989) which showed that visual perception of colour intensity increases with increased concentrations of astaxanthin in muscle. The high astaxanthin level (approximately 8.6 mg kg^{-1} wet weight) found in the fish at the end of this study is higher than the plateau level for astaxanthin in muscle (approximately 8 ppm) of Atlantic salmon found by Torrissen (1995).

HSI values in the present experiment were at the same level or lower (1.02 – 1.2) than those found in previous studies (Aksnes, 1995; Hillestad *et al.* 2001), which suggests that the dietary carbohydrate levels used in the diets did not appear to have any negative effect on the liver of fish of this size. VSI values for all groups were similar throughout the trail period. Any increase in dietary lipid level results in increased lipid deposition (Grisdale-Helland and Helland, 1997). This lipid is stored as subcutaneous fat, in muscle and as visceral fat (Hillestad and Johnsen, 1994).

3.5 – Conclusion

The primary objective of intensive farming of Atlantic salmon is to maximise growth at minimal cost. The development of feeds for salmonid fish has focussed on protein economy (Refstie *et al.* 2001). Primarily, there have been extensive efforts to define and develop cost-effective protein sources that can at least in part, substitute for high quality fish meals which are in increasing

demand. However, to fully maximise efforts into reducing reliance on fish meal, determination of the optimal time when Atlantic salmon can be fed lower protein diets is crucial to the development of protein economy and production of least-cost feed formulations. In addition, any production strategy that utilises less protein will minimise nitrogen excretion of fish and subsequently reduce the potential for eutrophication in coastal waters. The similar growth rates and flesh characteristics obtained throughout the trial period suggests that feeding fish low protein diets from approximately 330 g onwards does not affect growth performance. The reduction in crude protein content of the test diets ranged from 11 to 13 % at each diet switch compared to the control diet, with an overall reduction of 10 % in the use of crude protein throughout the trail period.

Based on the results the hypothesis "H_o: Introducing groups of Atlantic salmon to low protein feeds at different times throughout the marine phase of production will not result in differences in growth or dietary performance" may be accepted as fish fed low protein diets had similar growth and dietary performance to fish fed a standard commercial ration.

Chapter 4 – Assessment of the patterns of ammonia excretion from farmed Atlantic salmon (*Salmo salar* L.) fed different diet types.

4.1 - Introduction

Protein intake is the major factor affecting nitrogenous waste production from fish farms and the quantity and quality of dietary protein are factors that influence nitrogen excretion. It has been estimated that nitrogenous wastes account for between 52-95% of feed nitrogen depending on the species of fish and the diet (Wu, 1995).

Nitrogen excretion rates can be divided into two components: postprandial N excretion and endogenous N excretion rates. Postprandial N excretion reflects the catabolism of proteins from feeding and endogenous N excretion reflects catabolism and turnover of body proteins irrespective of the feed metabolism in fish (Forsberg, 1996).

4.1.2- Postprandial nitrogen excretion (PNE)

Branchial and urinary N excretion account for most of the losses per unit N uptake. Whilst most proteins are well digested and the digestive N losses are relatively low, losses through branchial and urinary N excretion can account for between 30 – 60% of N intake (Kaushik and Cowey, 1991). The ingestion of food has a marked effect on N excretion (Handy and Poxton, 1993). Studies have shown that there is an immediate increase in ammonia excretion rates after a meal and the amplitude and time of appearance of peak rates are dependant on fish size, amount of N intake and water temperature (Rychly and Marina, 1977). The peak ammonia excretion rate of salmonids occurs within 3-

5 hours after a meal and is usually 30-60% higher than daily mean rates and the interval needed for reaching routine or pre-feeding level is variable being both size and temperature dependent (Clarke *et al.* 1985; Kaushik and Cowey, 1991). In addition, urea excretion rates do not follow a definite pattern during the postprandial state (Kaushik and Cowey, 1991). This is consistent with a study on Atlantic salmon kept under a 24hr feeding period (Fivelstad *et al.* 1990).

4.1.3 - Endogenous Nitrogen Excretion (ENE)

Measurements of ENE are either made under fasting, using protein-free diets or based on regression of N intake to N excretion (Kaushik and Cowey, 1991). A study by (Forsberg, 1996) predicted the ENE rate in post-smolt Atlantic salmon to be about 36mg TAN kg $fish^{-1}$ day^{-1} and to contribute about 25% of the total amount of ammoniacal nitrogen excreted. This is double the ENE rate in fasting fish. However, studies on smaller fish have found ENE rates to be higher, usually in the range 100-200mg TAN kg $fish^{-1}$ day^{-1} (Rychly and Marina, 1977; Kaushik and Gomez, 1988).

4.1.4 – Reduction of dissolved wastes through diet formulation

The main factors affecting dissolved nitrogenous wastes outputs are those that influence the catabolism and deposition of ammonia acids by the fish (Cho. and Bureau, 2001). Elevated ammonia production by fish has been observed when the concentration of dietary protein is excessive relative to non-protein energy, such that a portion of dietary protein not used for protein accretion is broken down and used for energy. For example, Médale *et al.* (1995) found that higher digestible protein relative to digestible energy resulted in increased ammonia excretion in rainbow trout. Thus, increasing digestible energy content in the diet relative to the digestible protein content is the one of the nutritional strategies used to lower nitrogen excretion (Kaushik and Cowey, 1991). Another nutritional strategy to lower nitrogen excretion is to replace feed ingredients having low protein digestibility with those having high protein digestibility coefficients. This approach depends upon using the results of studies of the digestibility and nutritional value of various feed ingredients (Hardy, 1996). In addition to ingredient composition of diets, manufacturing procedures also may influence nutrient digestibility of diets. The material in extruded pellets is expanded and fused together, this reduces the amount of surface area of the pellet which comes into contact with water, the result is that extruded feeds are far more stable in water than standard dry pellets (Seymour and Bergheim, 1991). A further advantage of extruded pellets is that they can contain a higher fat content and these high energy diets reduce the feed requirement per unit of production and spare protein which is the major source of nitrogen pollution from fish farms (Seymour and Bergheim, 1991; Kaushik and Cowey, 1991). Where the protein content in fed was reduced from 45.6% to 41.5% and the fat

content was increased from 22% to 30% respectively, this resulted in 35-37% reduction in nitrogen load from post-smolt Atlantic salmon (Johnsen and Wandsvik, 1991).

Over the past several years, the potential for dissolved wastes to impact on the marine environment has become a matter of concern for the various levels of government, the public and for the aquaculture producers themselves. Any substantial and measurable increase in the concentration of dissolved nutrients may lead to an increase in phytoplankton growth and productivity if growth is limited by nutrients (Gowen and Bradbury, 1987). Minimising environmental impact is thus a key factor in insuring the long-term sustainability of the aquaculture industry.

The aim of the studies described here was to determine the ammonia excretion rates and patterns from intensively farmed Atlantic salmon fed different diet types. There has been little or no research undertaken on the patterns of ammonia excretion from intensively farmed salmonids in cages. In the first study, triplicate groups of Atlantic salmon were fed diets containing different inclusion levels and sources of dietary protein and oils. The diets fed were a high energy, nutrient dense (ND) diet rich in marine fish meal and fish oil. High energy diets have been shown to reduce solid and nitrogenous waste outputs (Cho *et al.* 1991). A low protein (LP) diet. Potentially, using lower protein diets will result in less ammonia being excreted by fish and as a result will reduce the potential ecological risk associated with dissolved wastes. A diet with partial replacement of fish meal with corn gluten and fish oil with rapeseed oil (SUS). Such diets have the potential to be more environmentally sustainable by

reducing the amount of marine fish derived products used. The last diet used was a control diet which has similar dietary levels of fishmeal and fish oil as normal commercial grower diet used in the industry at present. Each diet was assessed in terms of growth performance and efficiency of feed utilisation as described in chapter 2. The ultimate aim of this study was to determine whether there were differences in ammonia excretion patterns between fish fed different diet types and if there were differences in the timing and magnitude of food-related concentration peaks of ammonia.

In the second study, ammonia excretion patterns and food-related concentration peaks of ammonia were compared from triplicate groups of fish introduce low protein feeds to triplicate groups of fish at different sizes throughout the marine phase of the production cycle. The first low protein feed was introduced when fish had grown to approximately 330 g in size with subsequent introductions of low protein feeds as the fish grow. Each diet was assessed in terms of growth performance and efficiency of feed utilisation as described in chapter 3. The main hypothesis that was tested was: H_o: Feeding groups of Atlantic salmon either different diet types or introducing different groups of fish to low protein diets as they grew did not result in differences in the rate of or the patterns of ammonia excretion.

4.2 – Materials and methods

4.2.1 – Sampling of water for ammonia analysis from fish cages

Water samples were obtained from each triplicate group of fish throughout the period of the trials discussed in chapters 2 and 3. In the first study, four trial

cages (5 x 5 x 5 m) with 150 fish allocated to each cage were selected prior to each sampling day, with each population of fish in these cages being fed one of four experimental diets. The diets were a high energy diet (ND), a low protein (LP) diet, a partial replacement of fish meal and fish oil (SUS) diet and a control diet (Table 4.1).

A 220 W submersible pump with 7 m maximum head (ZEHNDER PUMPEN GmbH) was then placed into each cage and connected to the power supply on the cage block. Each pump had an 8 m section of 10 mm flexible hose attached and was positioned in the cage with the aid of ropes to facilitate positioning. The other end of the hose was attached to the edge of the cage rails to facilitate collection of water samples. The pumps were switched on and operated continuously till the end of the sampling period. The fish where then allowed to acclimatise to them before the beginning of sampling. Water samples were collected from the end of the hose attached to the cage edge and stored in fish boxes prior to filtering. This procedure was performed until all 12 cages had been sampled. Sampling of the cages occurred between the 25th and 27th October 2003 for the first analysis and between 2nd and 4th November 2003 for the second analysis (see Table 4.2). During the second analysis, cages 601; 602; 603 and 604 were not sampled due to the fish in these cages not feeding. Figure 4.1 shows the layout of the trail cages sampled and which diet was used for each cage.

Table 4.1 Dietary ingredient and chemical analysis of the experimental diets.

	Diet type			
	LP	SUS	ND	Control
Ingredient (%)				
Fish meal	20.0	20.0	42.8	28.1
Corn gluten	-	17.5	12.5	-
Fish oil	36.0	10.0	39.2	33.1
Rapeseed oil	-	23.4	-	-
Chemical composition (%)				
Protein	31.5	35.3	35.6	35.6
Oil	40.59	34.58	45.58	37.68
Calculations				
Gross energy (MJ kg^{-1})	26.45	25.35	27.97	26.22
Digestible energy (MJ kg^{-1})	21.22	22.44	23.97	22.56
Protein to energy ratio	11.9	13.9	12.7	13.6

Table 4.2 Dates when water sampling occurred throughout the trial period with mean fish biomass and estimated feed inputs.

Diet	Cage number	Sampling date	**Feed Input (g)	*Biomass (Kg)
Control	609	25/09/03	1946	601.85
	604	26/09/03	729	609.52
	607	27/09/03	2650	613.14
	607	02/11/03	1860	613.14
	609	04/11/03	1673	601.85
LP	606	25/09/03	1387	560.41
	601	26/09/03	573	584.40
	612	27/09/03	2494	591.81
	612	02/11/03	1432	591.81
	606	04/11/03	1035	560.41
ND	610	25/09/03	2044	625.99
	603	26/09/03	749	613.65
	608	27/09/03	1890	632.85
	608	02/11/03	2403	632.85
	610	04/11/03	2159	625.99
SUS	605	25/09/03	2577	621.19
	602	26/09/03	507	590.85
	611	27/09/03	1674	574.93
	611	02/11/03	1528	574.93
	605	04/11/03	1263	621.19

*Calculated at the end of the trail period (day 139) using a Marel M 2000 series, type M60 animal weighing scale. ** Calculated on a daily basis.

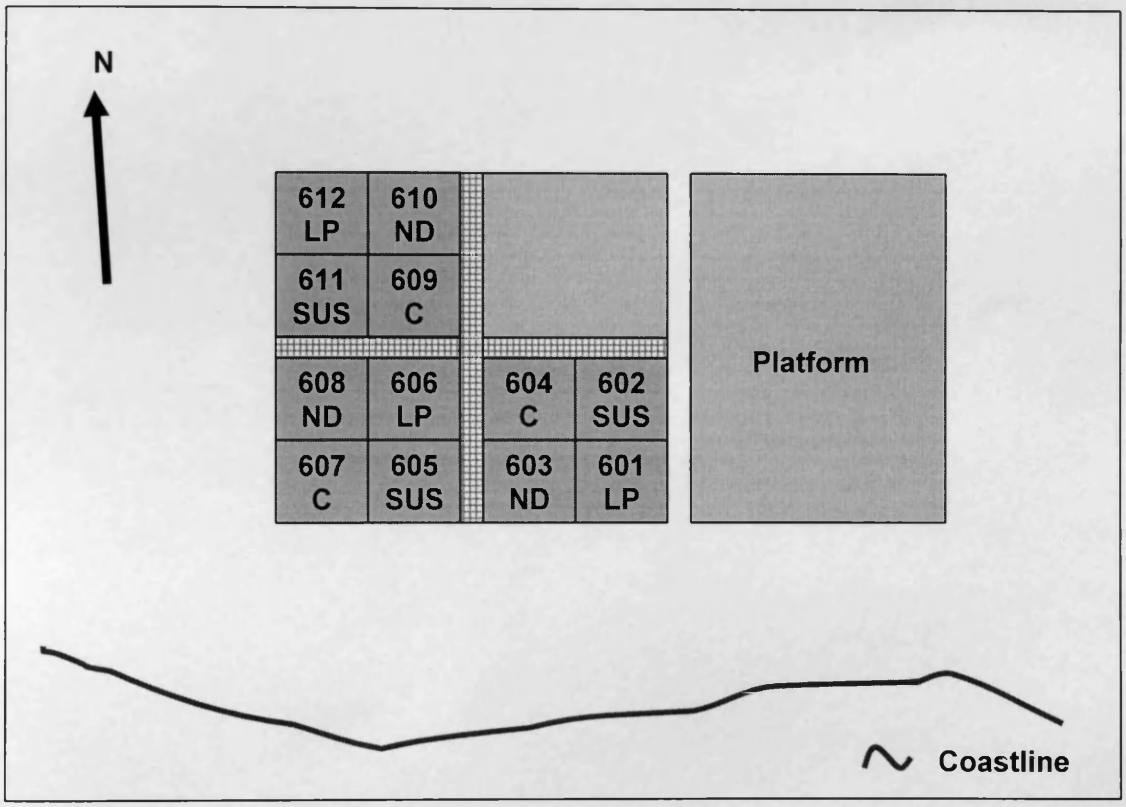


Figure 4.1 Schematic representation of the trial cages used during the trail. Each cage has an assigned number and diet type (ND – nutrient dense diet; LP – low protein diet; SUS – partial replacement diet and C – control diet).

For the second study, twelve groups of smolts were established in 60 m circular cages with equal amounts of fish in each cage (23 000 – 24 000). The sizes of the groups were kept unchanged throughout the experiment. The feeds were produced by BioMar AS, Norway. From smolt input until approximately 330 g weight, all groups were fed the BioOptimal Classic products C50 and C100 (Control feed). From this size a low-protein diet (Test diet C250) was introduced to three cages of fish while the remaining nine cages continued on the standard product (C250 control). At approximately 1000 g live weights another three cages of fish were transferred to the Test diet, while fish in the remaining six cages continue on the control diet (C800). The last three cages were given low-protein feed from 1600 g onwards, while fish in the control group continued on the standard product (C1500-C2000) until harvest. Table 4.3 shows the dietary composition, chemical analysis and calculated energy content of the experimental diets. By this procedure, nine cages of fish were transferred to the Test diet at 250g, 800g or 1500g, respectively; while three cages were kept on a standard feed control from smolt input to harvest.

Six cages were selected prior to each sampling day. A 220 W submersible pump with 7 m maximum head (ZEHNDER PUMPEN GmbH) was then placed into the centre of each cage at a depth of 6 m and connected to the power supply on the cage block. Each pump had a 12 m section of 10 mm flexible hose attached and was positioned in the cage with the aid of ropes to facilitate positioning. The other end of the hose was attached to the edge of the cage rails to facilitate collection of water samples. The pumps were switched on and operated continuously till the end of the sampling period. Sampling of the

cages commenced in September, 2003 prior to the first group of fish being fed a low protein diet. The next sampling occurred in November, 2003 after the first group of fish had been fed on a low protein diet for 28 days (see Table 4.4). Subsequent samplings were undertaken prior to and after each triplicate group of fish were introduced to low protein diets in order to allow comparisons between each group. Sampling continued until November, 2004. Figure 4.2 shows the layout of the cages sampled and which diet was used for each cage.

For both studies, water sampling was initiated the following day with duplicate samples take every thirty minutes from each of the cages. Sampling was initiated prior to feeding the fish and continued for 5 h after the cessation of feeding. The sampling time was reduced to every 15 minutes when 3 h had elapsed after the cessation of feeding. After sampling, the pumps were removed from the cages and repositioned into the next set of cages for subsequent sampling the next day. This procedure was performed until all 12 cages had been sampled.

Table 4.3 Dietary ingredient, chemical analysis and calculated values of the experimental diets.

	C50	C100	C250		C800		C1500		C2000a		C2000b		C2000c	
			Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test	Contr.	Test
Recipe														
Fishmeal, %	55.9	59.7	48.1	34.5	32.6	36.4	33.8	32.6	30.2	27.4	36.4	28.6	33.9	30.8
Fish oil, %	16.6	17.4	22.7	25.5	27.7	31.1	31.9	32.6	33.3	34.5	21.2	21.0	32.3	33.3
Analysed composition														
Crude protein, %	46.1	46.3	45.7	40.6	40.5	35.3	36.4	32.3	35.2	32.3	36.3	33.2	35.2	33.2
*Nitrogen content (%)	7.38	7.41	7.31	6.50	6.49	5.65	5.82	5.17	5.63	5.17	5.81	5.31	5.63	5.31
Crude fat, %	24.8	25.1	26.7	29.8	32.0	34.7	37.1	37.6	35.9	37.2	35.9	37.7	36.0	38.6
**Gross energy (GE), MJ kg^{-1}	22.7	22.8	23.3	23.9	24.7	24.9	25.8	25.6	25.5	25.6	25.4	25.8	25.4	26.0
Protein to energy ratio	20.3	20.3	19.6	17.0	16.4	14.2	14.1	12.6	13.8	12.6	14.3	12.8	13.8	12.8

* Calculated from the assumption that 16 % of the protein content is Nitrogen.
** Estimated from caloric values of 39.5, 23.6 and 17.2 kJ g-1 for fat, protein and carbohydrate, respectively.

Table 4.4 Dates when water sampling and diet changes occurred throughout the trial period with mean fish weights and estimated feed inputs.

Sampling date	*Mean fish weight (g)	**Feed Input (kg)	Diet switch date
29/09/2003	192	100.00	
	330		03/10/2003 – Test 250
31/10/2003	365	125.00	
20/11/2003	485	100.00	
	1000		17/12/2003 – Test 800
15/04/2004	1500	97.50	
	1600		18/04/2004 – Test 1500
22/04/2004	1650	97.50	
07/07/2004	2511	150.00	
15/08/2004	3400	100.00	
23/11/2004	5000	125.00	

* Approximate calculation based on monthly weight estimated using an Aqua-metric weigh frame system.
**Calculated using feed delivery settings on the porror spreaders fitted to Bitten hoppers on each cage.

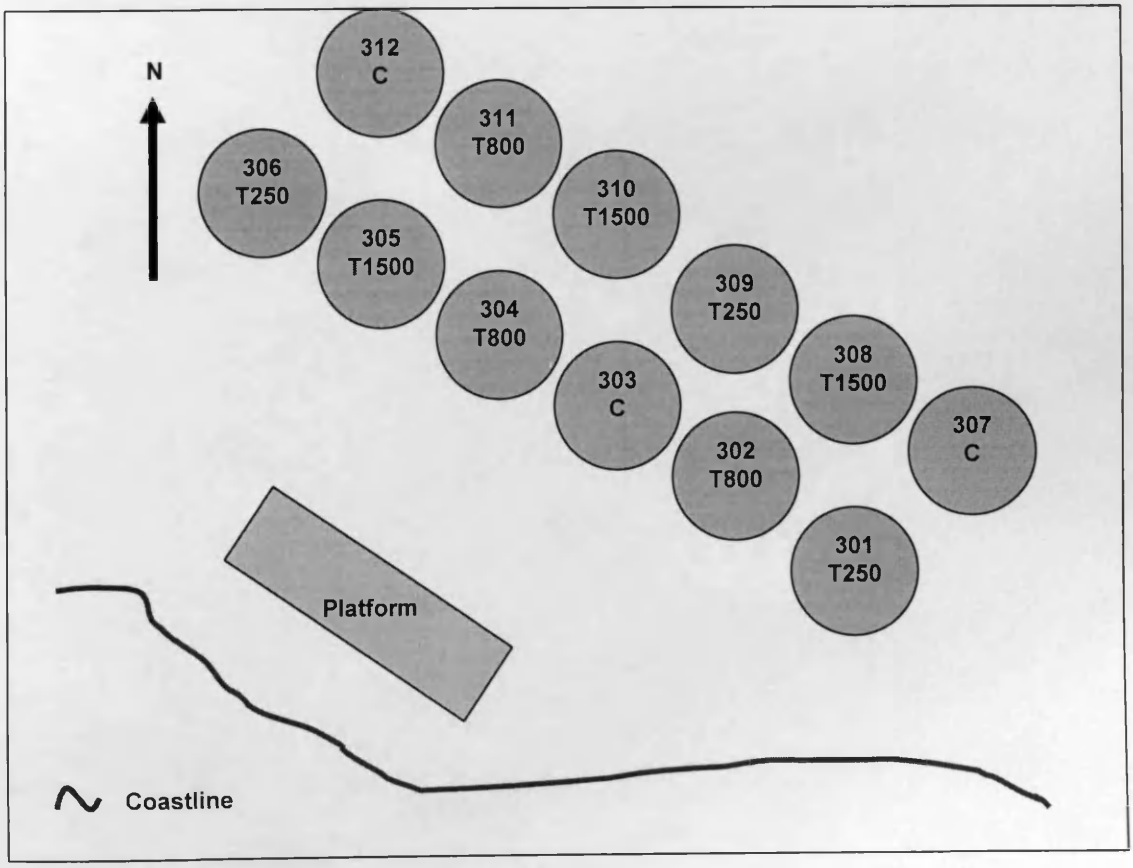


Figure 4.2 Schematic representation of the trial cages used during the trail. Each cage has an assigned number and diet type.

4.2.2 – Analysis of ammonia from water samples

Each water sample was filtered through Whatman GF/C filter paper using a hand-held Millipore filtering apparatus and stored frozen at – 20^0C in a 250 ml polyethylene container. From each water sample, 25 ml was added to a 100 ml Erlenmeyer flask using a 25 ml-measuring cylinder. 1 ml of phenol solution was then added to each sample using an automatic pipette and each flask was shaken to ensure mixing. 1 ml of nitroprusside was then added to each flask and again each flask was shaken to ensure mixing. 2.5 ml of oxidising solution was then added to each flask and the flasks were then stored in darkness for one hour. The blue indophenol colour that formed during storage was measured using a WPA S104D spectrophotometer. The extinction of the sample was read at 640 nm in the spectrophotometer using a 1 cm cuvette. Ammonia-nitrogen was calculated using the equation:

$$\text{Ammonia } (\mu\text{g-at N L}^{-1}) = F \times E$$

where F = factor derived from the calibration with a standard ammonia solution and E = the corrected extinction based on a specially prepared blank (Parssons *et al.*1984).

Ammonia concentration was calculated per kg of fish (wet weight) for all cages to take into account differences in biomass between cages and calculated per kg feed fed to the fish during each sampling day. For the graphs plotted, the area under the curve was calculated using NCSS/PASS statistical software (Utah, USA). The timing of any ammonia concentration peaks (Y max) was

recorded for each diet type. In addition, the area under each concentration peak of ammonia was calculated as a percentage of total ammonia detected over time ± 60 min.

4.2.3 – Feeding

For the first study, fish were fed by hand to satiation twice a day, with a minimum of four hours between meals. In order to facilitate accurate calculations of feed intake each cage was fitted with an air uplift system to allow accurate determination of food wastage. Feed intake can be seen in Table 4.2. During each meal, each population of fish was fed randomly to avoid any systematic differences among treatments. For fish fed the low protein diets during the second study, daily feed delivery was based on distinct meals. Each cage was equipped with a Betten automatic feeder fitted with a Porror spreader which was set at 4 seconds on and 20 seconds off. This is equivalent of delivering 65.0 Kg feed h^{-1}. Feeding was stopped after approximately one hour in all cages being sampled to allow for determination of the amount of feed delivered to each cage. Feed intake can be seen in Table 4.4.

4.3 – Results

Ammonia excretion patterns detected over time on the different sampling dates for the diets assessed in the first study can be seen in Figure 4.3 and Figure 4.4. From analysis of the data, concentration peaks of ammonia were detected for each diet type after the cessation of feeding. The timing and magnitude of

concentration peaks of ammonia differed between each diet type. Table 4.5 summarises the results. For the control diet, the ammonia concentration peaks (Y max) detected on each sampling date ranged from 0.016 to 0.046 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, with a mean concentration peak of ammonia of 0.034 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, which occurred on average, 258 minutes after the cessation of feeding. The area under each concentration peak of ammonia detected ranged from 52.5 to 88.8 % ± 60 min of total ammonia detected over time. For the LP diet, Y max detected on each sampling date ranged from 0.014 to 0.038 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, with a mean concentration peak of ammonia of 0.030 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, which occurred on average, 249 minutes after the cessation of feeding. The area under each concentration peak of ammonia detected ranged from 43.6 to 86.1 % ± 60 min of total ammonia detected over time. In contrast, Y max detected on each sampling date for fish fed the ND diet, ranged from 0.036 to 0.045 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, with a mean concentration peak of ammonia of 0.041 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, which occurred on average, 183 minutes after the cessation of feeding. The area under each concentration peak of ammonia detected ranged from 59.4 to 72.8 % ± 60 min of total ammonia detected over time. For fish fed the SUS diet, Y max detected on each sampling date ranged from 0.024 to 0.040 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, with a mean concentration peak of ammonia of 0.034 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$, which occurred on average, 246 minutes after cessation of feeding. The area under each concentration peak of ammonia detected ranged from 50.9 to 82.2 % ± 60 min of total ammonia detected over time.

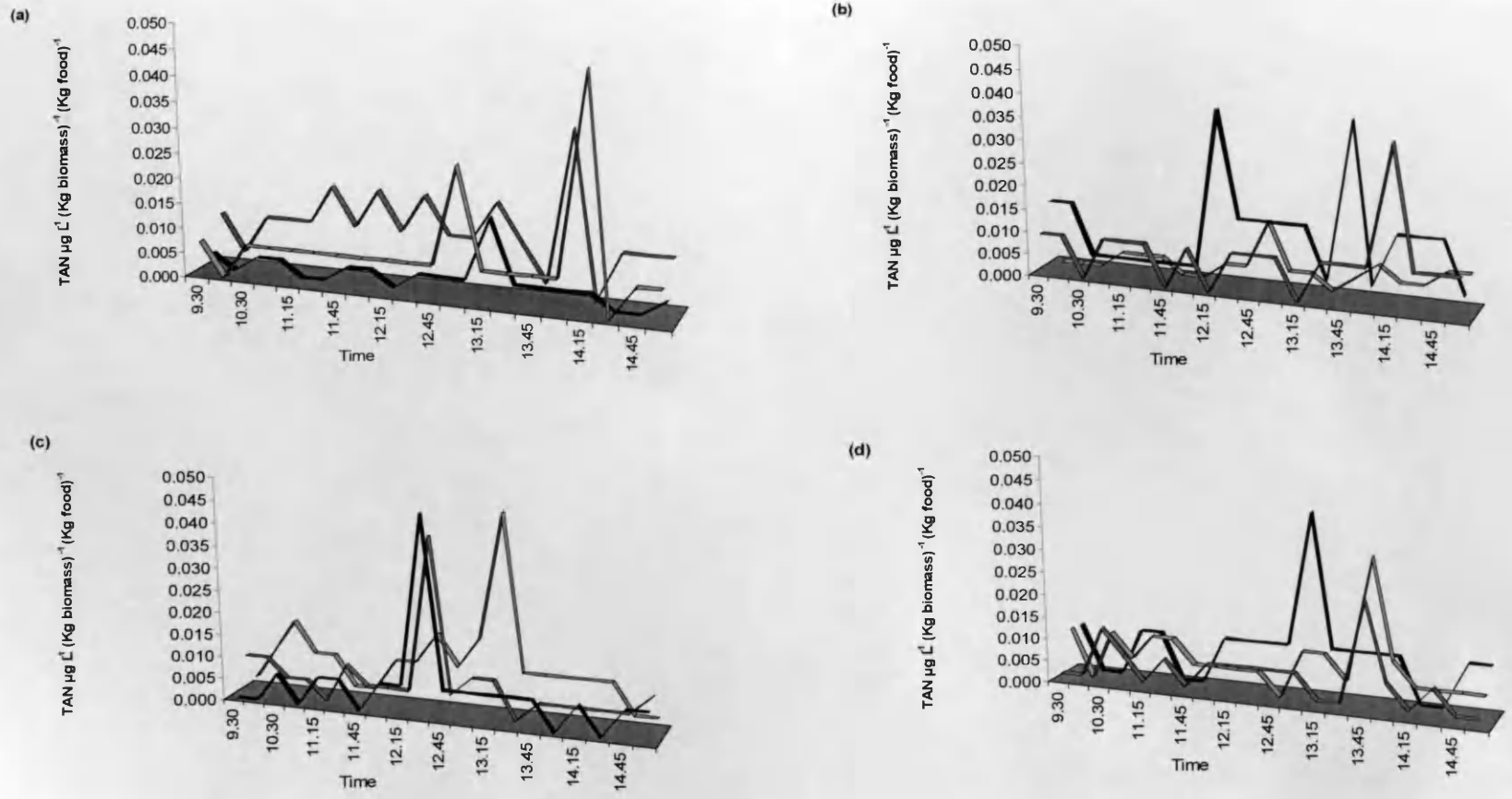


Figure 4.3 Ammonia excretion from farmed Atlantic salmon fed µg L^{-1} (kg biomass)$^{-1}$(kg feed)$^{-1}$. (a) control diet; (b) LP diet; (c) ND diet and (d) SUS diet. Each series of data represents sampling from a different cage on 25th, 26th and 27th October 2004 for each diet.

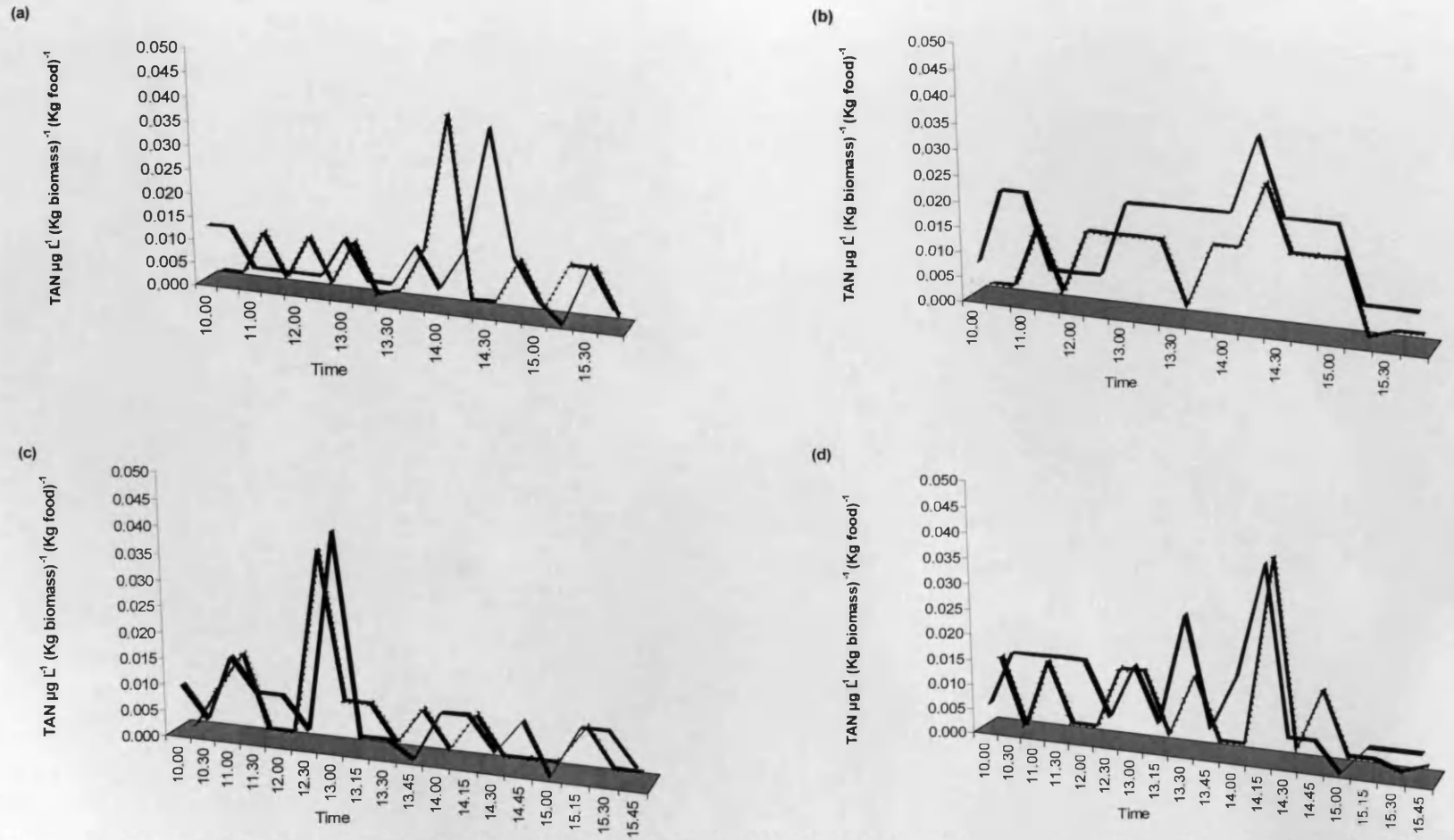


Figure 4.4 Ammonia excretion from farmed Atlantic salmon fed µg L^{-1} (kg biomass)$^{-1}$(kg feed)$^{-1}$. (a) control diet; (b) LP diet; (c) ND diet and (d) SUS diet. Each series of data represents sampling from a different cage on 2nd and 4th November 2004 for each diet.

Table4.5 Summary of magnitude and timing of ammonia concentration peaks detected on the different sampling dates.

Diet	Date	Ammonia concentration peak (Y max) $\mu g\ L^{-1}$ (kg biomass) (kg Food)$^{-1}$	Ammonia concentration peak $(\mu g\ L)^{-1}$	Area under curve $(\mu g\ L)^{-1}$	Time at Y max after cessation of feeding $(min)^{-1}$	Percentage area under peak of total ammonia detected over time ± 60 min
Cont	25/09/03	0.033	38.45	3.64	270	52.5
	26/09/03	0.046	20.45	2.85	285	53.3
	27/09/03	0.016	25.79	0.89	225	74.2
	02/11/03	0.038	44.41	3.03	240	88.8
	04/11/03	0.039	38.38	2.33	270	82.8
LP	25/09/03	0.038	29.58	3.99	255	43.6
	26/09/03	0.035	11.84	2.74	285	59.8
	27/09/03	0.014	20.61	1.01	195	51.5
	02/11/03	0.027	22.96	3.53	255	82.7
	04/11/03	0.037	21.40	5.82	255	86.1
ND	25/09/03	0.044	56.20	3.51	225	68.7
	26/09/03	0.045	20.45	2.02	180	72.8
	27/09/03	0.039	46.49	2.23	180	62.3
	02/11/03	0.036	55.14	2.60	150	71.9
	04/11/03	0.041	55.36	3.21	180	59.4
SUS	25/09/03	0.024	38.45	2.06	255	50.9
	26/09/03	0.040	11.84	2.58	210	82.2
	27/09/03	0.032	30.95	2.56	255	62.5
	02/11/03	0.038	33.69	2.61	255	80.1
	04/11/03	0.038	29.89	4.38	255	69.6

Ammonia excretion patterns detected over time on the different sampling dates for the diets assessed in the second study can be seen in Figure 4.5 and 4.6 below with the data summarised in Table 4.6. There were no clear trends in ammonia excretion patterns detected on the first sampling date (Figure 4.5a). The maximum concentration peak of ammonia (Y max) detected ranged from 0.00005 to 0.00007 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$. The area under the highest peak detected for all groups of fish ranged from 31.7 to 68.1 % ± 60 min of total ammonia detected over time. On the second sampling date (Figure 4.5b), fish fed the first low protein diet (T250) from 03/10/2003 onwards had a Y max detected of 72.53 μg L^{-1} which corresponds to 0.00009 μg L^{-1}(kg biomass)$^{-1}$(kg food)$^{-1}$.This was lower than the concentration peaks of ammonia detected (119.29; 105.93 and 125.97 μg L^{-1}) for the control group, T800 and T1500 groups respectively. The time at Y max for the T250 group was 255 minutes after the cessation of feeding compared to 300, 285 and 225 minutes for the control group, T800 and T1550 groups respectively. The area under each concentration peak of ammonia detected for each group of fish ranged from 69.9 to 74.6 % ± 60 min of total ammonia detected over time.

On the third sampling date (Figure 4.5c), this trend continued with the T250 group having a peak concentration of ammonia detected of 65.85 μg L^{-1} which corresponds to 0.00005 μg L^{-1}(kg biomass)$^{-1}$(kg food)$^{-1}$. This was lower than the concentration peaks of ammonia detected (82.55; 85.89 and 109.27 μg L^{-1}) for the control group, T800 and T1500 groups respectively. . The time at Y max for the T250 group was 240 minutes after the cessation of feeding compared to 225, 240 and 255 minutes for the control group, T800 and T1550 groups

respectively. The area under each concentration peak of ammonia detected for each group of fish ranged from 61.8 to 76.3 % ± 60 min of total ammonia detected over time.

The second diet switch occurred on 17/12/2003 with the T800 group being fed a low protein diet along with the T250 group. For both groups, ammonia concentration peaks detected on the fourth sampling date (Figure 4.5d) were lower than (99.25 and 102.59 μg L^{-1} respectively) compared to ammonia concentration peaks detected from the control and T1500 groups (125.97 and 132.65 μg L^{-1} respectively). The time at Y max for the T250, T1500 and control groups was 240 minutes after the cessation of feeding compared to 225 minutes for the T800 group. The area under each concentration peak of ammonia detected for each group of fish ranged from 65.9 to 72.9 % ± 60 min of total ammonia detected over time.

The third diet switch occurred on 18/04/2004 with the T1500 group being fed a low protein diet along with the T250 and T800 groups from this point onwards. Sampling undertaken on the 22/04/2004 (Figure 4.6a) showed that all three groups being fed the low protein diet had lower concentration peaks of ammonia detected (89.23; 89.21 and 95.91 μg L^{-1} respectively) compared to a concentration peak of ammonia of 115.95 μg L^{-1} being detected for fish fed the control diet. The time at Y max for the control group was 255 minutes after the cessation of feeding compared to 270 minutes for the T250 and T800 groups and 240 minutes fro the T1500 group. The area under each concentration peak of ammonia detected for each group of fish ranged from 60.3 to 68.1 % ± 60 min of total ammonia detected over time.

The trend for each of the groups of fish fed lower protein diets to have smaller concentration peaks of ammonia detected continued on 07/07/2004 with ammonia concentration peaks of ammonia of 149.35, 162.71 and 152.69 μg L^{-1} being detected for the T250, T800 and T1500 groups respectively compared to the control group (Figure 4.6b) which had a concentration peak of ammonia of 206.13 μg L^{-1}. The time at Y max for the control group was 255 minutes after the cessation of feeding compared to 195 minutes for the T250 group whilst Y max for the T800 and T1550 groups was 210 minutes. The area under each concentration peak of ammonia detected for each group of fish ranged from 62.1% for the T250 group to 72.4 % for the control group ± 60 min of total ammonia detected over time.

Similarly for sampling on 15/08/2004 and 23/11/2004, fish fed the control diet has higher concentration peaks evident on both sampling dates (182.75 and 172.73 μg L^{-1}) compared to fish fed the lower protein diets (Figure 4.6c and 4.6d). For sampling undertaken on the 15/08/2004, the time at Y max for the control group was 255 minutes after the cessation of feeding compared to 195 minutes for the T250 group whilst Y max for the T800 and T1550 groups was 225 minutes. The area under each concentration peak of ammonia detected for each group of fish ranged from 57.7 % for the T1500 group to 6 8.6 % for the control group ± 60 min of total ammonia detected over time. For the sampling undertake in November, 2004, the time at Y max for the control, T800 and T1500 groups was 255 minutes after the cessation of feeding compared to 225 minutes for the T250 group. The area under each concentration peak of ammonia detected for each group of fish ranged from 54.1 to 80.7 % ± 60 min of total ammonia detected over time.

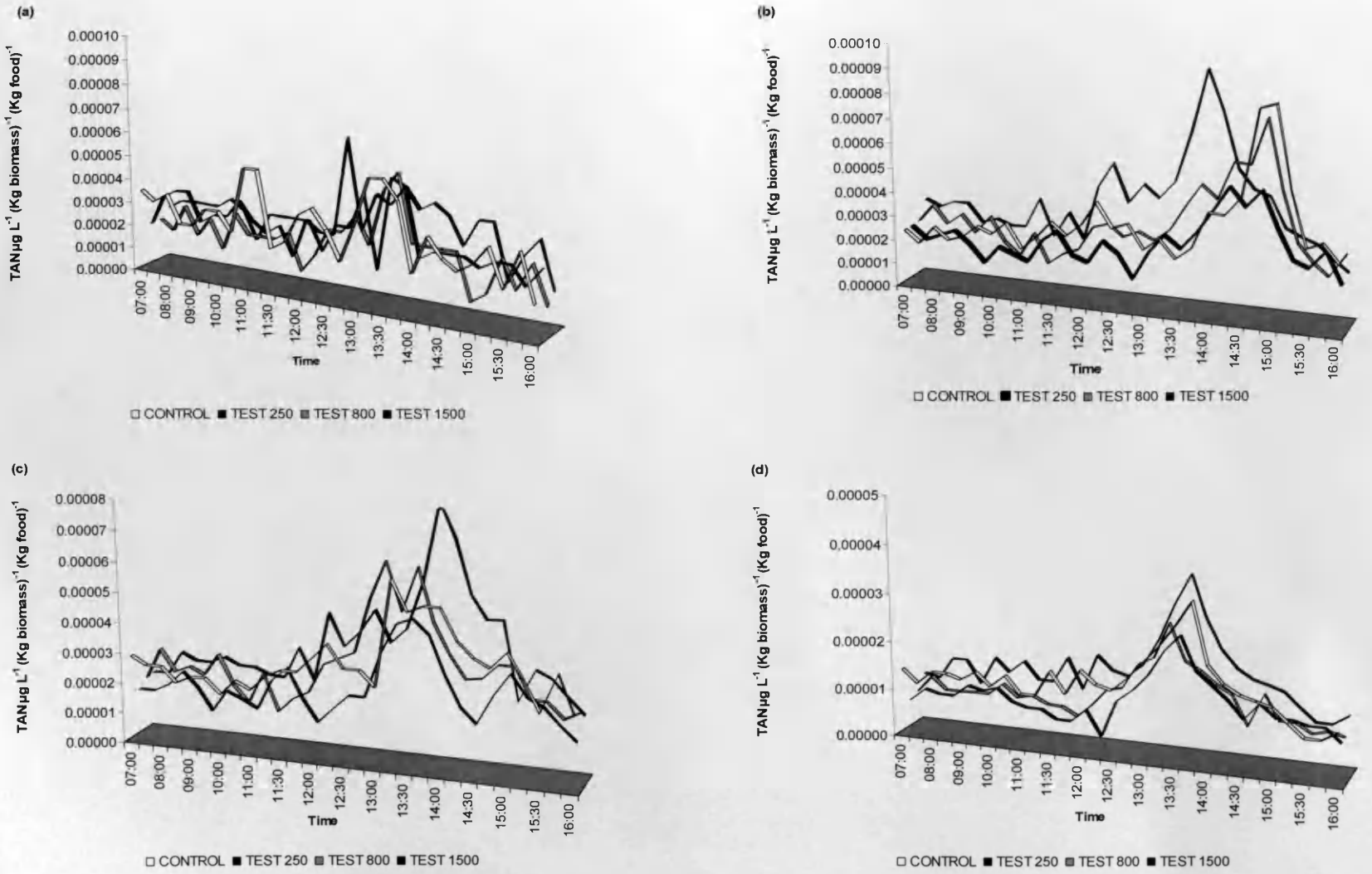


Figure 4.5 Ammonia excretion patterns detected from farmed Atlantic salmon µg L-1 (kg biomass)$^{-1}$(kg feed)$^{-1}$ on (a) 29/09/2003; (b) 31/10/2003; (c) 20/11/2003 and (d) 15/04/2004.

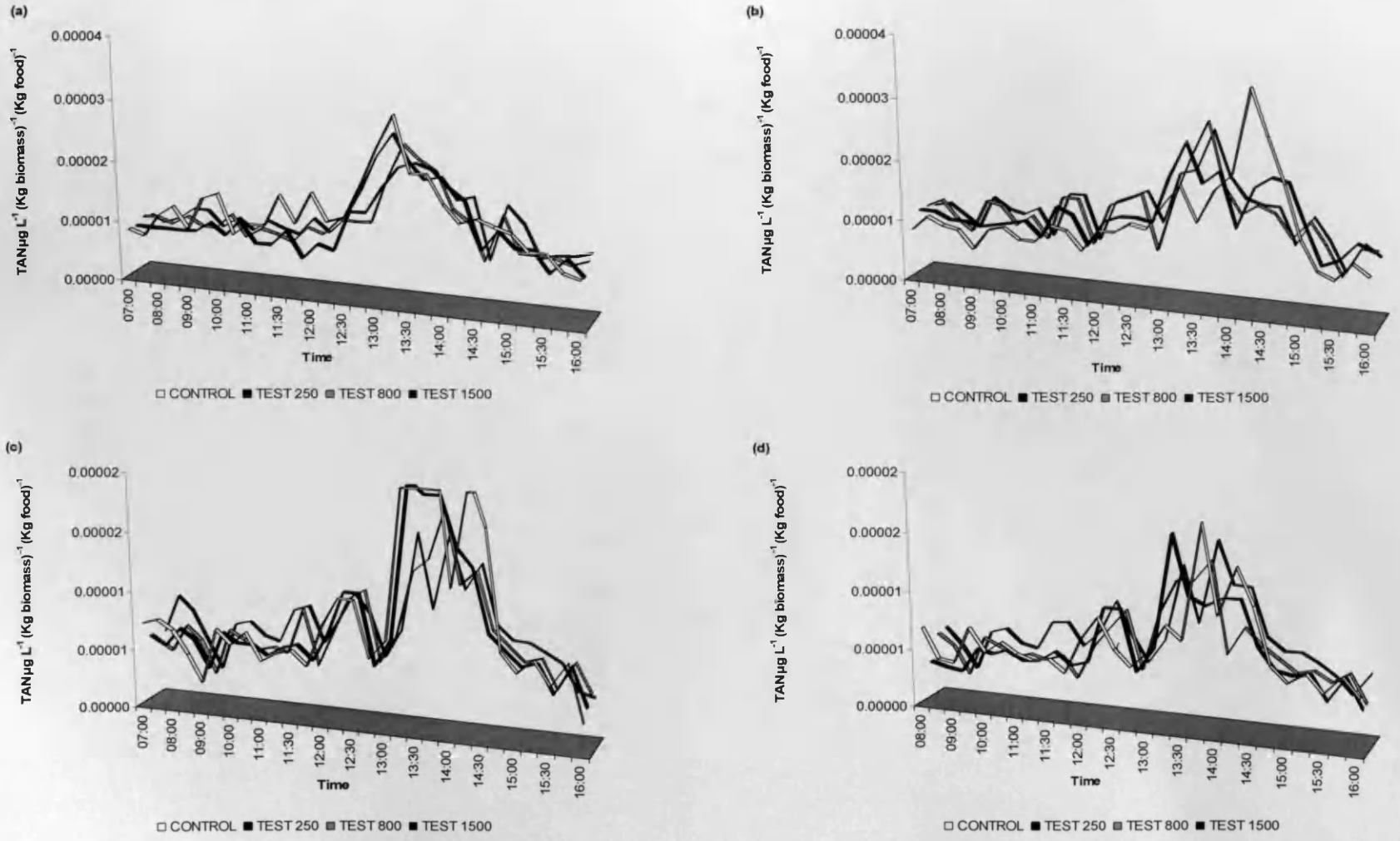


Figure 4.6 Ammonia excretion patterns detected from farmed Atlantic salmon μg L^{-1} (kg biomass)$^{-1}$(kg feed)$^{-1}$ on (a) 22/04/2004; (b) 07/07/2004; (c) 15/08/2004 and (d) 23/11/2004.

Table 4.6 Summary of magnitude and timing of ammonia concentration peaks detected on the different sampling dates.

Diet	Date	Ammonia concentration peak (Y max) $\mu g\ L^{-1}$ (kg biomass) (kg Food)$^{-1}$	Ammonia concentration peak $(\mu g\ L)^{-1}$	Area under curve $(\mu g\ L)^{-1}$	Time at Y max after cessation of feeding $(min)^{-1}$	Percentage area under peak of total ammonia detected over time ± 60 min
Control	29/09/2003	0.00005	42.47	0.0161	225	31.7
	31/10/2003	0.00009	119.29	0.0178	300	69.9
	20/11/2003	0.00006	82.55	0.0165	225	61.8
	15/04/2004	0.00003	125.97	0.0072	240	72.9
	22/04/2004	0.00003	115.95	0.0074	255	64.9
	07/07/2004	0.00004	206.13	0.0069	255	72.4
	15/08/2004	0.00002	182.75	0.0051	255	68.6
	23/11/2004	0.00002	172.73	0.0038	255	63.2
T250	29/09/2003	0.00007	55.83	0.0165	195	56.3
	31/10/2003	0.00005	72.53	0.0131	255	73.3
	20/11/2003	0.00005	65.85	0.0123	240	65.9
	15/04/2004	0.00003	99.25	0.0062	240	67.7
	22/04/2004	0.00002	89.23	0.0063	270	60.3
	07/07/2004	0.00003	149.35	0.0066	195	62.1
	15/08/2004	0.00002	172.73	0.0055	195	65.5
	23/11/2004	0.00002	149.35	0.0026	225	80.7

Table 4.6 continued.

Diet	Date	Ammonia concentration peak (Y max) $\mu g\ L^{-1}$ (kg biomass) (kg Food)$^{-1}$	Ammonia concentration peak $(\mu g\ L)^{-1}$	Area under curve $(\mu g\ L)^{-1}$	Time at Y max after cessation of feeding $(min)^{-1}$	Percentage area under peak of total ammonia detected over time ± 60 min
T800	29/09/2003	0.00005	45.81	0.0119	240	68.1
	31/10/2003	0.00008	105.93	0.0161	285	71.7
	20/11/2003	0.00006	85.89	0.0149	240	73.8
	15/04/2004	0.00003	102.59	0.0065	225	69.2
	22/04/2004	0.00002	89.23	0.0063	270	60.3
	07/07/2004	0.00003	162.71	0.0068	210	66.2
	15/08/2004	0.00002	152.62	0.0046	225	58.7
	23/11/2004	0.00001	142.67	0.0025	255	-
T1500	29/09/2003	0.00005	42.42	0.0159	225	55.7
	31/10/2003	0.00009	125.97	0.0197	225	74.6
	20/11/2003	0.00008	109.27	0.0173	255	76.3
	15/04/2004	0.00004	132.65	0.0084	240	69.6
	22/04/2004	0.00003	95.91	0.0069	240	68.1
	07/07/2004	0.00003	152.69	0.0069	210	69.6
	15/08/2004	0.00002	132.65	0.0052	225	57.7
	23/11/2004	0.00001	140.50	0.0037	255	54.1

4.4 Discussion

Quantitative assessment of nitrogenous compounds released into the aquatic environment is extremely difficult especially when considering intensive culture conditions. The flow of water through a fish cage whilst delivering oxygen to the cultured animals will also help to dilute the ammonia excreted by the fish thus avoiding build-up of toxic levels in most cases. The data obtained from the ammonia analysis is difficult to assess quantifiably as the data was obtained from open cages which were subject to water flow. In addition, any feed related concentration peaks of ammonia detected are subjected to dilution immediately after excretion by fish and duplicate samples may show one with a feed related concentration peak of ammonia evident and one without a concentration peak evident. However, given that for each diet, differences in biomass and feed intake were taken into account on each sampling date, several conclusions can be made.

For fish fed the diets assessed in the first study, there was an increase in ammonia excretion after a meal for fish fed each diet type. For fish fed the control, LP and SUS diets, the increase in ammonia was detected approximately 4 hours after feeding and for fish fed the ND diet, an increase in ammonia was detected on average, 3 hours after feeding. These results are in agreement with previous studies on salmonids were it has been shown that the peak ammonia excretion occurred within 3-5 hours after a meal and that the amplitude and time of appearance of concentration peak rates are dependant on fish size, amount of N intake and water temperature (Rychly and Marina, 1977).

High energy (nutrient dense) diets such as the ND diet used in this study have been widely used in salmon and trout farming, to improve protein retention and reduced nitrogen excretion (Cho *et al.* 1994) due to high levels of non-protein energy (fats or digestible carbohydrate). However, for fish fed the ND diet, the peak ammonia excretion consistently occurred earlier compared to fish the other diet types and the ammonia concentration peaks were highest in fish fed this diet. Fish fed the LP diet had the lowest concentration peaks of ammonia with fish fed the SUS and control diets has similar ammonia concentration peak outputs.

It may well be that the ND diet used in this study had higher inclusion levels of fish meal (48.2%) than required for optimal growth and this resulted in an increase in feed-related concentration peaks of ammonia which were the result of excess protein degradation. In a study on the use of high energy diets, Cho *et al*, 2001 showed that feeding high energy diets with a lower dietary fish meal inclusion of 35 % to salmonids did indeed result in a reduction of nitrogenous dissolved wastes compared to fish fed a standard commercial ration. The strategy of increasing the fat content to spare protein for growth along with a reduction in the amount of dietary protein required for optimal growth results in lower DP:DE ratios. One of the most beneficial effects of a reduction in the DP:DE ratios is the reduction in total N loss, due to optimisation of protein utilisation (Kaushik and Oliva-Teles, 1985). Fish fed the ND diet with a higher DP:DE ratio of 12.7 compared to the LP diet with a DP:DE ratio of 11.9 had higher concentration peaks of ammonia detected. However, both the SUS and control diets had higher DP:DE ratios (13.9 and 13.6) compared to the ND diet but this did not result in fish being fed these diets discharging greater

concentration peaks of ammonia compared to fish fed the ND diet. This may be explained by the fact that improvement in protein utilisation due to a higher supply of DE is less marked when diets are rich in protein (Cho *et al.* 1991).

For all four diets, approximately 68 % of the total ammonia detected over time was excreted as a food-related concentration peak when the area under each curve was calculated. Thus over a short time period of only two hours, a large amount of the total ammonia was excreted by feeding fish. This may have the potential to impact on the marine environment. The input of soluble nitrogenous waste is known to cause hypernutrification (Ryther and Dunstan, 1970) and several authors have considered the possibility that the discharge of soluble nitrogenous wastes from fish farms could have the same effects (Gowen and Bradbury, 1987; Ackefors and Enell, 1990; Gowen and Ezzi, 1992; Ross *et al.* 1994).

For fish fed the diets assessed in the second study, again there was an increase in ammonia excretion detected after a meal for fish fed each diet type. The increase was detected on average between 3 h and 4 h 30 min after cessation of feeding for all diets throughout the trail period which is again in agreement with Rychly and Marina (1977). There were no clear trends for the peak ammonia excretion to occur earlier for any particular diet fed to fish as seen with fish fed the ND diet from the first study. There were however, clear trends in the magnitude of the response with ammonia concentration peaks being between 17 and 42 % lower than fish fed the control diet. For all four diets, approximately 65 % of the total ammonia detected over time was

excreted as a food-related concentration peak when the area under each curve was calculated. These results are similar to results obtained for the first study.

Clearly, the ammonia excretion patterns detected suggest that fish fed low protein diets excrete less ammonia as a feed related concentration peak. This has obvious environmental benefits as concern exits over the effect of excretion of nitrogenous compounds on marine pelagic ecosystems.

The compositions of the feed changes as fish grow. Smolt diets generally contain higher inclusion levels of protein compared to diets for larger fish. However, the fish assessed in the second study were introduced to low protein diets throughout the growth period to assess the optimal time for introduction to low protein feeds without compromising on growth and performance.
Introducing fish to low protein diets from approximately 300 g onwards does not necessarily result in significantly poorer growth compared to commercial grower diets used in the industry at present (as discussed in chapter 3). The FCR values obtained in that study ranged from 1.04 for fish fed the control diet, T250 and T1500 diets to 1.06 for fish fed the C800 diet. Cleary there is potential to maximise use of low protein diets, which are clearly more sustainable in terms of the amounts of marine fishmeal that would be required to achieve reasonable performance. This would represent a significant economic saving as well as reducing reliance on fishmeal.

4. 5 - Conclusions

Prior to setting goals for reducing waste outputs, there is a need for objective estimates of the amounts of waste produced. It has been shown in these

studies that assessment of the amount of nitrogenous compounds released into the aquatic environment is extremely difficult to quantify when considering intensive fish culture conditions. However, the present study has shown that a large portion of the daily ammonia excretion occurred during a two hour period from a single meal only. Given that intensively farmed salmon are generally fed more than one meal per day, large concentration peaks of ammonia will be repeatedly excreted over relatively short time periods. Given that there is growing concern that nitrogenous compounds excreted from intensively farmed fish may be the "trigger" for phytoplankton blooms, there is a need to determine whether these concentration peaks of ammonia have the potential to impact on the marine environment. In chapter 6, a mesocosm study was undertaken to assess this.

The present study has shown that there is potential for aquaculture to be more environmentally sustainable by reducing the present pressure on the requirement for marine fishmeal and oil used in diets fed to intensively farmed Atlantic salmon without affecting growth and performance. Fish fed low protein diets were shown to have different ammonia excretion patterns compared to fish fed a normal commercial ration. Fish fed the lower protein diets appeared to have lower feed related concentration peaks of ammonia throughout the marine phase of the production cycle. As a result, the hypothesis "H_0: Feeding groups of Atlantic salmon either different diet types or introducing different groups of fish to low protein diets as they grew did not result in differences in the rate of or the patterns of ammonia excretion" may be rejected.

Chapter 5 – Assessment of the sustainability of different diet types fed to farmed Atlantic salmon (*Salmo salar* L.).

5.1 – Introduction

Food conversion ratios (FCR) are continuously improving through the understanding of the dietary requirements of the cultured species. Feed wastage has been reduced through the use of advanced pellet monitoring systems such as Storvic™ systems (Storvik AS, Norway) and feedback loop systems such as AQI systems (AQ1 Systems Ltd. Australia) due to economic and to a lesser extent, environmental pressures placed on the industry. This is particularly the case in the Scottish farmed salmon industry, where FCR values of less than one have been obtained under commercial conditions due to advances in husbandry practices and the optimisation of the protein:energy ratios. Despite these improvements, it still requires between 3 and 5 kg wild fish to produce 1 kg of fish-meal fed cultured fish (Naylor *et al.* 2000; Tidwell and Allan, 2001). This has lead some authors to suggest that aquaculture can be seen as a net consumer of fish (Naylor *et al.* 1998; Naylor *et al.* 2000), whilst others, using the same data, suggest that farming salmon, for example, represents a significant ecological advantage based on classic energy flows. (Forster, 1999) states that 10 kg of forage fish are required to produce 1 kg of a carnivore in capture fisheries, such as a wild salmon. If by-catch values are taken in to account this increases to 15 kg of forage fish for 1 kg of caught wild salmon.

Fishmeal supplies the largest part of dietary protein for salmonid culture and as the industry continues to expand so does the need for high quality protein sources (Hardy, 1996). Fishmeal is an increasingly expensive component of commercial salmon feeds and as demand for the world fisheries catch

increases, higher global demand for fish landings may increase the price of fishmeal which was recently demonstrated during the decline in catches due to El Niño (Sargent and Tacon, 1999; Vielma *et al.* 2000). There have been numerous investigations to find alternative protein sources, and in particular the use of vegetable protein such as soybean and gluten. Soybean is now widely used to partially replace some fishmeal in salmonid feeds (Carter, 2000). Other plant proteins offer potential but lower feed costs may not reduce production costs if growth is reduced and feed conversion ratio impaired due to anti-nutritional factors, unfavourable amino acid profiles and low protein content (Opstveldt *et al.* 2003).

Much of the fish oil used in the aquaculture industry comes from wild-caught pelagic fisheries which are fully fished or overexploited and alternatives to its use in the diets of farmed fish should be found (Sargent and Tacon, 1999). Marine fish oils in "oily fish", such as Atlantic salmon, contain considerable amounts of polyunsaturated fatty acids (PUFAs), which have considerable human health benefits (De Deckere *et al.* 1998). In farmed fish these are provided in the diet by fish oils from capture fisheries. Any substitute for this fish oil in the diets of farmed salmon must ensure that the fish produced continue to give these same health benefits.

A number of recent studies have shown that vegetable oil inclusion in diets for salmon do not result in reduced growth performance, feed conversion or development of histopathology in the fish (Bell *et al.* 2001; Bell *et al.* 2003). Studies have shown that salmon can grow normally on diets containing high levels of vegetable oils and that they are able to convert 18:3n-3 and 18:2n-6 to

their longer chain, highly unsaturated fatty acid (HUFA) products including 20:5n-3 and 20:4n-6 (Tocher *et al.* 2000). Fish feed substituted with plant oils have already been used commercially in Norway but the main issue affecting the plant oil option is consumer opinion and the affect that this may have on the final product which is regarded as high quality. To produce a cultured fish that has a nutritional quality as near to wild fish as possible has lead some researchers to focus on the dilution of vegetable oils in the fish flesh for a period of time before harvest when the fish are fed diets containing 100% marine fish oils. Studies have shown that by feeding salmon 100% fish oil diets for 12 weeks that have been previously fed on diets containing high levels of vegetable oils restores the n-3 highly unsaturated fatty acids (HUFA), 20:5n-3 and 22:6n-3 to levels comparable to fish fed normal diets (Bell *et al.* 2003).

The aim of this study was to determine the amounts of marine fish meal and fish oil required to produce 1 Kg intensively farmed Atlantic salmon fed different diet types based on inclusion levels of fish meal and fish oil in each of the diets and on growth performance. In the first study, the amount of fish meal and fish oil required was calculated for each of four different diet types fed to large Atlantic salmon over a period of 139 days. The four diets were a nutrient dense diet, a low protein diet, a vegetable oil and terrestrial protein substitution diet and a standard commercial diet. In the second study, the amounts of fish meal and fish oil required was calculated from fish introduced to low protein diets at different sizes throughout a full marine phase of production.

The main hypothesis that was tested was:

H_O: Feeding groups of Atlantic salmon either different diet types or introducing different groups of fish to low protein diets as they grew did not result in differences in the amounts of marine fish meal and/or fish oil required

5.2 – Materials and methods

A simple spreadsheet model was developed to allow for the determination of the amount of wild pelagic fish that would be required to produce 1 Kg farmed Atlantic salmon. The model integrates information on the amounts of marine fish meal and fish oil incorporated into the diets assessed in chapters 2 and 3 and given the known inclusion levels of fish meal and fish oil in feeds used and the FCR values obtained during both studies, it was possible to estimate how much wild fish would be required to produce 1 kg farmed salmon for each feed type. The calculations were based on both the wild fish required to produce the fishmeal or to produce the fish oil in the feeds.

In the first study, the diets described in chapter 2 were assessed. Triplicate groups of Atlantic salmon were fed diets containing different inclusion levels and sources of dietary protein and oils. The diets fed were a high energy, nutrient dense (ND) diet, a low protein (LP) diet, a diet with partial replacement of fish meal with corn gluten and fish oil with rapeseed oil (SUS). The last diet used was a control diet which has similar dietary levels of fishmeal and fish oil as normal commercial grower diet used in the industry at present.

In the second study, the diets described in chapter 3 were assessed. From smolt input until approximately 330 g weight, all groups were fed the BioOptimal Classic diets (C50 and C100 - Control feed). From this size a low-protein diet

(Test diet C250) was introduced to three cages of fish while the remaining nine cages continued on the standard product (C250 control). At approximately 1000 g live weights another three cages of fish were transferred to the Test diet, while fish in the remaining six cages continue on the control diet (C800). The last three cages were given low-protein feed from 1600 g onwards, while fish in the control group continued on a standard product (C1500-C2000) until harvest. The feeds were produced by BioMar AS, Norway

For both studies, Feed conversion ratio (FCR) was calculated on a dry weight basis using the formula described in chapter 2. The amount of fish meal incorporated into each diet was calculated using the equation:

[(FCR*1000) / 100 (% fish meal in diet)]

Similarly, the amount of fish oil incorporated into each diet was calculated using the equation:

[(FCR*1000) / 100 (% fish oil in diet)]

It has been estimated that feed fish produce approximately 22% fishmeal and between 6 and 8% fish oil of the total raw body weight (FAO, 2002b). Thus from 1000 Kg wild pelagic fish, 220Kg of fish meal and between 60 and 80 Kg of fish oil is produced. Using this and the amount of fish meal and fish oil incorporated into each diet, it was possible to calculate the amount of feed fish required to produce 1 Kg farmed salmon by dividing the amount of fish meal / fish oil in each diet by the amount of fish meal produced from 1000 Kg pelagic fish. The amount of fish oil produced was based on the assumption that 8 % fish oil will be produced from total raw body weight.

5.3 – Results

For the diets assessed in the first study, for fish meal: the SUS diet had an inclusion level of 20% fish meal and an overall FCR of 1.00 (Table 5.1), using the values cited approximately 1 kg of wild fish would be required to produce 1 kg farmed salmon. For the ND, LP and control feeds, with fish meal inclusions of 43%, 20% and 28% and FCR of 0.97, 1.09 and 1.09 respectively, approximately 1.9, 1.0 and 1.4 kg of wild fish would be required to produce 1 kg farmed salmon (Figure 5.1).

For fish oil (assuming 8% fish oil is obtained from raw body weight – (FAO, 2002a): The ND, LP and control feeds, which had fish oil inclusions of 39.2%, 36% and 33.1% respectively (Table 5.1), would require between 4.5 to 5 kg of wild fish to produce 1 kg of farmed salmon. The SUS feed, with fish oil inclusion of 10%, would utilise approximately 1.3 kg of feed fish to produce 1 kg farmed salmon.

The data for fish meal and fish oil inclusion levels, calculated FCR and mean fish weights for the diets assessed in the second study from smolt input to harvest is summarised in Table 5.2. Based on this data, for fish meal, the amount of wild fish required to produce 1 kg farmed salmon for fish fed the C50 and C100 diets with fish meal inclusion levels of 55.9and 59.7% respectively, would be between 1.7 and 1.9 kg. For fish oil, with inclusion levels of 16.6 and 17.4 %, the amount required would be between 1.4 and 1.6 kg.

Table 5.1 Calculated FCR, mean fish weight and percentage fish meal and fish oil incorporated into each diet.

	Diet type			
	LP	SUS	ND	Control
FCR	1.09	1.00	0.97	1.09
Mean fish weight (g)	3738	3810	4020	3999
% Fish meal in diet	20.0	20.0	42.8	28.1
% Fish oil in diet	36.0	10.0	39.2	33.1

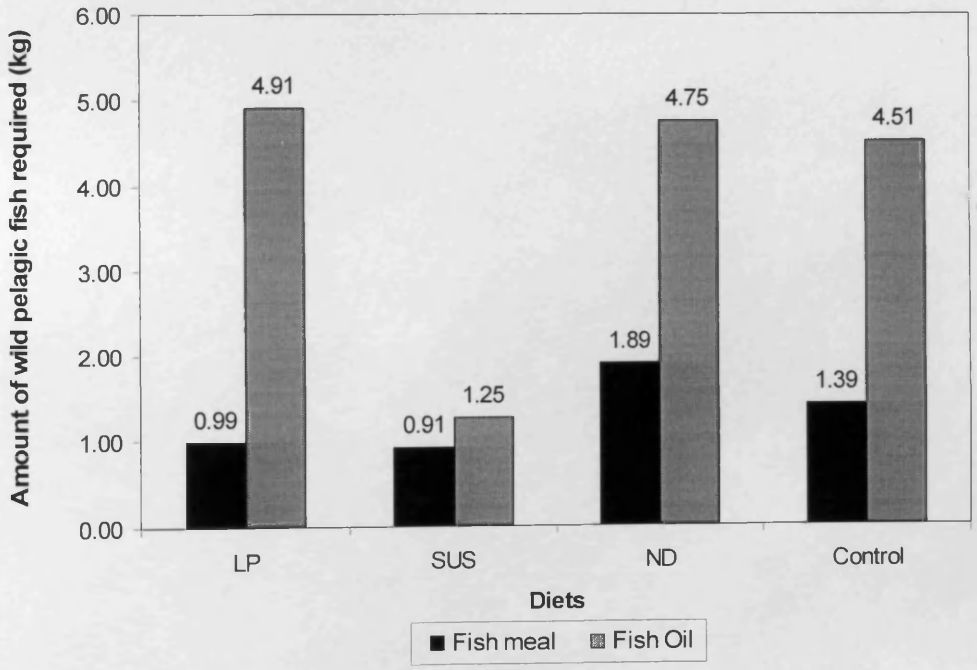


Figure 5.1 Amount of wild pelagic fish required (Kg) to produce 1 Kg farmed Atlantic salmon for each diet type. Numbers in graph refer to actual amount of wild fish required based on both dietary fish meal and fish oil inclusion levels for each diet type.

After the first diet switch which occurred on 03/11/2003, the amount of wild fish required to produce 1 kg farmed salmon for fish fed the control diet with fish meal and fish oil inclusions of 48.1 and 22.7 % respectively, would be 1.6 kg for fish meal used and 2.1 kg for fish oil used. In comparison, for fish fed the C250 diet with fish meal inclusion level of 34.5 % and fish oil inclusion level of 25.5 %, it would take 1.2 kg and 2.3 kg of wild fish to produce 1 kg farmed salmon based on fish meal and fish oil used in the diets along with calculated FCR for each group (Table 5.2).

After the second diet switch, fish meal and fish oil inclusion levels in the lower protein diets (C250 and C800 diets) were 32.6 and 31.1 % respectively, using these values, the amount of wild fish required to produce 1 kg farmed salmon would be 1.2 and 2.9 kg based on fish meal and fish oil used. In comparison, the control diet with a fish meal and fish oil inclusion levels of 36.4 and 27.7 % respectively, the amount of wild fish required would be 1.1 kg based on fish meal used and between 2.4 and 2.5 kg based on fish oil used (Table 5.2).

At the third diet switch, fish meal and fish oil inclusion levels in the lower protein diets (C250, C800 and C1500) were both 32.6 %, using these values and calculated FCR, the amount of wild fish required to produce 1 kg farmed salmon would be 1.3 and between 3.6 and 3.7 kg based on fish meal and fish oil used. In comparison, the control diet with fish meal and fish oil inclusion levels of 33.8 and 31.9 % respectively, the amount of wild fish required would be 1.4 and 3.7 kg for fish meal and fish oil used.

Prior to the end of the trial, fish meal and fish oil inclusion levels in the lower protein diets were 27.4 ands 34.5 % respectively. Using these values, the

amount of wild fish required to produce 1 kg farmed salmon would be 1.3 kg based on fish meal used and between 4.5 and 4.6 kg based on fish oil used. In comparison, the control diet had fish meal and fish oil inclusion levels of 30.2 and 33.3 %. Using these values, the amount of wild fish required to produce 1 kg farmed salmon would be 1.4 kg based on fish meal used and 4.3 kg based on fish oil used (Table 5.2).

At harvest, taking the mean values for fish meal and fish oil used throughout the trail period and calculated FCR for each group, the overall amount of wild fish required to produce 1 kg farmed salmon can be seen in Figure 5.2 . For fish meal: the control diet had an average inclusion level of 41.8 % and overall FCR of 1.0. Using these values, 1.98 kg of wild fish would be required to produce 1 kg farmed salmon. For the C250, C800 and C1500 diets, with fish meal inclusion of 38.2 % and FCR of 1.04, 1.06 and 1.04 respectively, 1.79, 1.82 and 1.78 kg of wild fish would be required to produce 1 kg farmed salmon.

For fish oil (assuming 8% fish oil is obtained from raw body weight – (FAO, 2002a): The T250, T800 and T1500 feeds, which had an average fish oil inclusion of 36.5 %, would require between 3.43 and 3.51 kg of wild fish to produce 1 kg of farmed salmon. The control feed, with fish oil inclusion of 25.4 %, would utilise approximately 3.31 kg of feed fish to produce 1 kg farmed salmon (Figure 5.2).

Table 5.2 Calculated FCR, mean fish weight and amount of fish meal and fish oil required to produce 1 Kg farmed Atlantic salmon throughout the marine phase of the production cycle.

	DIETS											
	C50				C100				C250			
Groups	**Cont.**	**T250**	**T800**	**T1500**	**Cont.**	**T250**	**T800**	**T1500**	**Cont.**	**T250**	**T800**	**T1500**
FCR	0.69	0.71	0.70	0.67	0.66	0.67	0.72	0.67	0.73	0.73	0.74	0.74
Mean fish weight (g)	74.4	74.4	74.4	74.4	191.9	193.6	193.1	193.0	316.0	432.0	413.7	435.3
% Fish meal in diet	55.9	55.9	55.9	55.9	59.7	59.7	59.7	59.7	48.1	34.5	48.1	48.1
% Difference in FM used between groups	-	-	-	-	-	-	-	-		< 28.3	-	-
% Fish oil in diet	16.6	16.6	16.6	16.6	17.4	17.4	17.4	17.4	22.7	25.5	22.7	22.7
% Difference in FO used between groups	-	-	-	-	-	-	-	-	-	> 11.0	-	-
Fish meal use	384.12	395.31	393.44	374.80	396.01	399.99	427.85	398.00	352.73	253.00	357.54	355.94
Fish Oil used	113.77	117.09	116.54	111.01	115.42	116.58	124.70	116.00	166.47	187.00	168.74	167.98
Pelagic fish required based on FM use (kg/kg)	**1.75**	**1.80**	**1.79**	**1.70**	**1.80**	**1.82**	**1.94**	**1.81**	**1.60**	**1.15**	**1.63**	**1.62**
Pelagic fish required based on FO use (kg/kg)	**1.42**	**1.46**	**1.46**	**1.39**	**1.44**	**1.46**	**1.56**	**1.45**	**2.08**	**2.34**	**2.11**	**2.10**

Table 5.2 Continued.

	DIETS											
	C800				C1500				C2000			
Groups	**Cont.**	**T250**	**T800**	**T1500**	**Cont.**	**T250**	**T800**	**T1500**	**Cont.**	**T250**	**T800**	**T1500**
FCR	0.73	0.73	0.74	0.74	0.92	0.90	0.90	0.88	1.04	1.04	1.06	1.04
Mean fish weight (g)	1018.7	1007.3	1024.3	987.0	1671.0	1727.3	1714.3	1667.3	3676.0	3475.7	3479.7	3630.7
% Fish meal in diet	36.4	32.6	32.6	36.4	33.8	32.6	32.6	32.6	30.2	27.4	27.4	27.4
% Difference in FM used between groups	-	< 10.4		-			< 3.6				< 9.3	
% Fish oil in diet	27.7	31.1	31.1	27.7	31.9	32.6	32.6	32.6	33.3	34.5	34.5	34.5
% Difference in FO used between groups		> 10.9					> 2.15				> 3.5	
Fish meal used	239.07	266.93	270.57	241.24	309.83	292.31	293.40	287.97	315.09	284.96	290.44	284.05
Fish Oil used	203.13	228.07	231.18	204.98	292.42	292.31	293.40	287.97	347.43	358.80	365.70	357.65
Pelagic fish required based on FM use (kg/kg)	**1.09**	**1.21**	**1.23**	**1.10**	**1.41**	**1.33**	**1.33**	**1.31**	**1.43**	**1.30**	**1.32**	**1.29**
Pelagic fish required based on FO use (kg/kg)	**2.54**	**2.85**	**2.89**	**2.56**	**3.66**	**3.65**	**3.67**	**3.60**	**4.34**	**4.49**	**4.57**	**4.47**

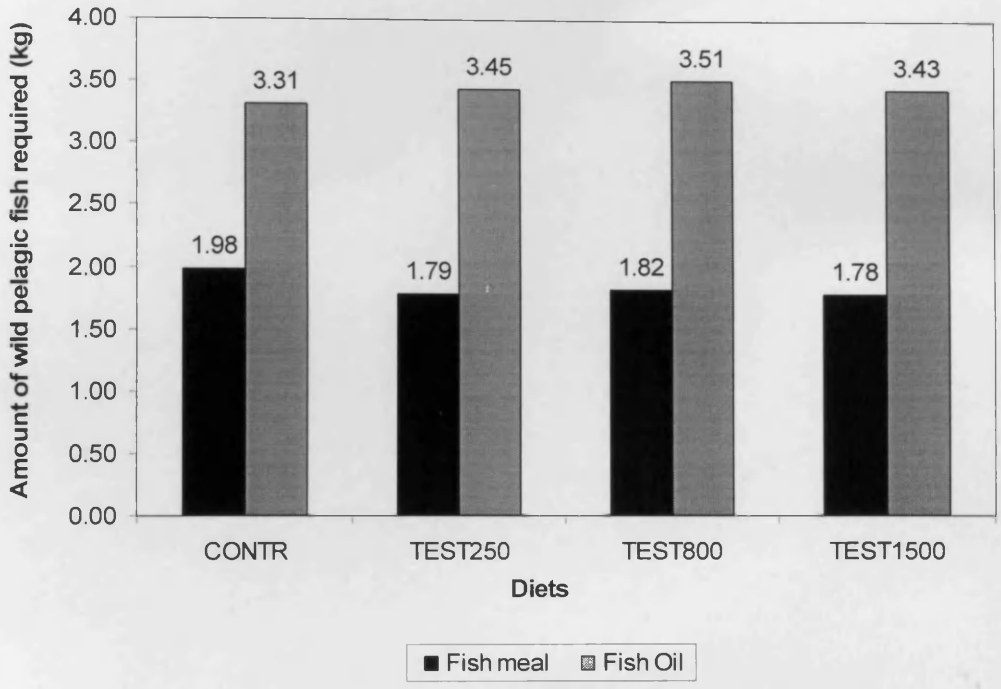


Figure 5.2 Amount of wild pelagic fish required (Kg) to produce 1 Kg farmed Atlantic salmon for each diet type. Numbers in graph refer to actual amount of wild fish required based on both dietary fish meal and fish oil inclusion levels for each diet type.

5.4 - Discussion

It is suggested that it takes 3 – 5 kg of pelagic fish to produce 1 kg of farmed salmon (Naylor *et al.* 1998). For the diets assessed in the first study, levels of conversion of wild to farmed fish for the ND and control feeds are similar to those quoted in the literature. However, the conversion value for the SUS feed is considerably lower than that quoted for both fishmeal and fish oil, with an overall conversion of 1.3:1 for the latter. The LP feed has a low conversion if using fishmeal (1:1) as an indicator of source but would still require levels of wild fish similar to those quoted to provide the fish oil needed to formulate this feed.

It is known that wild fish used to produce fish oil varies considerably in oil content both between species and with season (Barlow and Windsor, 1984), which will cause the conversion values given above to vary. For example, assuming 6 % fish oil is produced from total raw body weight rather than 8 % used in this study, the amount of wild fish required to produce 1 kg farmed salmon would increase to between 4.58 and 4.68 kg for fish fed the T250, T800 and T1500 low protein feeds assessed in the second study whilst the amount required for the control feed increased to 4.41 kg. Similarly, 6.01, 6.34 and 6.54 kg of wild fish would be required for fish fed either the control diet, nutrient dense diet or low protein diet respectively from the first study. However, even using 6 % for fish oil, the SUS diet utilises considerable less feed fish to produce 1 kg farmed salmon than values cited (1.67 kg). Clearly, such a diet is much more environmentally sustainable for industrial fisheries than diets used in the industry at present and has considerable potential, in terms of fish

growth, nutrition and food conversion ratio, to be used in the production of farmed salmon.

The low protein diets fed to fish (second study) resulted in levels of conversion of wild to farmed fish similar to those quoted in the literature with conversions of 1.8 to 1.9:1, if using fishmeal as an indicator of source and conversions of between 3.3 and 3.5:1 if using fish oil as indicator of source. The lower protein diets used during the trial did use less fish meal compared to fish fed the control diet but the reductions were not significant compared to the control diet. However, partial replacement of the fish meal used for the low protein diets with corn gluten as an additional protein source would reduce the conversions considerably. Replacing 30 % of the fish meal with corn gluten would result in conversion of wild fish to farmed fish of 1.03:1 for fish fed the lower protein diets using fish meal as an indicator of source. These conversions are similar to the conversion obtained for fish fed the SUS diet type assessed in the first study. It has been shown that corn gluten is a good alternative to fishmeal in Atlantic salmon diets. Even at high inclusion levels, fish fed diets containing corn gluten show good growth and feed efficiency (Anderson *et al.* 1992). Similarly, replacing fish oil with 40 % rapeseed oil in the lower protein diets would result in conversions of wild fish to farmed fish of 1.2:1 using fish oil as indicator of source. It has been shown in chapter 2 that replacing fish oil with rapeseed oil does not result in poorer growth compared to fish fed a normal commercial ration and a study by Bell *et al* (2003) has shown that rapeseed oil can be a successful substitute for fish oil in diets for Atlantic salmon with no adverse effects at levels below 50 % inclusion.

5. 5 - Conclusions

In terms of sustainability, it has been clearly shown that feeding fish modified diets containing partial replacement of fish meal and fish oil results in lower conversions of wild fish to farmed fish compared to conversions achieved with standard commercial rations used in the industry at present. In addition, using low protein diets does not result in significant differences in growth and performance and conversions achieved using low protein diets may be significantly lowered by partially replacing fish meal and fish oil with corn gluten and rapeseed oil.

Based on the results the hypothesis "H_O: Feeding groups of Atlantic salmon either different diet types or introducing different groups of fish to low protein diets as they grew did not result in differences in the amounts of marine fish meal and/or fish oil required" may also be rejected as there were clear differences in the amount of fish meal and fish oil used. The SUS diet required less fish meal and fish oil to produce 1 kg farmed salmon compared to either the LP, ND and control diets whilst the low protein feeds in the second study required less amounts of fish meal but more fish oil to produce 1 kg farmed salmon.

Chapter 6 – The potential effects of postprandial excretion rates of ammonia from farmed salmonids on plankton communities within marine enclosures.

6.1 Introduction

In northern Europe cultivation of Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorhynchus mykiss*) predominates and occurs mainly in the fjordic systems, such as sea lochs on the west coast of Scotland. These sea lochs have long been considered as pristine environments with exceptional water quality and little or no anthropogenic impacts (Gillibrand, 2001). As discussed in chapter 1(section 1.4) the release of nitrogenous compounds from intensively farmed fish has lead to considerable concern and speculation regarding the effects that these nutrients may have on receiving water bodies. As the industry continues to expand production and plans to intensively farm other species such as Atlantic cod and halibut in the near future, methods for assessing the potential for dissolved wastes to impact on the environment are required.

It has been estimated that approximately 30% of ingested nitrogen is excreted at the gills of rainbow trout as ammonia (Dosdat *et al.* 1996) and work by (Davies, 2000; Davies and Slaski, 2003) using a mass balance approach has estimated that the predicted total amount of dissolved waste over a full growth cycle for Atlantic salmon and halibut to be 35.6 and 47.8 kg N tonne fish produced respectively. However, even though the values cited above derived from laboratory and simulation work may be accurate, they do not take into account fluctuations in the daily excretion rates of ammonia as discussed in chapter 4. To this end it was decided to use mesocosms to assess the potential for the absolute levels of dissolved nitrogenous nutrients released from fish cages to impact on plankton communities.

Use of mesocosms to study marine and freshwater plankton environments has been increasing over last ten years (Watts and Bigg, 2001). They have been used to examine the effect of controlled changes to the environment such as pH (Chen and Durbin,1994), mesozooplankton (Levasseur *et al.* 1996), nutrients (Aksnes *et al.* 1994; Escaravage *et al.* 1999) and pollutants (Medina, 2002)

In situ marine mesocosms are essentially large bags enclosing plankton communities in natural waters and can bridge the gap between laboratory and natural conditions (Jacobsen *et al.* 1995), allowing a clearer extrapolation of results to environmental impacts, which is a failing of laboratory derived data (Calow, 1989). The enclosures are controlled in terms of water movement and nutrient composition, but follow the natural fluctuations of light and temperature thus making them suitable for phytoplankton investigation (Egge and Aksnes, 1992). Within mesocosms different levels of the planktonic community and food web can be investigated under conditions of excess nutrients and the enclosed treatment allows for observation of direct and indirect effects of added nutrients on plankton populations by monitoring changes in physical and biological parameters. For example, (Escaravage *et al.* 1999) showed that using land-based mesocosms filled with sea water from the Dutch coast, an excess of one nutrient activated by the limitation by another nutrient induced a shift in phytoplankton species dominance from diatom to flagellate with a shift from phosphorus to silicon limitation.

The potential for ammonia excreted from intensively farmed fish to impact on the marine environment has become a matter of concern as any substantial

and measurable increase in the concentration of dissolved nutrients may lead to an increase in phytoplankton growth and productivity if growth is limited by nutrients (Gowen and Bradbury, 1987).

The present study attempted to determine whether an ecological risk existed between discharges of dissolved ammonia from intensively farmed rainbow trout and Atlantic salmon and uptake of these excess nutrients by phytoplankton communities. The study attempted to determine whether phytoplankton community composition and densities would be affected by addition of excess nutrients within enclosed mesocosms. This involved two experiments. In the first study, the range of concentrations of dissolved nutrients added to the mesocosms was based on postprandial excretion rates of ammonia detected from a rainbow trout farm situated in Loch Etive (Reynolds, 2002). The upper values reflected possible postprandial ammonia excretion from larger cage farms whilst the lower values represent concentrations from smaller scale cage farms. In the second study, the range of concentrations of nutrients added was based on postprandial excretion rates detected from an Atlantic salmon farm situated in northern Norway (see chapter 4). The upper values reflected postprandial ammonia excretion from fish fed a nutrient dense diet whereas the lower concentrations of nutrients used reflected postprandial excretion rates from fish fed a low protein diet. The main hypotheses that were tested were:

H_o^1: Feed related concentration peaks of ammonia from farmed rainbow trout have no effect on marine phytoplankton community composition and densities.

H_o^2: Feed related concentration peaks of ammonia from farmed Atlantic salmon fed a nutrient dense diet or a low protein diet do not effect marine phytoplankton communities differently.

6.2 Materials and methods

6.2.1 – Mesocosms

The mesocosm enclosures were located at Ardchattan fish farms on Loch Etive operated by Aquascot Group Ltd. Experiments were conducted during May 2003 and May 2004.

The mesocosms described in Figure 6.1 employed changeable 125 µm thick polyethylene (500 gauge), which is biologically inert as well as having good mechanical strength. The mesocosms were built using two layers of polyethylene which was closed at one end to form a bag with a double layer. The bags were suspended from a triangular galvanised frame and attached to it with the aid of an adjustable collar made from connected jubilee clips.

A Perspex sheet cover was placed over the bag mouth to prevent rainwater from entering and also to exclude possible nutrient enrichment from bird excreta. They are designed to hold a portion of the natural water column, including its assemblage of biota and to hold it isolated from the environment (Medina *et al.* 2003). Six mesocosms were used in each study and each enclosure had a volume of 0.797 m^3. The mouth diameter was 0.58 m and the length 3 m (Table 6.1).

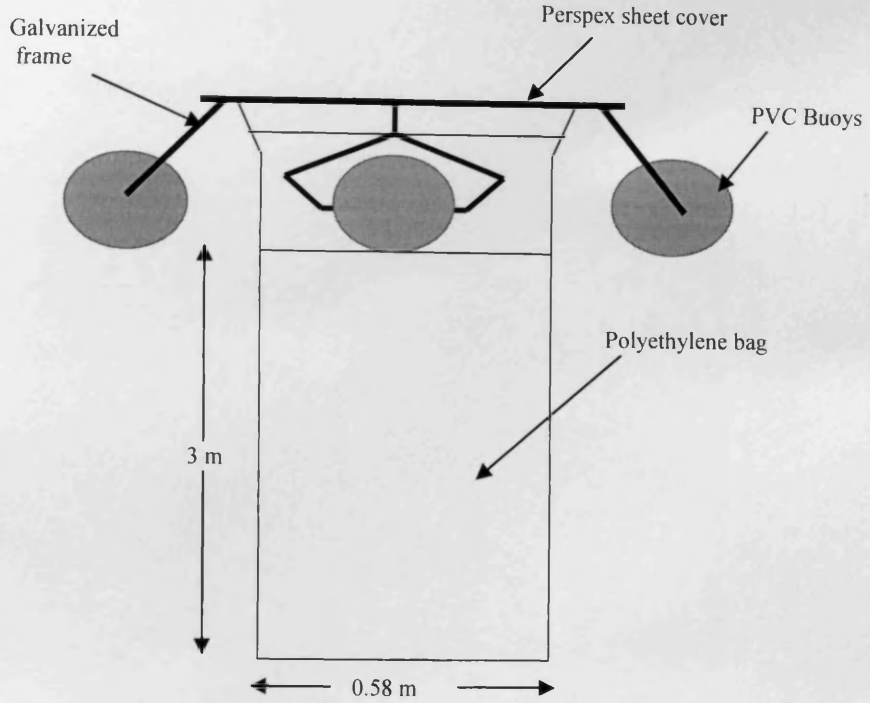


Figure 6.1 Diagram of the mesocosm used in this study.

Table 6.1 Mesocosm dimensions and characteristics (From Medina, 2002).

Characteristics	Dimensions
Bag mouth circumference	1.828 m
Bag mouth diameter	0.582 m
Bag mouth radius	0.290 m
Bag mouth area	0.266 m^2
Bag length (depth)	3.000 m
Bag volume	0.797 m^3 (797 L)
Bag material	Polyethylene
Frame size	1.36 m
Frame material	Galvanised steel
Buoy size	0.5 m diameter

The mesocosms were weighed down with the aid of large stones, with an approximate weight of 10 kg. Bags were filled with seawater taken from the middle of the loch at 6 m depth using a pump attached to a hose with a 1.5 mm (pore) mesh on the end to avoid inclusion of macroplankton (2 – 200 mm Cnidaria, Ctenophora, fish larvae and cephalopods) in order to avoid rapid depletion of mesoplankton by these larger predators (Kuiper, 1977). The mesocosms were roped together in two groups of three and anchored on the shore side of the farm in order to provide adequate shelter from natural perturbations such as wind and storms (Figure 6.2). For each experiment, the bags were filled in pairs. Each pair was taken to the centre of the loch and filled then they were immediately towed carefully back to their respective frames and attached to the frame with the jubilee clip collar. After attachment each enclosure was covered with a disc of Perspex sheeting. All six bags were filled within the hour from the same position in the Loch. Once bags were filled they were left for 5 days to allow any suspended material to settle and for the community to stabilise (Medina *et al.* 2003)

6.2.2 – Experiment 1

The first experiment assessed the effects of the addition of ammonia, based on the postprandial excretion rates of farmed rainbow trout (*Onchoryncus mykiss*). The concentrations used were based on data obtained from ammonia studies on a rainbow trout facility situated on loch Etive, Scotland. Data derived from a previous study at the site (Reynolds, 2002) showed that significant food-related concentration peaks of total dissolved ammoniacal nitrogen (TAN) occurred

several hours post-feeding. The largest concentration peak detected was 0.051 μg L^{-1} kg-biomass^{-1} following a feed input of 75.0 kg. Two smaller feeding peaks were detected following feed inputs of 66.7 kg and 33.3 kg respectively. Using this data, it was possible to predict how much ammoniacal nitrogen would be discharged as a food related concentration peak from a theoretical 500 tonne and 1000 tonne production facility. For 500 tonnes production, the expected food related concentration peak would be 25.5 mg L^{-1} (0.051 μg L^{-1} kg-biomass^{-1} x 500, 000 kg) and conversely, for 1000 tonnes production the expected food related concentration peak would be 51.0 mg L^{-1}. To simulate the effect of these concentration peaks on plankton communities and given the molecular weight of NH_4, two of the bags received 43.5 g NH_4Cl based on 500 T production (LN bags) and two bags received 69.0 g NH_4Cl based on 1000 T production (HN bags). Two bags as controls received no additional chemicals.

6.2.3 – Experiment 2

The second experiment followed the same protocols as for the first experiment but the addition of nutrient compounds was based on ammonia excretion patterns of Atlantic salmon (*Salmo salar*) fed either a nutrient dense (ND) diet or a low protein diet (LP) (chapter 4). With fish fed on the ND diet, the mean ammonia concentration peak detected over time was 0.041 μg L^{-1} kg-biomass^{-1}, whereas, the mean ammonia concentration peak detected over time for the LP diet was 0.030 μg L^{-1} kg-biomass^{-1}.

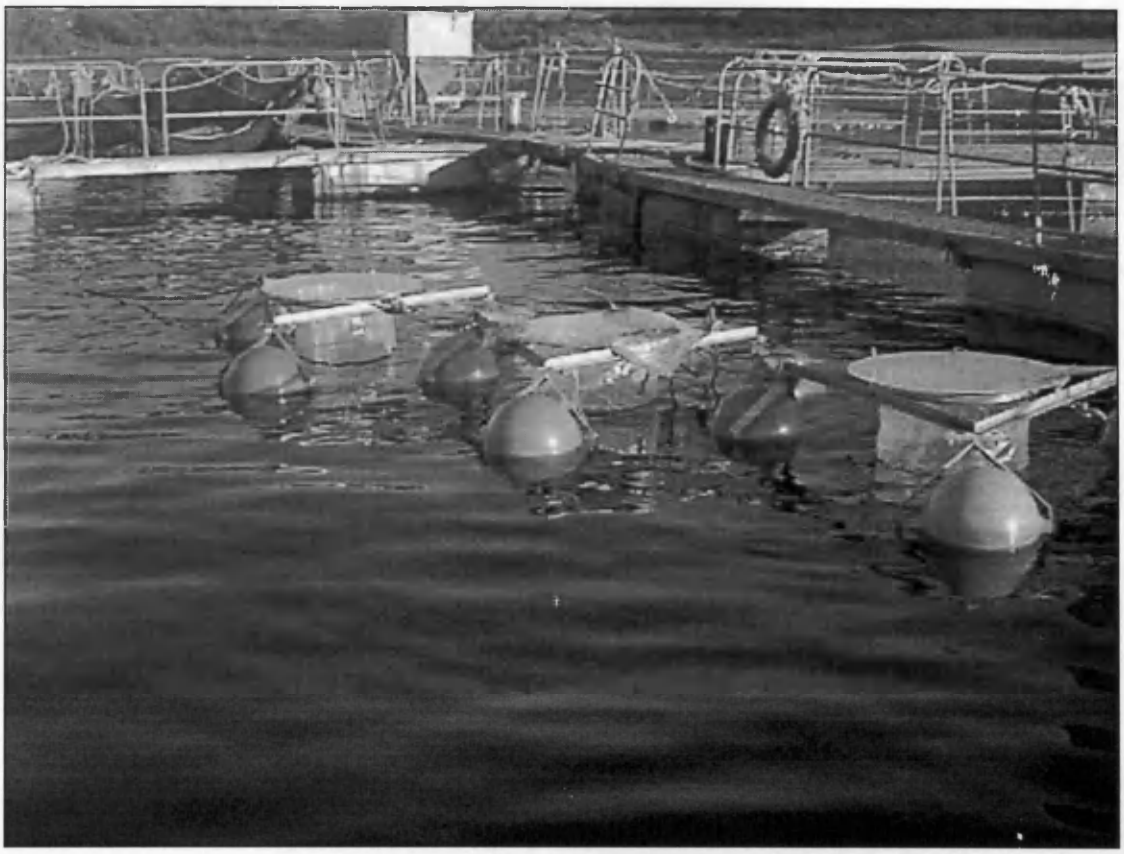


Figure 6.2 Mesocosms moored to Aquascot fish farm, loch Etive, Scotland

To simulate the effect of these concentration peaks on plankton communities and given the molecular weight of NH_4, two of the bags received 48.0 g NH_4Cl (LP bags) and two bags received 59.0 g NH_4Cl based on 1000 T production (ND bags). Two bags as controls received no additional nutrients.

6.2.4 - Sampling Procedures / end points

For each experiment, the bags were left for five days before the addition of nutrients. Sampling occurred on days 1, 5, 8, 11, 17 and 26 for Experiment 1 and days 1, 5, 8, 12, 16 and 21 for Experiment 2. Day 1 represented the day of nutrient addition to the LN and HN bags for the first experiment and the LP and ND bags for the second experiment. Water samples were taken on day 1 to allow investigations into the natural abundance of assemblages in the bags immediately prior to the addition of nutrients. End-Points that were measured can be seen in Table 6.2.

Table 6.2 End-points measured during each experiment.

Biological parameters	Water quality parameters
Phytoplankton group composition	Temperature (^{0} C)
Phytoplankton abundance (cells mL^{-1})	Salinity (psu)
Zooplankton group composition	pH
Zooplankton abundance (animals L^{-1})	Dissolved oxygen (mg L^{-1})
	Chlorophyll *a* (µg L^{-1})
	Ammonia (NH_3 and NH_4) (µg L^{-1})
	Nitrate, nitrite, phosphate (µg L^{-1})

6.2.5 – Sampling

Water samples were collected using a PVC pipe (3.9 cm diameter; 2.5 m long) that had a ball valve closing mechanism (Medina, 2002). For each sample, five integrated vertical sub-samples of the water column (2.5 m) were pooled together. Each of the five sub-samples was collected from random sites within the mesocosm to reduce possible effects of plankton patchiness (Stephenson *et al.* 1984). Water sampling commenced immediately prior to addition of NH_4Cl on day 1.

Pooled sub-samples had a final sample volume of 14.9 L^{-1}. From the pooled sample (14.9 L^{-1}) a 2 L^{-1} sub-sample was taken and from this sub-sample, 500 ml was filtered through Whatman GF/C filter paper and the filter paper was wrapped in foil and kept in deep-freeze for later chlorophyll *a* analysis. From the filtered water, 250 ml was retained in a polypropylene bottle and stored in a dark deep-freeze for analysis of dissolved inorganic nutrients at a later date. From the 2 L^{-1} sub-sample, 500 ml was also taken and fixed with 2 ml of Lugols iodine solution acidified with glacial acetic acid for phytoplankton species and abundance analysis. The remaining 12.9 L^{-1} pooled sample was filtered through 60 µm mesh and the filtered material (zooplankton) collected and fixed with 4% formaldehyde in seawater (10% formalin).

6.2.6- Sample analysis

The fixed phytoplankton samples (500 mL) were placed in measuring cylinders and left for 48h to allow sedimentation of the cells. After this time period, 450 mL of the supernatant was siphoned off leaving a concentrated sample of algal

cells in the remaining 50 mL. This 50 mL was transferred to a 50 mL measuring cylinder and the sample was shaken and again left again for 48h for sedimentation. After 48h, 40 mL of the supernatant was siphoned off and the remaining 5 mL was transferred to a samples flask until analysis. The species composition was first analysed under a microscope by taking one drop from the bottom of the flask after 1h sedimentation. Once species in the sample were identified, the concentration of cells was determined by counting the number of cells with a haemocytometer (NeuBauer Improved 1/400 mm^2). Here, five sub-samples were counted after shaking. The concentration of the algae in the mesocosm was calculated considering the volume of the haemocytometer and the initial and final volume of the sample left for sedimentation (500 mL to 5mL). In addition, phytoplankton composition was determined to genus level using the taxonomic keys proposed by Newell and Newell (1979) and Bellinger (1992) (Appendix 1).

The development of zooplankton samples was determined by counting and identifying each sample using an Olympus SZ 40 stereo-microscope (Olympus Microscopes Ltd, Japan). All individuals present in the samples were counted. Determination of different groups was performed using the taxonomic keys proposed by Newell and Newell (1979) and Todd *et* al (1991) at the lowest possible level (Appendix 2).

Chlorophyll *a* concentrations in the samples were determined from the Whatman GF/C filter papers using the method proposed by by Golterman and Clymo (1970). The filter papers were placed in test-tube filled with 13 ml of 90% acetone (technical-grade) and left for 24h. After 24h the test tubes were shaken

and centrifuged twice at 2,200 r.p.m on a Mistral 3000i centrifuge. Supernatants were decanted into a 4 cm path-length spectrophotometer cuvette and extinction was measured at 665 nm and 750 nm:

$$\text{Chl-a (ug/L)} = \frac{(E_{665} - E_{750}) \times A \times Va}{V \times L}$$

Where: E = absorbance at quoted wavelength

A = absorbance coefficient of Chl-a in acetone (11.9)

Va = volume of acetone used (ml)

V = volume of water filtered (L)

L = path length of cuvette (cm)

Temperature, dissolved oxygen and salinity readings were taken *in-situ* at the surface, 1, 2 and 3 metres depth from each bag on each sampling day. Temperature and dissolved oxygen was recorded with a WTW – OXI 197 probe and salinity recorded with a WTW LI-196 microprocessor conductivity meter. pH was recorded from the water samples in the laboratory using a PW9409 digital pH meter. Ammonia and nitrate were determined using an auto analyser (Bran + Lubbe Digital calorimeter Autoanalyser 3; Parc de Ste Apolline, France) and phosphate was determined using the method described by Strickland and Parsons (1972).

6.2.7 – Statistical analysis

Multivariate analysis of the data was undertaken using the multivariate statistical package (MVSP) version 3.13c (Kovach Computing Services, Anglesey). Biological and water quality data were integrated and relationships found using Canonical Correspondence Analysis (CCA).

Analysis of variance (ANOVA) was performed on biological data to determine significant differences among treatments using MINITAB (version 13.1). Normality and homogeneity of variance of all data sets was checked prior to parametric statistical analysis. Normality was checked by graphic examination of probability plots and the Anderson-Darling test. Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$).

6.3 – Results

6.3.1 – Experiment 1

6.3.1.1 – Water quality

Figure 6.3 depicts variations with time of water quality in each enclosure. Similar trends over time in physico-chemical parameters (D.O., salinity and temperature) were observed among treatments and controls as they showed the same trends in variation with time, except for pH which was lower in the control enclosures until d 8 and then followed similar patterns to both LN and HN enclosures. There was a constant decrease in dissolved oxygen until d 11 followed by an increase from d 12 to d 17 in all enclosures. This was followed by a slight decrease until the end of the experiment. Temperature increased

initially in the enclosures then decreased between d 8 and d 11. This was followed by an increase until the last sampling day. Salinity decreased with time in all enclosures apart from a small increase noted at d 11.

6.3.1.2 – Chemical parameters

Figure 6.4 shows changes in nutrient concentration with time. Total ammoniacal nitrogen (TAN) concentration measured before the addition of nutrients on day 1 showed that all enclosures had similar low values of TAN present. There was a clear increase in TAN concentration immediately after addition of nutrients to the LN and HN enclosures (2061.95 μg L^{-1} and 10,391.34 μg L^{-1} respectively) as well as a small increase in TAN in control enclosures. This was followed by a decrease in TAN in all enclosures from day 5 post-fertilisation until day 30 when concentrations in all treatments and controls were similar to those prior to the addition of nutrients to the enclosures. There was an increase in nitrate concentration in both LN and HN enclosures between day 1 and day 5, followed by a decrease in concentration until day 26 when values were similar to values obtained at the start of the experiment. Nitrate concentration in control enclosures were higher at the start of the experiment but after addition of nutrients were lower than both LN and HN enclosures until the end of the trial.

There were no clear trends in phosphate concentration with time in any of the enclosures.

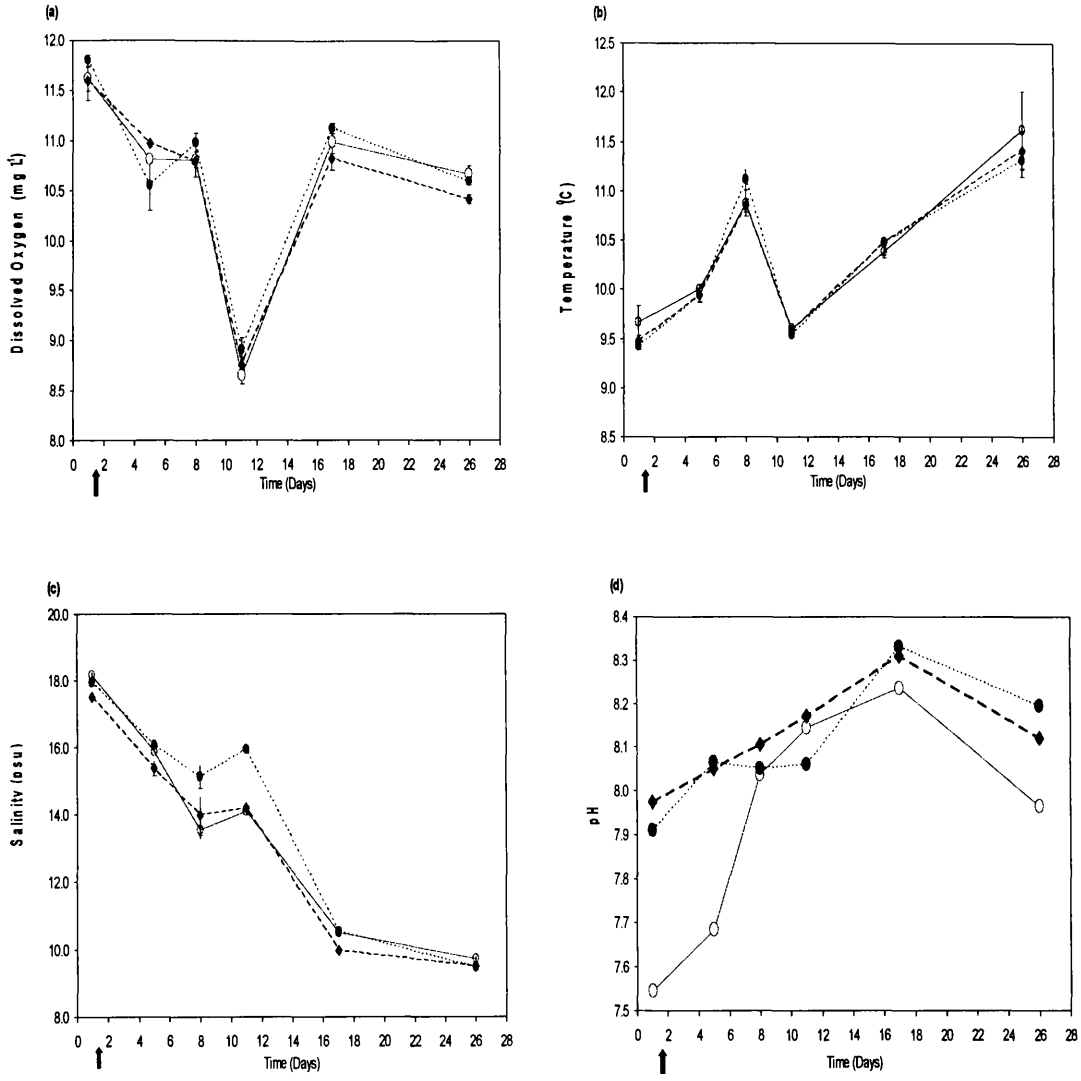


Figure 6.3 (a) Dissolved oxygen (mg L^{-1}), (b) Temperature (^{0} C), (c) salinity (g L^{-1}) and (d) pH readings obtained over the duration of the experiment. (○) Control, (◆) LN enclosures and (●) HN enclosures. Data are shown as mean values from each duplicate with 95% confidence intervals (n = 4). Arrows indicate time of nutrient addition (day 1).

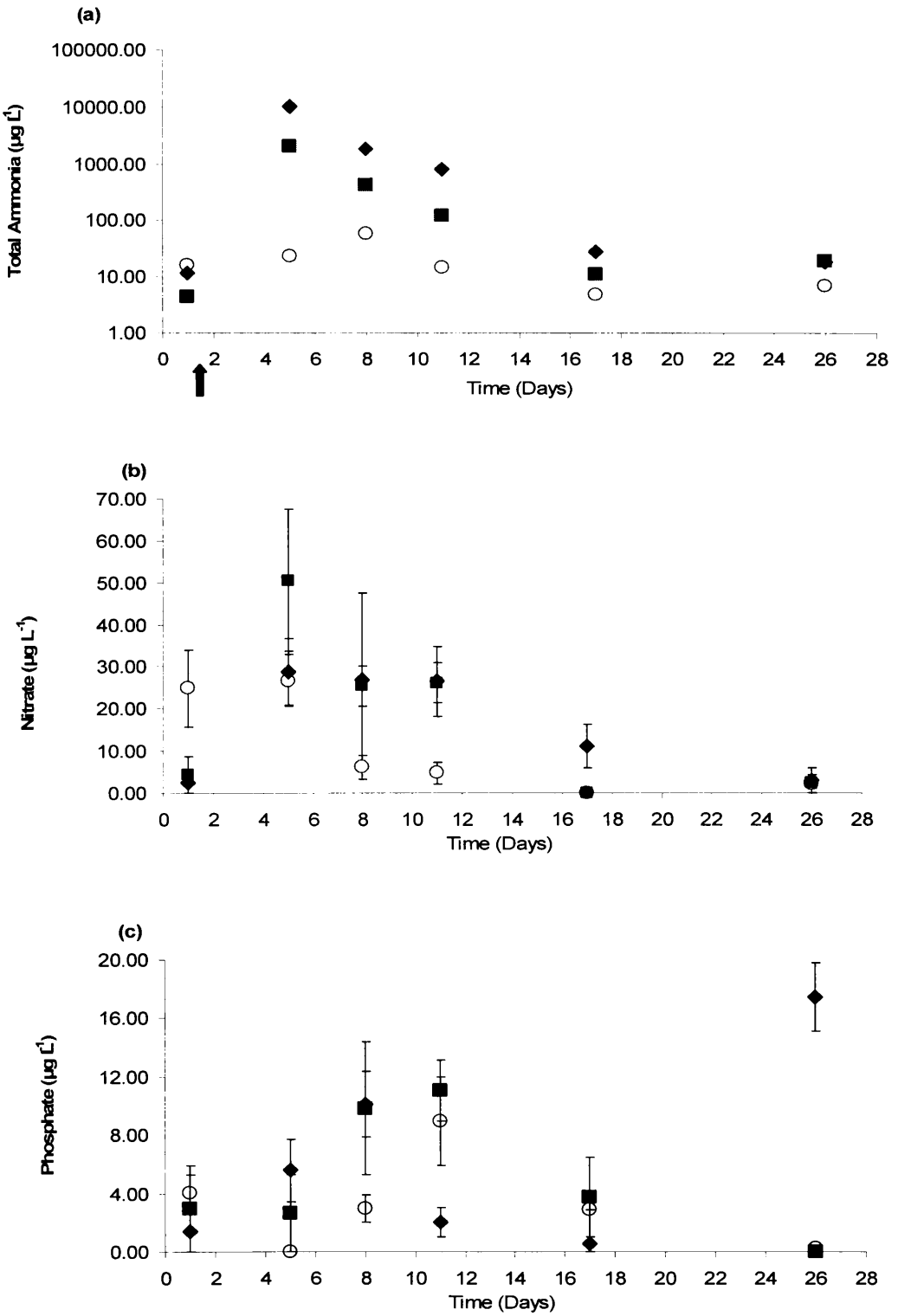


Figure 6. 4 Mean concentrations in (a) total ammonia (µg L-1) plotted on log scale; (b) Nitrate (µg L-1) and (c) Phosphate (µg L-1) detected during the course of the experiment. (○) Control; (■) LN and (◆) HN enclosures. Arrow indicates time of addition of nutrients to the enclosures (day 1). Data are shown as mean values from each duplicate with 95% confidence intervals.

6.3.1.3 - Biological Parameters

Chlorophyll *a* values, which are indicative of total plankton productivity, (Figure 6.5) showed a significant increase with time in all enclosures until day 11 ($F_{17.18}$ = 7.56; $p < 0.05$). There were no clear trends between chlorophyll *a* production and cell density with time. By day 17, chlorophyll *a* values in the HN enclosures were higher than both the LN and control enclosures which had lower values compared to day 11 values. At the end of the experiment (day 26 post-fertilsation), the HN enclosures still showed higher values compared to the other enclosures. However, there were no significant differences in chlorophyll *a* between treatments and controls during each sampling day ($F_{2.3} < 8.81$; $p > 0.05$).

Figure 6.6 shows total phytoplankton estimated in enclosures over the experiment. Although there was a similar pattern of increase of phytoplankton abundance in all enclosures, addition of nitrogen appeared to have affected an increase in cell density with time. There were significant increases in cell numbers with time in all enclosures ($F_{17.18}$ = 22.33; $p < 0.05$) until day 11 after which, there was a decline in cell numbers in treatments and controls until the end of the experiment. LN enclosures had more cells mL^{-1} than control enclosures and similarly HN enclosures had more cells mL^{-1} than either LN or control enclosures. These differences in cell numbers, however, did not occur until day 8 even though nutrients were added on day 1. Although there were more cells present in both LN and HN enclosures, there were no significant differences between enclosures on each sampling day ($F_{2.3} < 3.82$;$p > 0.05$).

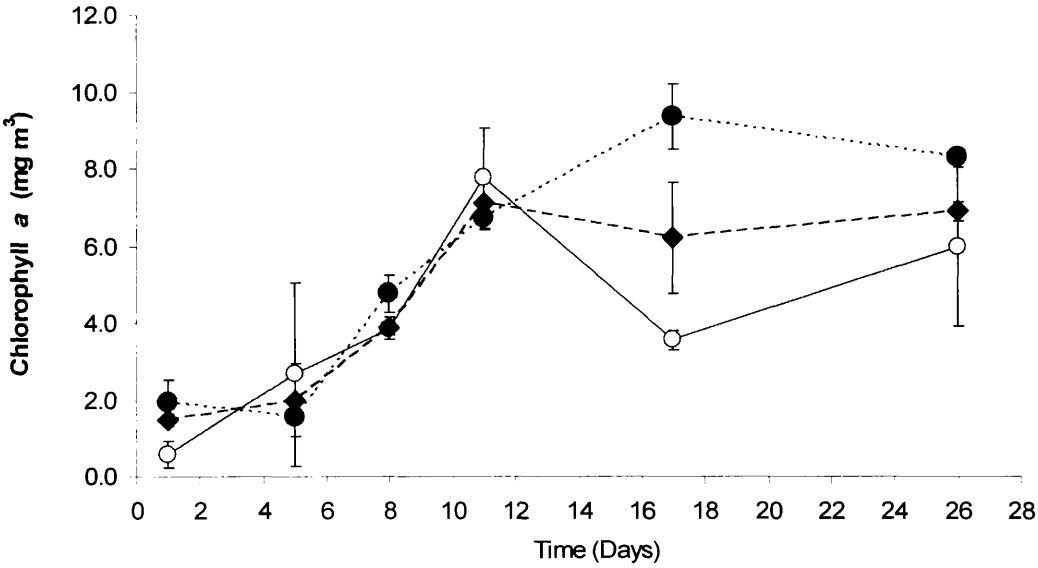


Figure 6.5 Changes in chlorophyll a concentration (mg m3) measured in the. (○) Control, (◆) LN enclosures and (●) HN enclosures. Data are shown as mean values from each duplicate treatment with 95% confidence intervals (n = 2).

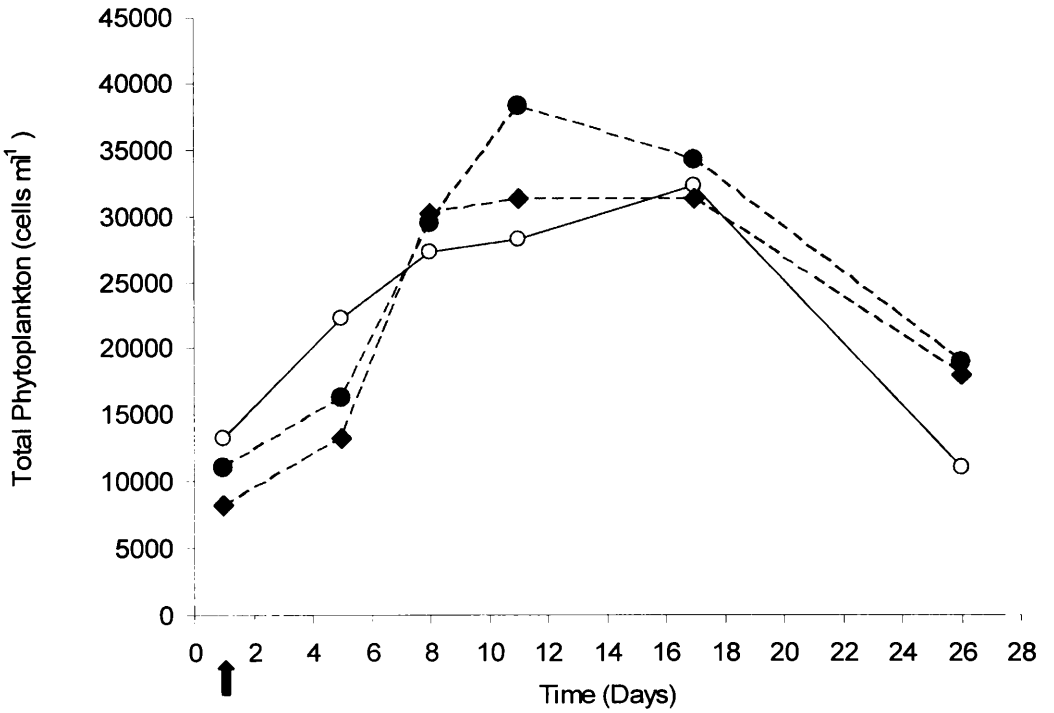


Figure 6.6 Total Phytoplankton estimated with time (cells mL-1). Control (○); LN (◆) and (●) HN enclosures. Arrow indicates time of nutrient addition (day 1).

Figure 6.7 shows the main groups of phytoplankton identified were the classes Bacillariophyceae (diatoms), Chrysophyceae, Chlorophyceae and Chryptophyceae (flagellates) and Phytomastogophora (dinoflagellates). Diatoms dominated throughout the duration of the experiment with eight families of the sub-classes Centricae dominating the diatom population. Flagellates were the next most dominant group identified in all enclosures with time followed by the.dinoflagellates.

Figure 6.8 shows total zooplankton in the enclosures over the duration of the experiment. There was a significant increase in zooplankton with time in the LN enclosures until day 8 and the control and HN enclosures until day 11. This was followed by a decline in the number of zooplankton detected until the end of the experiment ($F_{5.28}$ 29.60; $p < 0.05$). By day 5 there were significantly more animals recorded in HN enclosures than in the control enclosures ($F_{2.3}$ 18.72; $p < 0.05$) but on all other sampling days there was no significant difference in numbers between treatments ($F_{2.3} < 8.14$; $p > 0.05$). Representatives of phyla were recorded during zooplankton analysis with Arthropoda dominating throughout the experiment (see Figure 6.9). Within this phylum, calanoids predominated in all enclosures throughout the experiment with 5 families identified. Harpacticoids were the next most numerous order.

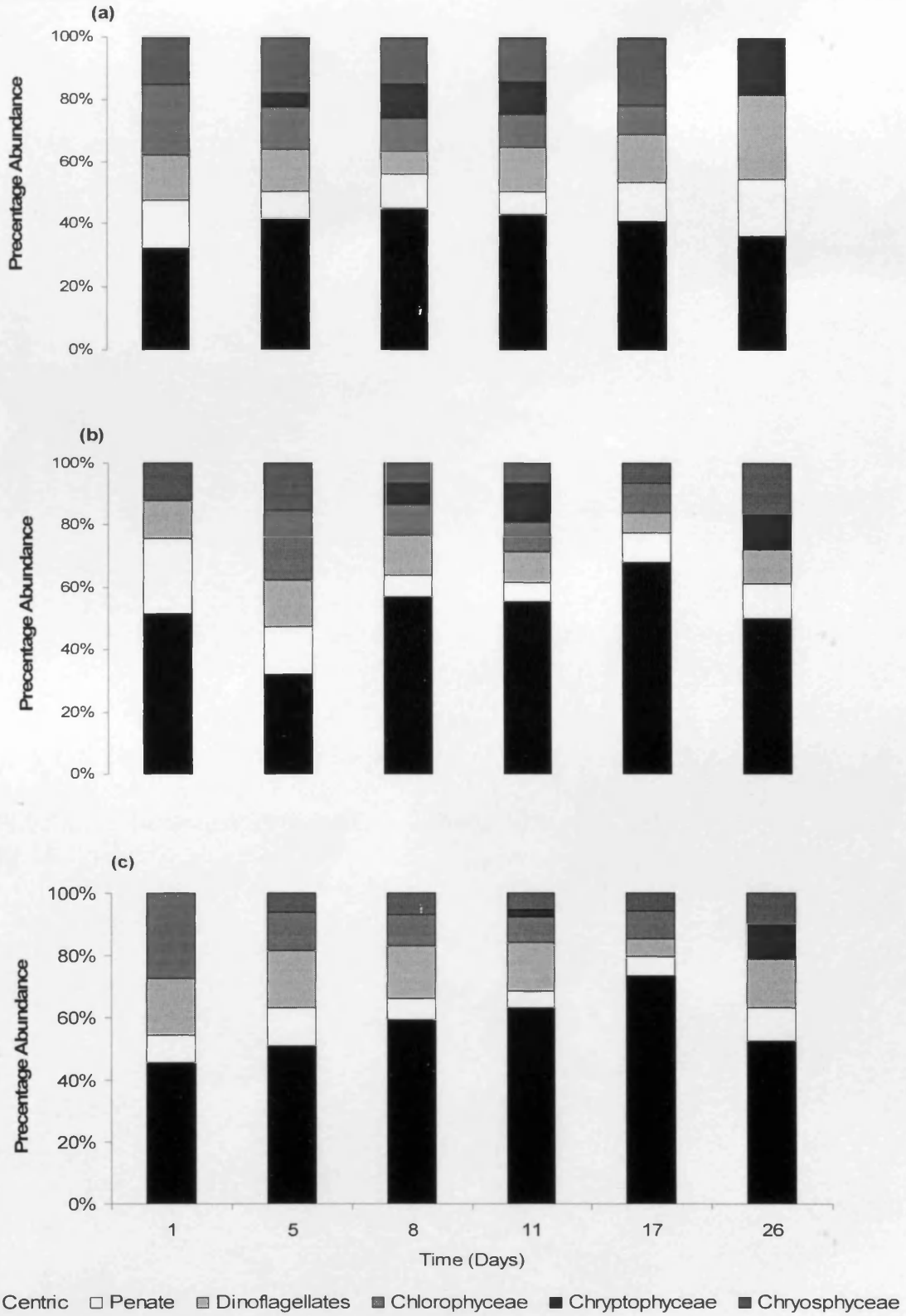


Figure 6.7 Percentage abundance of phytoplankton recorded with time. (a) Control, (b) LN and (c) HN enclosures.

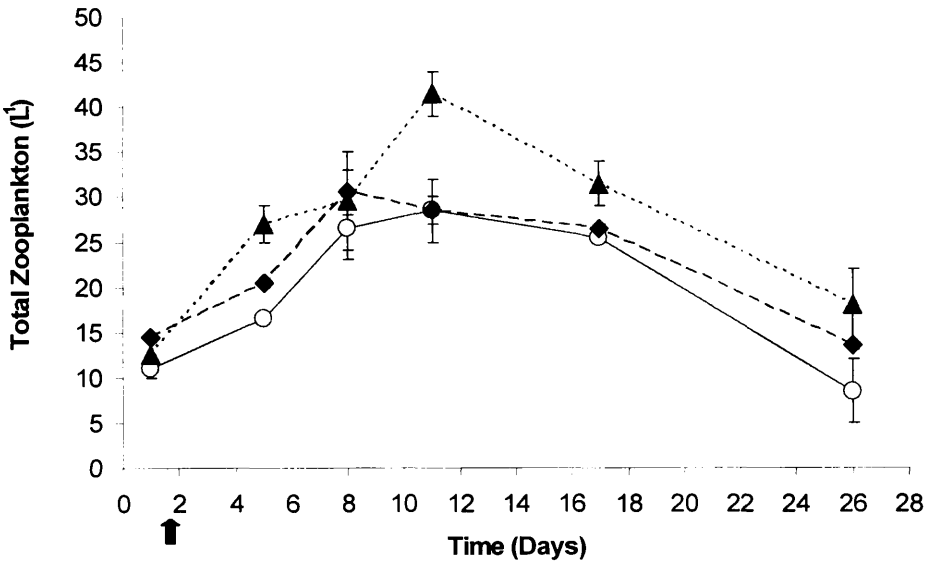


Figure 6.8 Total Zooplankton counted with time. Control (○); LN (◆) and (●) HN enclosures. Data are shown as mean values from each duplicate treatment with 95% confidence intervals (n = 2). Arrow indicates time of nutrient addition (day 1).

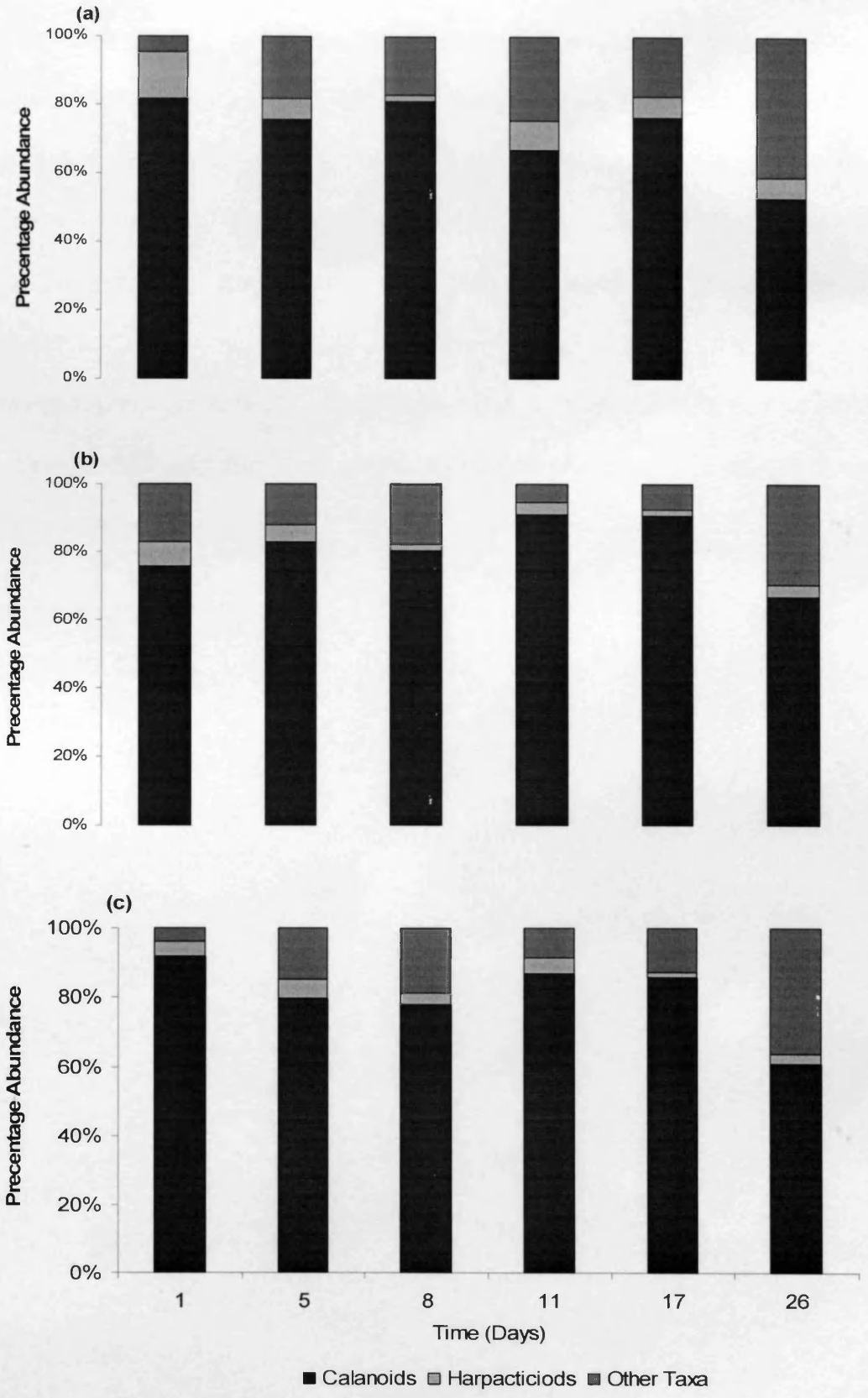


Figure 6.9 Percentage abundance of zooplankton recorded with time. (a) Control; (b) LN and (c) HN enclosures.

Figure 6.10 summarises temporal changes in phytoplankton populations in the three treatments expressed as CCA results. The first axis was the most significant trend, accounted for 16.2% of total variance in the data. The second axis only accounted for 0.3% of total variance, indicating any real trends in the data are exhibited along the first axis. The treatments are grouped along axis one in two clusters representing predominantly high nutrient treatments and low nutrient/control treatments respectively. The direction of separation of these clusters is defined closest by ammonia, nitrate and dissolved oxygen and to a lesser extent salinity.

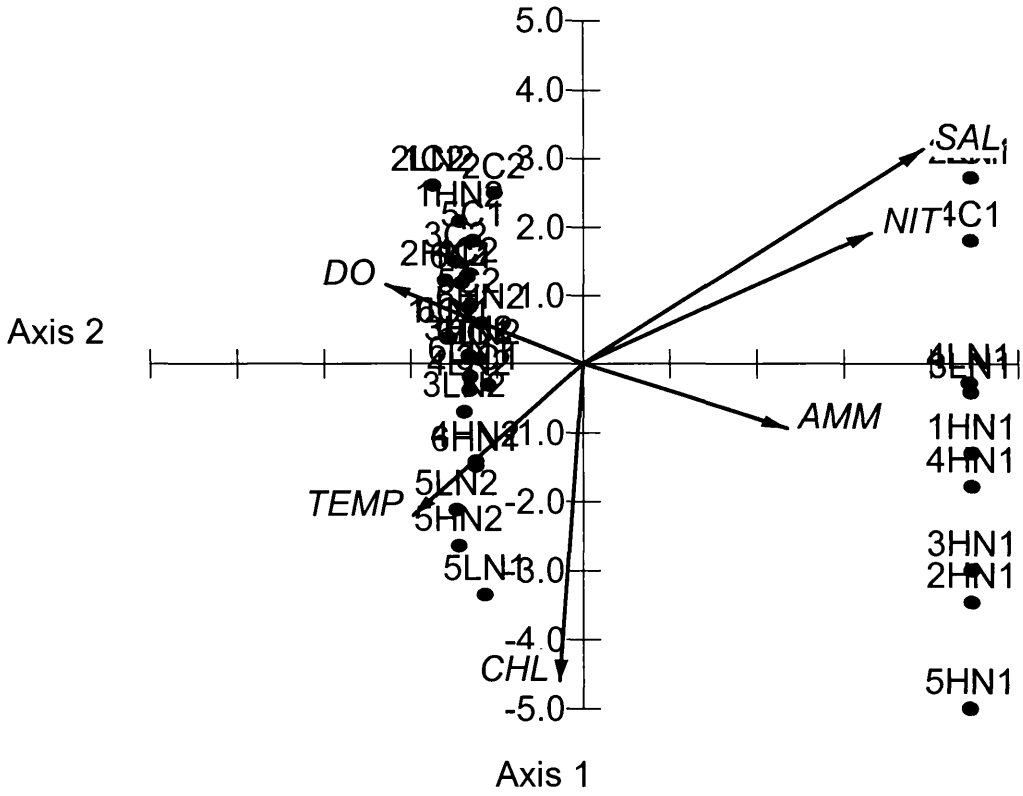


Figure 6.10 Canonical correspondence analysis based on phytoplankton abundance (different groups) in the six enclosures. Temperature (TEMP); salinity (SAL); ammonia (AMM); chlorophyll (CHL) and dissolved oxygen (DO).

6.3.2 – Experiment 2

6.3.2.1 – Water quality

Figure 6.11 depicts temporal variations of water quality in each enclosure. Dissolved oxygen concentration was similar in all enclosures until day 12 after which, values recorded were higher in all enclosures with significantly higher concentrations present in both the LP and ND enclosures compared to the control enclosures at day 16. This was followed by a slight decrease in concentration in all enclosures recorded at the end of the experiment. Temperature remained similar in all enclosures until day 5 after which, the temperature recorded was higher in both the LP and ND enclosures compared to the control enclosures at day 8. At day 12, the temperature recorded in the LP and ND enclosures had decreased and was similar to levels recorded in the control enclosures. Between day 12 and day 21, temperature remained fairly constant in all enclosures. Salinity decreased with time in all enclosures from day 1 to day 21, decreasing from an average of 22.5 psu to 9.6 psu in all enclosures. The pH values were similar in all enclosures until day 8 after which, pH increased in all enclosures until day 16, after which, a decrease in pH was recorded at the end of the experiment.

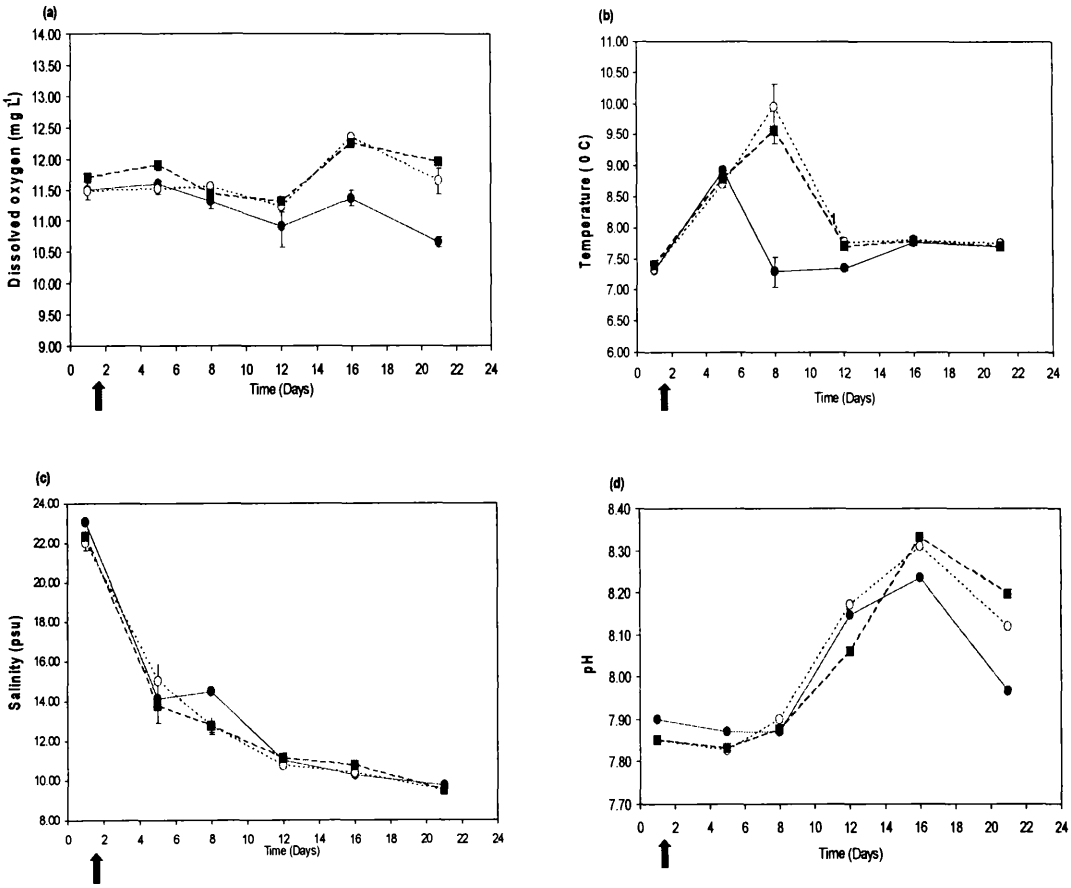


Figure 6.11 Dissolved oxygen (mg L^{-1}), (b) Temperature (^{0} C), (c) salinity (psu) and (d) pH readings obtained over the duration of the experiment. (●) Control, (○) LP and (■) ND enclosures. Data are shown as mean values from each duplicate with 95% confidence intervals (n = 4). Arrows indicate time of nutrient addition.

6.3.2.2 – Chemical parameters

Figure 6.12 shows changes in nutrient concentration with time. Total TAN concentration measured before the addition of nutrients on day 1 showed that all enclosures had similar low values of TAN present. There was a clear increase in TAN concentration immediately after addition of nutrients to the LP and ND enclosures (524.96 and 1380.05 μg L^{-1} respectively). This was followed by a constant decrease in TAN after day 5 in the ND enclosures, whilst TAN concentrations remained similar in the LP enclosures until day 12 before a decrease was observed. At day 21, TAN concentrations in both the LP and ND enclosures had decreased to values that were slightly higher before the addition of NH_4Cl. TAN concentrations in the control enclosures remained similar throughout the experiment.

There was an increase in nitrate concentration in all enclosures until day 5 with higher concentrations being present in the ND enclosures where the trend was for the concentration to decrease until day 16. This was followed by a slight increase in concentration to values which were similar before the addition of NH_4Cl. Nitrate concentration in the LP enclosures increased until day 8, this was followed by a decrease until day 21 were values were similar before the addition of NH_4Cl. Nitrate concentration remained similar in the control enclosures until the end of the experiment.

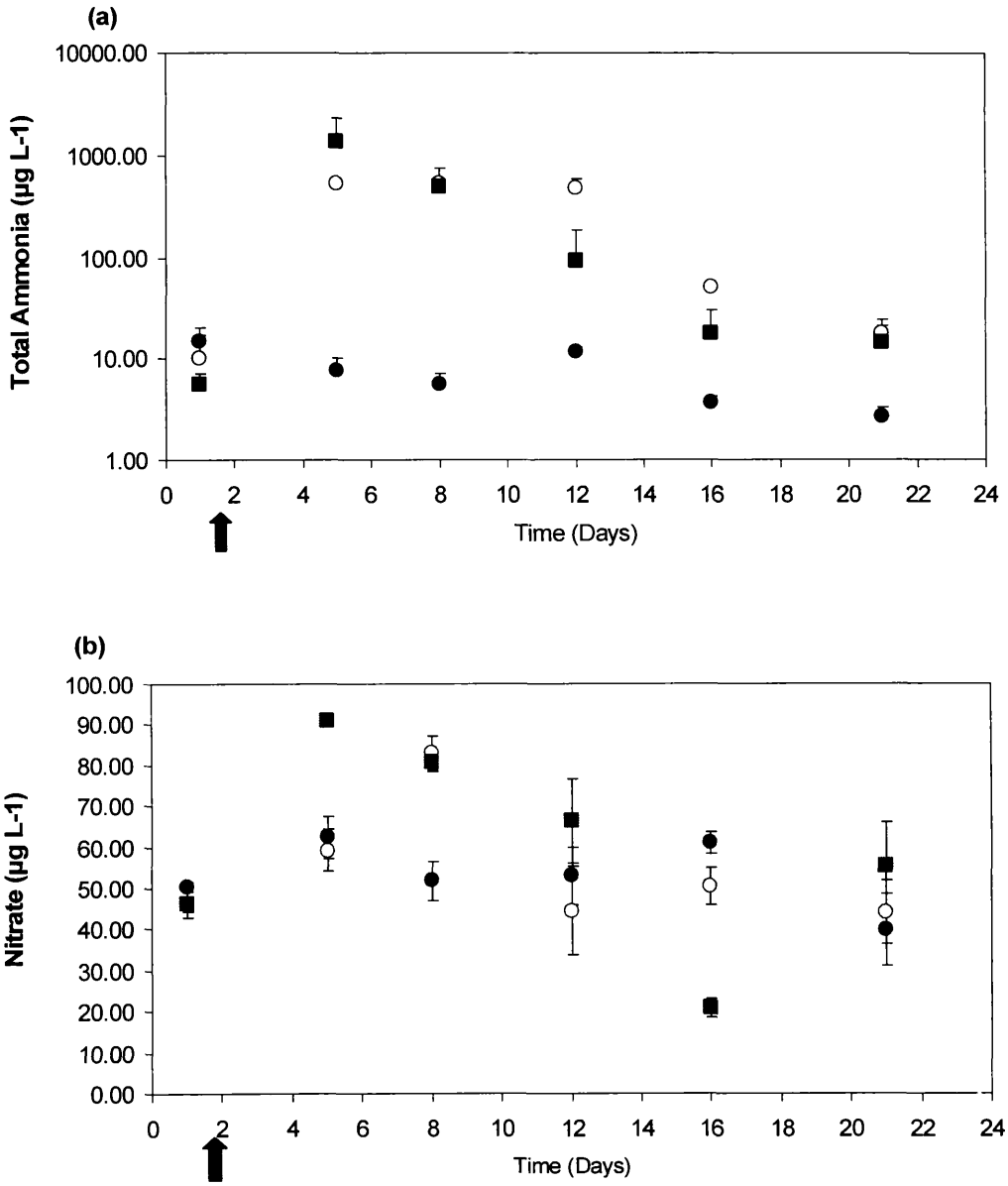


Figure 6.12 Changes in (a) total ammonia (µg L^{-1}) plotted on log scale and (b) Nitrate (µg L^{-1}) detected during the course of the experiment. (●) Control, (○) LP and (■) ND enclosures. Arrows indicate time of addition of nutrients to the enclosures (day 1).

6.3.2.3 – Biological parameters

There were clear trends between chlorophyll *a* production and cell density with time. Chlorophyll *a* values (Figure 6.13) showed a significant increase with time in the control and LP enclosures after day 5 and after day 8 in the ND enclosures ($F_{5.8}$ 14.06; $p < 0.05$). By day 12, chlorophyll *a* values in the LP and ND enclosures were significantly higher than the control enclosures ($F_{2.3}$ 18.07; $p < 0.05$). This trend continued until the end of the experiment with significantly higher values recorded in the ND enclosures compared to values obtained from the control enclosures at day 21 ($F_{2.3}$ 10.20; $p < 0.05$). There were no significant differences between the LP and ND enclosures.

Figure 6.14 shows total phytoplankton estimated in enclosures over the experiment. Although there was a similar pattern of increase and decrease of phytoplankton biomass in all enclosures, addition of NH_4Cl appeared to have affected an increase in with time. There were significant increases in cell numbers with time in all enclosures ($F_{5.28}$ 7.03; $p < 0.05$) until day 16. This was followed by a decrease in all enclosures. Both the LP and ND enclosures had more cells mL^{-1} than control enclosures after day 5 with cell numbers being higher in the LP enclosures compared to the ND enclosures until day 16 after which there were more cells present in the ND enclosures. Although there were more cells present in both LP and ND enclosures, there were no significant differences between enclosures on each sampling day ($F_{2.3} < 4.11$; $p > 0.05$).

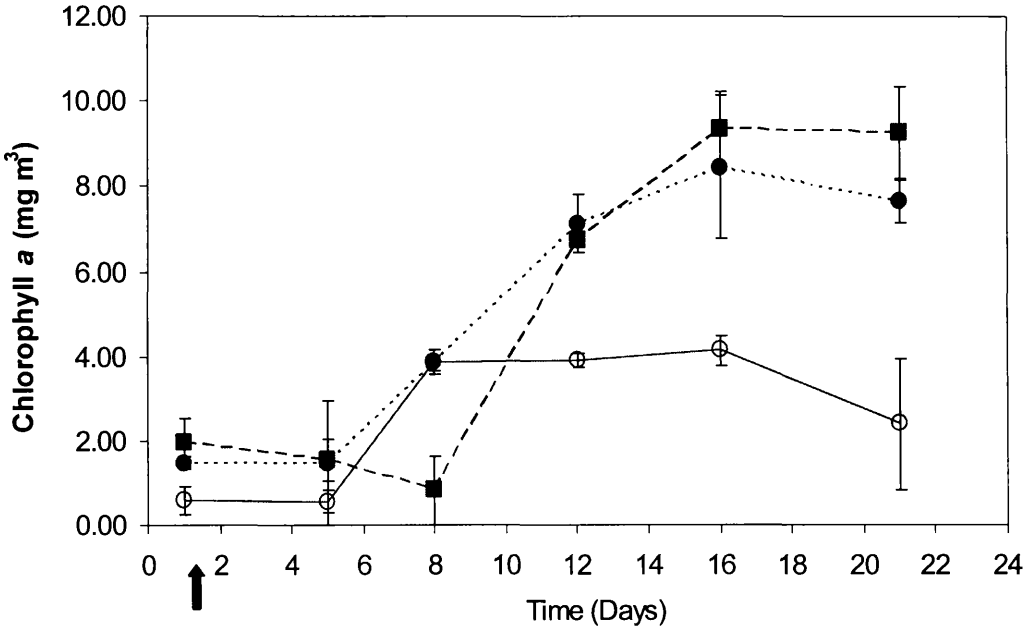


Figure 6.13 Changes in chlorophyll *a* concentration (mg m^3) measured in the. (○) Control, (●) LP and (■) ND enclosures. Data are shown as mean values from each duplicate treatment with 95% confidence intervals (n = 2).

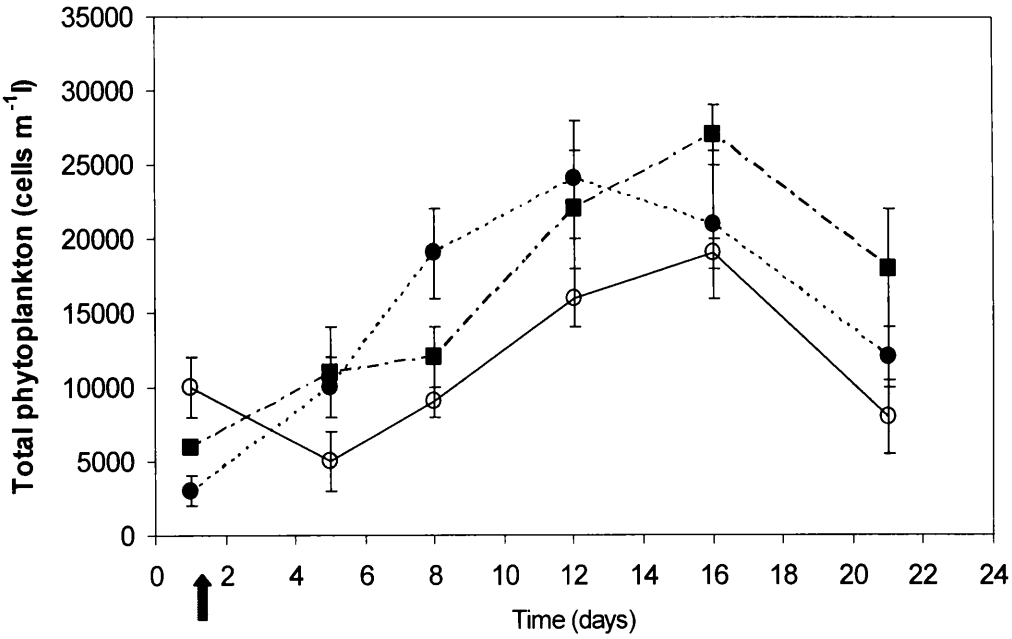


Figure 6.14 Total Phytoplankton estimated with time (cells mL^{-1}). (○) Control, (●) LP and (■) ND enclosures. Data are shown as mean values from each duplicate treatment with 95% confidence intervals (n = 2). Arrow indicates time of nutrient addition (day 1).

Figure 6.15 shows the main groups of phytoplankton identified were the classes Bacillariophyceae, Chrysophyceae, Chlorophyceae and Chryptophyceae and Phytomastogophora. Diatoms dominated throughout the duration of the experiment with eight families of the sub-classes Centricae dominating the diatom population in all enclosures. This dominance was evident in all enclosures at the start of the experiment, but in the ND enclosures, centric diatoms were noted to decrease with time after day 5. This decrease in percentage abundance of centric diatoms was accompanied by an increase in penate diatoms and to a lesser extent, dinoflagellates. In both the control and LP enclosures, centric diatom populations fluctuated throughout the experiment with both treatments having less numbers present at the end of the experiment.

Figure 6.16 shows total zooplankton in the enclosures over the duration of the experiment. There was a significant increase in zooplankton with time in all enclosures after day 5 ($F_{5.28}$ 7.03; $p < 0.05$) until d 16, when both the LP and ND enclosures had significantly more animals recorded compared to the control enclosures ($F_{2.3}$ 27.33; $p < 0.05$). There were 9 separate phyla recorded with Arthropoda dominating throughout the experiment (see Figure 6.17). Within this phylum, calanoids predominated in all enclosures throughout the experiment with 5 families identified except at day 5 when no calanoids were recorded in the ND enclosures. Harpacticoids were the next most numerous order with the remaining phyla present in lower numbers.

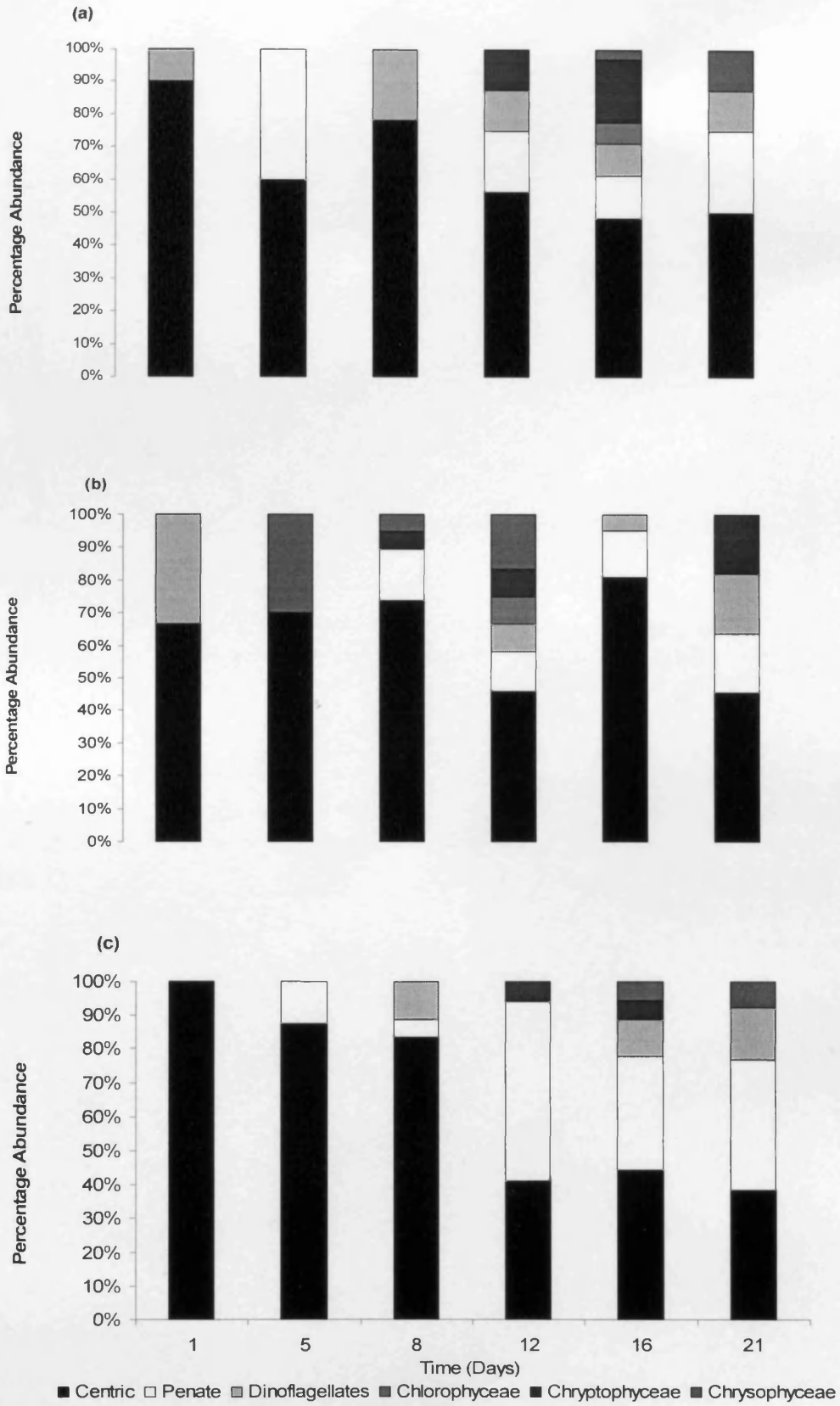


Figure 6.15 Percentage abundance of phytoplankton recorded with time. (a) Control, (b) LP and (c) ND enclosures.

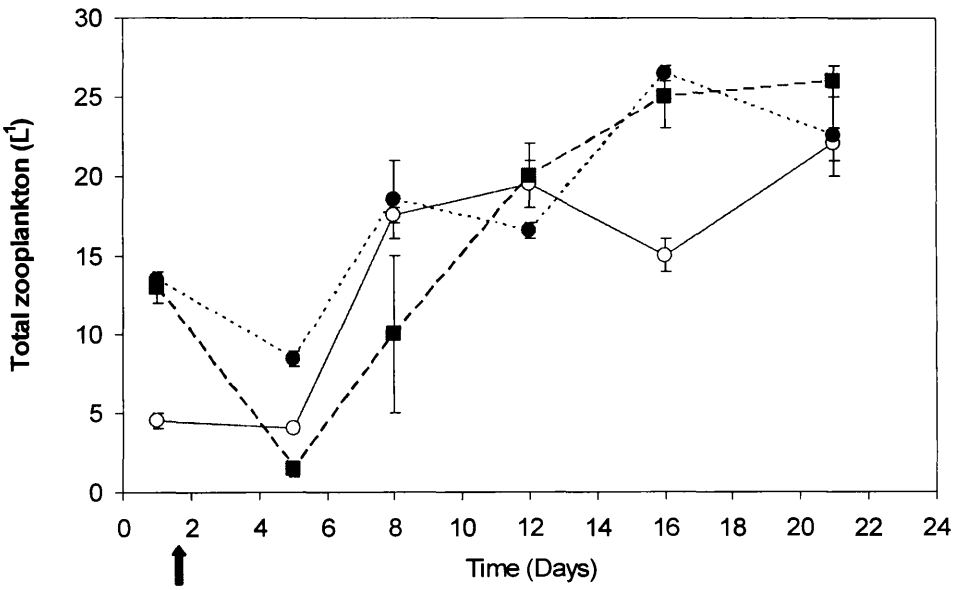


Figure 6.16 Total Zooplankton counted with time. (○) Control, (●) LP and (■) ND enclosures. Data are shown as mean values from each duplicate treatment with 95% confidence intervals (n = 2). Arrow indicates time of nutrient addition (day 1).

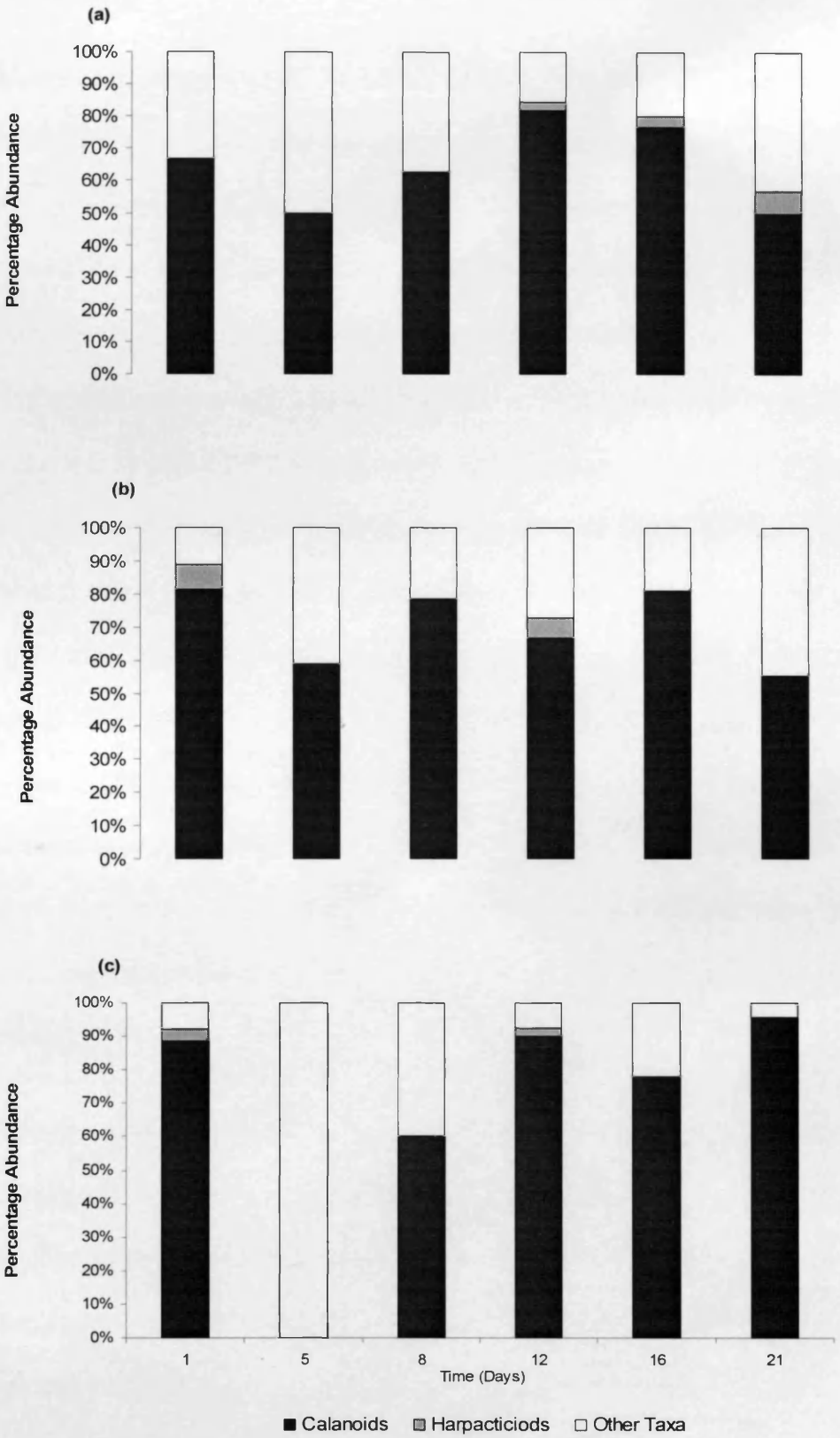


Figure 6.17 Percentage abundance of zooplankton recorded with time. (a) Control; (b) LP and (c) ND enclosures.

6.4- Discussion

Mesocosm experiments, in which varying volumes of seawater are held in floating plastic bags and subjected to various treatments, provide a valuable tool in simulating larger scale ecosystem effects (Jacobsen *et al.* 1995). The results from this study showed that adding nitrogen (as NH_4Cl) to enclosures resulted in population expansion after day 8 in both the LN and HN enclosures in the first experiment and by day 5 in the second experiment. However, the increase in cell numbers was small even though enclosures that received the highest concentration of nitrogen in the form of NH_4Cl had consistently more cells present throughout the experiment after day 8 and 5 for both experiments, even after nutrient depletion had occurred. This suggests that phytoplankton were nitrogen limited as the population began to decrease once nutrients were utilised. This is consistent with previous studies undertaken in temperate coastal waters (Ryther and Dunstan, 1970; Graneli and Sundback, 1985; Hein and Rietmann, 1995). The results from the first study also suggest that phytoplankton were PO_4 limited as the concentration of this nutrient decreased from day 11 which is when cell depletion started.

Added nutrient depletion in all of the treated enclosures started after day 5 and continued until the end of the experiment in both experiments when concentration levels were similar to levels in the control enclosures. This suggests that phytoplankton populations within the enclosures utilised the excess nutrient added to sustain an increase in abundance but once nutrients became depleted further growth could not be sustained and populations began to decrease to levels not dissimilar to levels in the control enclosures found at the end of the experiment. The increase in cell numbers in the control

enclosures was possibly supported by the presence of sufficient concentrations of nitrogenous compounds occurring naturally in the water inside the mesocosms. However, it was expected that by adding excess nutrients to the other enclosures that this would effect a significantly higher population expansion compared to the control enclosures and this was not the case.

This decrease in salinity was unexpected and could not be caused by rainfall entering the bags due to Perspex lids being fitted. The decrease in salinity may have been caused by infiltration by surface water as a result of wave action as there was a 3 cm gap between the mesocosm and lid. The surface waters surrounding the farm site have been found to have low salinity due to fresh water influx from rivers.

CCA results suggested that of all physico-chemical parameters measured in the first study, ammonia seemed to have the strongest effect on phytoplankton populations within the enclosures. Indeed, when NH_4Cl was added to the enclosures there were marked increases in populations in both LN and HN enclosures. Phytoplankton were able to utilise this added nitrogen to sustain increased populations even in the presence of protozoan and mesozooplankton consumers.

Zooplankton populations showed similar patterns of growth when compared to phytoplankton populations with time in the first experiment. There was an initial increase in abundance in all enclosures until d 15, followed by a sudden decline in numbers. This suggests that zooplankton communities, dominated by calanoid copepods, initially expanded as algal populations expanded and once algae populations started to decline, they could no longer support high numbers

of zooplankton. However, no clear trends were evident from the second experiment, even though zooplankton populations did increase in the ND enclosures until the end of the experiment and until days 20 and 16 in both the LP and control enclosures respectively. It is widely accepted that phytoplankton growth in coastal waters and sea-lochs is controlled by availability of light, nutrients and predation in a threshold manner (Droop *et al.* 1982). Thus it is assumed that zooplankton control was weak probably due to insufficient numbers being present in the enclosures. In some sea lochs, spring blooms may have already become established before populations of phytoplankton grazers begin to increase (Tett and Edwards, 2002). This is one of the most effective controls that regulate phytoplankton growth in most systems and if this control is weak or missing and in conditions where there has been an input of nutrients, there is concern that harmful algal blooms (HABs) may develop (Tett and Edwards, 2002).

Mesocosm experiments in the eutrophic Seton Sea of Japan show that silicon (Si) depletion leads to shifts of dominant species from larger to smaller diatoms or flagellates; and that nutrient stocks remaining in deeper water after depletion of those in the surface layer lead to the dominance of flagellates (Harada *et al* 1996). By increasing the concentration of nutrients, in this case nitrogen, the natural balance between nutrients would be disrupted and since the enclosures were not fertilised with Si, Si-limitation would be expected. Diatoms normally dominate the spring bloom (Gowen, 1994) and they are dependant on Si in addition to N and P for growth (Jacobsen *et al.* 1995). Once Si depletion occurs, the result would be a shift in community dominance to flagellates as it is accepted that dinoflagellates rarely form a significant part of the spring bloom in

Scottish coastal waters (Gowen, 1994). The results from the present experiment showed that there were no changes in community dominance and centric diatoms dominated the phytoplankton population throughout.

Intensive fish farming undoubtedly contributes to the pool of dissolved nutrients in coastal waters where this activity exists (Heath *et al.* 2002). The concentration added to each enclosure represented a single point discharge of nitrogen in the form of ammonia, which represents a single meal delivered to fish. However, as fish are normally fed more than one meal a day for most of the year, more than one food-related concentration peak of ammonia will be excreted. This has been shown previously after 3 h (Reynolds, unpublished data) and 5 h (Kaushik and Cowey, 1991). The present experiment showed that a single pulsed release of TAN equivalent to that excreted from a theoretical 500 or 1000 T fish farm could potentially affect phytoplankton population changes by increasing cell numbers even in the presence of zooplankton. This has obvious environmental implications in that if phytoplankton populations came into contact with several pulses, with short time durations between pulses, there may be a sustained increase in phytoplankton population growth which could in theory result in nuisance blooms occurring or, if potentially toxic species were present in sufficient numbers, toxic blooms may develop if there was sufficient amounts of PO_4 and Si present.

However, it is worth bearing in mind that any ammonia excreted by farmed fish would be subjected to a dilution factor that would possibly lessen its effect on phytoplankton growth.

Within coastal embayments, additional factors influence the ability of phytoplankton to utilise excess nutrients (Black, *et al.* 1997). The volume, surface area, rate of water renewal and vertical stratification all influence the ability of phytoplankton to utilise excess nutrients (Aure and Stigebrandt 1990). The time scale of water movements has an important influence on phytoplankton growth; this is particularly true for sea lochs (Gowen, *et al.* 1983). A study by Gowen and Ezzi, (1992) showed that during the neap period of the tidal cycle, there was localised enhancement of ammonia surrounding a fish farm. This is the time when dispersal would be minimal and could result in rapid uptake by phytoplankton. Indeed, during this study it was shown that after addition, rapid depletion occurred and that by d 30 (25 days after the addition of nutrients) concentrations had returned almost to levels detected before addition of nutrients. However, although it has been shown that there is rapid uptake of nutrients by phytoplankton during the spring bloom when conditions favour growth and when growth is controlled by nutrient availability, it would be expected that this would not be the case during times of the year when growth is limited by light or in the presence of stratification.

In addition, Gowen *et al.* (1983) suggest that differential timescales of loch flushing time and phytoplankton growth can control the maximum achievable biomass within a sea-loch. The study by Gowen *et al,* (1983) on Loch Ardbhair - a Scottish sea loch -showed that even though the loch was sufficiently shallow to allow for positive net photosynthesis, rapid flushing (1 - 2 days) ensured that phytoplankton did not remain on the loch long enough to achieve significant growth. Thus, even with excess nutrients being released by farmed fish as long as the timescale of flushing is less than or similar to the timescale of

phytoplankton growth (which is estimated to be approximately three days for the population to double), it is unlikely that there would be an increase in biomass as a result of *in-situ* growth. However, sea lochs such as Loch Etive, were the study was done has a longer flushing time (14 d) (Edwards and Sharples, 1986) and in addition has a low near surface salinity around the farm site due to fresh water input from river discharges (Reynolds, 2002). These factors providing a well-illuminated upper layer for photosynthesise as well as time to increase growth, may increase the probability that phytoplankton can come into contact and utilise areas of nutrient enrichment as a result from ammonia excretion from farmed fish.

6.5 - Conclusions

Although fish farming can lead to nutrient enrichment, significant amounts of nutrients would be required to cause hypernutrification in many sea lochs where intensive fish farming is practised. However, it has been demonstrated that even simulated nutrient enhancement representative of a single concentration peak of ammonia can effect change by increasing cell numbers within marine enclosures. Given that there would be several concentration peaks of ammonia released each and every day that feeding occurred in practical situations, there is the potential to alter the natural balance within phytoplankton populations. Fish fed a nutrient dense diet (experiment 2) have an ammonia excretion pattern that results in larger ammonia concentration peaks occurring earlier compared to fish fed a normal commercial grower diet. In contrast, fish fed a low protein diet excrete much lower concentration peaks

of ammonia. This reflects the lower protein inclusion level of such a diet. These results have obvious environmental ramifications.

Based on the results, the hypotheses "Feed related concentration peaks of ammonia from farmed rainbow trout have no effect on marine phytoplankton community composition and densities" may be rejected as there more cells present in both fertilised enclosures compared to the control. There was also more phytoplankton present in the enclosures that simulated feed related concentration peaks of ammonia from 1000 tonnes production than from 500 tonnes production.

Based on the results, the hypothesis "Feed related concentration peaks of ammonia from farmed Atlantic salmon fed a nutrient dense diet or a low protein diet do not affect marine phytoplankton communities differently" may also be rejected as there were more cells present in enclosures fertilised with NH_4Cl based on ammonia excretion from fish fed a nutrient dense diet compared to fish fed low protein diet after day 12 post fertilisation. However, further research is required to fully elucidate the effects of dissolved wastes on marine phytoplankton communities.

Chapter 7 - Estimation of waste outputs from Atlantic salmon (*Salmo salar* L) using a mass balance approach.

7.1 Introduction

One of the principal challenges currently facing the aquaculture industry from an environmental point of view is the reduction of wastes. In particular, wastes due to discharges of dissolved and solid material derived from feeding fish in intensive culture operations. Prior to setting goals for the reduction of these wastes, there is a need for objective estimates of the amount of different wastes produced by the culture organism (Cho and Bureau, 2001).

There are several methods for quantifying wastes lost to the environment (Beveridge, 1996). Namely, direct through the sampling and analysis of the water column and of particulate material from the sediments and indirectly usually involving the use of a mass balance approach. The former can be expensive and may be nearly impossible for certain facilities such as cage culture operations (Cho and Bureau, 2001). It has only been practical for estimating total uneaten food and faecal material via the use of sediment traps suspended below cages. The latter has been used extensively to estimate waste outputs and there are various ways of using this particular method.

A mass balance approach can be useful especially when used in conjunction with laboratory and field data. Uneaten food, faecal material and excretory looses can be estimated using data on the quantities of food given as well as the quality of such food. FCR, digestibility and faecal composition data can also be used (Beveridge, 1996).

An example of a mass balance approach by Beveridge, 1996, shows how the method can be used. Assuming the N content of fish to be 3% and the N content of food to be is 8%. With an FCR of 1.6 per tonne of fish produced, approximately 98Kg of N is lost to the environment. In the author's own words, a more detailed picture emerges if food losses are incorporated into the calculation. If food losses are 20%, then 102.4 kg of N is ingested. In addition, if 360g of faeces with an N content of 4% is produced per kg food consumed, then 18.4 kg of N is voided in the faeces, 3 kg is retained in the flesh and the remaining 54 kg is excreted as ammonia and urea.

A biological method for the prediction of aquaculture waste outputs (BMPAWO) is a simple method for estimating waste outputs that had been developed as an economical alternative to limnological methods, which can be very expensive (Cho *et al.* 1991). It uses a simple nutrient balance approach to estimate waste outputs (Cho *et al.* 1994). Digested protein, lipid and carbohydrate obtained from digested feedstuffs are potentially available for energy and nutrients for maintenance, growth and reproduction of the animal (Cho and Bureau, 2001). Undigested feed is excreted in the faeces as solid waste (SW) and the by-products of metabolism (ammonia, urea, carbon dioxide and phosphates) are excreted as dissolved wastes (DW). The total aquaculture wastes (TW) are made up of SW and DW together with apparent feed wastes (AFW). This method uses estimates of AFW by comparing with theoretical feed requirements obtained with bioenergetic models (Cho and Bureau, 1997) due to the difficulty in obtaining direct estimation of AFW (Cho and Bureau, 1998) Although this approach offers reasonable estimates as to the potential outputs generated by cultured fish, it does not take into account any environmental

variables. Also the ADC is only an estimation of digestibility and will obviously differ from true digestibility, which in itself is subjected to many variables even within a single population of fish.

Ackefors and Enell, (1990), developed an equation to describe discharge loadings for nitrogen. Using the equation:

$$Kg\ N = (A \times Cdn) - (B \times Cfn)$$

Where A is wet weight of dry pellets fed to fish per year, B is the wet weight of fish produced, Cdn is the nitrogen content of the pellets, expressed as a percentage of wet weight and Cfn is the N content of fish. However, this method only gives a total waste N output and does not partition the waste output between dissolved and particulate N fractions.

There have been few recent studies of the production of wastes from farmed Atlantic salmon. A review of the literature on mass balance approaches show that estimates for waste output can vary widely from author to author. For example, when considering nitrogen output from farmed Atlantic salmon, values range from 36-60% being excreted into the water column, mainly as ammonia and urea; this is the equivalent of 60 – 120 kg N/tonne of fish. Between 10-23% settles in the sediments as particulate material from waste feed and faeces, 27-40% is retained in fish tissues and is harvested and benthic flux (release from sediments) is estimated to be 1-4% (Ackefors and Enell, 1990; Seymour and Bergheim, 1991; Hall *et al.* 1992; Talbot and Hole, 1994; GESAMP, 1996; Cho and Bureau, 1997).

However, there have been considerable improvements in feed technology over the past few years, accompanied by improvements in FCR. As a consequence, utilisation of nitrogen by cultured fish is now much more efficient. For example, using a mass balance approach, a study by Davies, (2000) estimated that dissolved N released was 35.6 kg N/tonne of fish. This is much lower than values cited above and clearly demonstrates a need to estimate waste outputs based on current farming practices.

The aim of this experiment was to present estimates of the rate of nitrogen released from (a) large Atlantic salmon fed one of four different diet types for a period of 139 days and (b) Atlantic salmon introduced to low protein diets at different sizes throughout a complete marine phase of production. The experiment integrates information on the protein and nitrogen content of each diet used along with feeding practices with a mass balance model to provide estimates of waste production.

The main hypothesis that has been tested was:

H_o: Feeding Atlantic salmon different diet types did not result in differences in the rate of discharge of particulate and dissolved wastes.

7.2 - Materials and methods

7.2.1 – Experimental design

A simple mass balance model was developed to allow for the determination of waste outputs from Atlantic salmon. An example of the spreadsheet model used can be seen in Appendix 3.

In the first study, the mass-balance model was used to predict and compare the production of dissolved and particulate waste from four triplicate groups of large Atlantic salmon fed different diet types for a period of 139 days. The diets (assessed for growth performance in chapters 2) were a high energy diet (ND), a low protein (LP) diet, a partial replacement of fish meal and fish oil (SUS) diet and a control diet. There were 450 fish assigned to each group with 150 fish allocated to one of four trial cages (5 x 5 x 5 m). Estimates of waste production were calculated in time steps of 2 weeks for each group of fish

In the second part of the simulation, the FCR values obtained for each of the four diets were used to estimate waste N outputs from a theoretical farm producing 1000 tonnes of salmon from smolt input to harvest. The model integrated information on the protein content of each diet fed, the digestibility of protein for each of the diets, FCR values for each of the diets and the model was based on the assumption that 1000 T^{-1} of salmon were produced over a period of 20 months (Table 7.1).

In the second study, real averaged farm records were used to predict waste outputs from Atlantic salmon introduced to low protein diets at different sizes throughout the marine phase of the production cycle (the diets were assessed

for growth performance in chapters 3), Four triplicate groups of smolts were established in 60 m circular cages with equal amounts of fish in each cage (23 000 – 24 000). From smolt input until approximately 330 g weight, all groups were fed the BioOptimal Classic products C50 and C100 (Control feed). From this size a low-protein diet (Test diet C250) was introduced to three cages of fish while the remaining nine cages continued on the standard product (C250 control). At approximately 1000 g live weights another three cages of fish were transferred to the Test diet, while fish in the remaining six cages continue on the control diet (C800).

The last three cages were given low-protein feed from 1600 g onwards, while fish in the control group continued on the standard product (C1500-C2000) until harvest. By this procedure, nine cages of fish were transferred to the Test diet at 250g, 800g or 1500g, respectively; while three cages were kept on a standard feed control from smolt input to harvest. The feeds were produced by BioMar AS, Norway. The model integrated information on the nitrogen content of each diet fed, mortality rates for each of the groups and FCR values for each of the diets (Table 7.2).

Estimates of waste production were calculated in time steps of 4 weeks for each group of fish.

Table 7.1 Assumptions, input data and modelled waste outputs for the four diets assessed. Numbers in parenthesis are percentage values.

Parameter	Diet type			
	LP	ND	SUS	C
FCR[2]	1.09 ± 0.02	0.97 ± 0.02	1.00 ± 0.02	1.09 ± 0.01
% Protein in diet	31.5	35.6	35.3	35.6
% Nitrogen in diet[2]	6.27 ± 0.05	6.59 ± 0.04	6.29 ± 0.10	6.20 ± 0.26
% Protein Digestibility[2]	85.57 ± 0.72	85.99 ± 0.38	85.45 ± 0.11	85.42 ± 0.31
N input[1]	68.34	63.92	62.90	67.58
Waste feed N (2%)	1.37	1.28	1.26	1.35
Total N consumed	66.97	62.64	61.64	66.23
Faeces[1]	9.86	8.96	9.2	9.85
Total N discharge[1]	32.97	28.64	27.64	32.23
Total particulate N[1]	11.23	10.24	10.46	11.20
Total dissolved N[1]	23.11	18.40	17.18	21.03
% Particulate N waste	16.4	16.0	16.6	16.6
% Dissolved N waste	33.8	28.8	27.3	31.1
% N retained as growth	49.8	53.2	54.1	50.3

[1]Values are Kg^{-1} NT^{-1} Production. [2]Values represent means ± S.E

Table 7.2 Assumptions, input data and modelled waste outputs for fish fed low protein and control diets.

Parameter	Diet Types			
	Control	C250	C800	C1500
FCR	1.05	1.11	1.10	1.08
[1]N content of diet (%)	6.43	5.99	5.99	5.99
N input	67.52	66.49	65.89	64.69
N waste feed (5%)	3.38	3.32	3.29	3.23
Total N consumed	64.14	63.16	62.60	61.46
Faeces	6.75	6.65	6.59	6.47
[2]Mortality (%)	5.13	5.01	5.38	4.06
[3]Total N discharge	33.45	32.48	31.91	30.77
[3]Total particulate N discharge	10.13	9.97	9.88	9.70
[3]Total dissolved N discharge	23.32	22.51	22.02	21.07
% Particulate N waste	15.0	15.0	15.0	15.0
% Dissolved N waste	34.5	33.9	33.4	32.6
% N retained as growth	50.4	51.1	51.6	53.6

[1]Mean N content of diet from smolt input to harvest.
[2]Mortality calculated from removal of dead fish on a daily basis from smolt input to harvest.
[3] Values are Kg^{-1} NT^{-1} Production

7.2.2 – Mass balances

The theoretical mass balance for nitrogen (N) was calculated for each diet type (Figure 7.1). Nitrogen input was calculated using the equation:

[(Feed input/100* %N content of diet)]

Where feed input was calculated using FCR values obtained for each diet type (on a wet weight basis). Feed conversion ratio (FCR) was calculated as in chapters 2 and 3. The percentage N content of each diet type assessed in the first study was calculated as a percentage of total dry weight using a Perkin Elmer 2400 Elemental Analyser. The percentage N content of each diet type assessed in the second study was calculated on the assumption that 16 % of the protein content was N as no proximate analysis of N was performed on these diets.

Total N consumed was calculated as the difference between total N input and N waste feed. The amount of N retained in fish was calculated as the bulk nitrogen composition of farmed fish (C_{fn}), expressed as a percentage of wet weight. C_{fn} is constant for both salmon and rainbow trout and is reported to be 3.4 % (8.1 % N as dry weight) (Ackefors and Enell, 1990). The model takes into account mortalities. A retrieval system for dead fish was installed in every cage so that dead fish can be taken out of cages on a daily basis. Dead fish were counted and weighed in bulk.

Undigested faecal material was calculated using the equation:

$$N_{faeces} = (N \text{ input} * \% \text{ undigested protein})$$

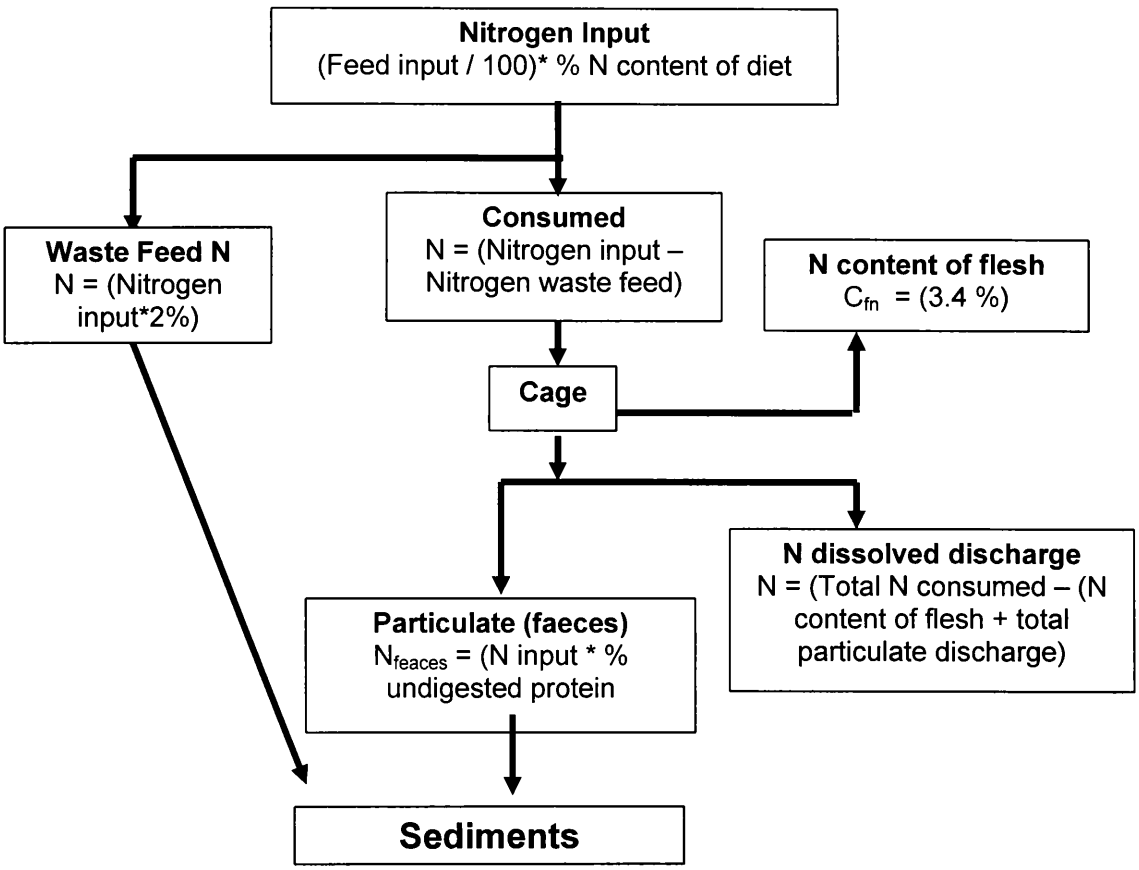


Figure 7.1 Theoretical mass balance for nitrogen used to calculate waste outputs from the different diet types.

Where % digestibility of protein was determined for each diet type in the first study using yttrium oxide (see section 2.2.7). As no digestibility study was undertaken on the fish assessed in the second study and all four diet types assessed in the first study had similar protein digestibility, digestibility was set at 85 %. The amount of dissolved N released was calculated as the difference between total N consumed and the sum of the N composition of the fish and total particulate waste. The total nitrogen discharge rate is therefore the sum of the dissolved and particulate rates.

The basis of the models assumed that the major source of N to the fish was derived from the diet. That is, they receive a negligible proportion of their nutrition from natural sources. It was assumed that a proportion of the feed would be lost from the system as uneaten feed pellets. Estimated losses associated with pelleted feeds used on cage salmon farms are reported to be around 3 – 5 % (Gowen and Bradbury, 1987; Davies, 2000; Brooks *et al* 2002). To this end, fed waste was set at 2 % for the diets assessed in the first study due to the fish being hand fed to satiation and most of the uneaten pellets were collected from the system with the aid of an air-uplift system thus allowing for estimation of feed waste. Feed waste was set at 5 % for the diets assessed in the second study due to these diets being fed to fish in large-scale production polar circles with no air -uplift system in operation.

Although current farming practices in Scotland limits feed wastage to no more than 5 % of the added feed (Davies, 2000), feed wastage was set at 2 % for the diets assessed in the first study due to the fish being hand fed to satiation and most of the uneaten pellets were collected from the system with the aid of an

air-uplift system (Reynolds *pers comm.*). Feed waste was set at 5 % for the diets assessed in the second study due to these diets being fed to fish in large-scale production polar circles with no air -uplift system in operation.

7.3 – Results

7.3.1 – Study 1

Calculated feed input for each of the four diets can be seen in Figure 7.2. Maximum rate of feed input occurred between weeks 6 and 8 as well as between weeks 16 and 18 for all of the diets. This corresponds to late July/August for the first period and October for the later period. Actual weekly biomass gain for fish fed each of the different diet types can be seen in Figure 7.3. Biomass gain was similar for each group with fish fed the LP, SUS, ND and control diets having a final peak biomass of 1.74, 1.69, 1.71 and 1.75 tonnes respectively.

Total N discharge in time periods of two weeks can be seen in Figure 7.4. Fish fed the LP diet had consistently lower total N discharge throughout the trail period. Total N discharge rates for fish fed the SUS diet were lower compared to fish fed the ND and control diets during week 2 and from week 12 onwards. Fish fed the ND and control diets had the highest total N discharge rates from week 12 onwards. Peak discharge rates for all groups occurred between weeks 6 and 8 as well as between weeks 16 and 18. This corresponds to the highest feed input rates for the same time periods.

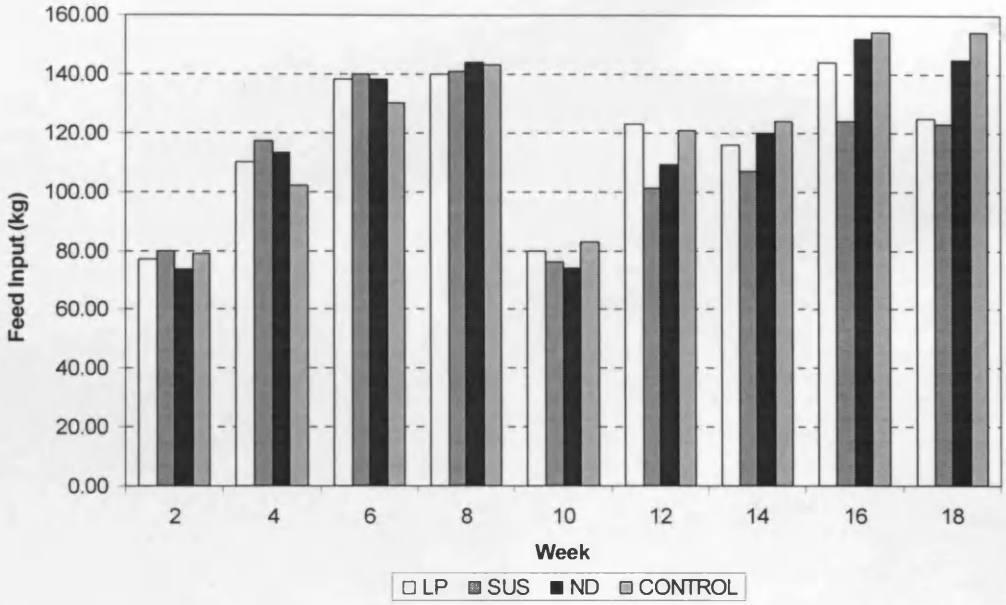


Figure 7.2 Actual feed input (kg) in time intervals of two weeks over the trial period for each of the four diets.

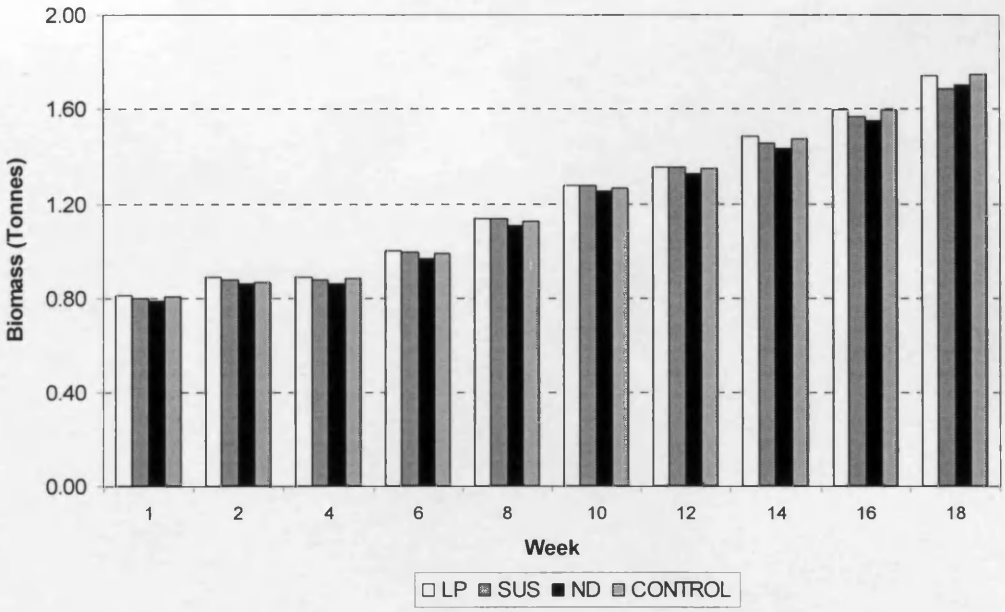


Figure 7.3 Actual weekly biomass gain in time intervals of two weeks over the trial period for each group of fish fed the different diet types.

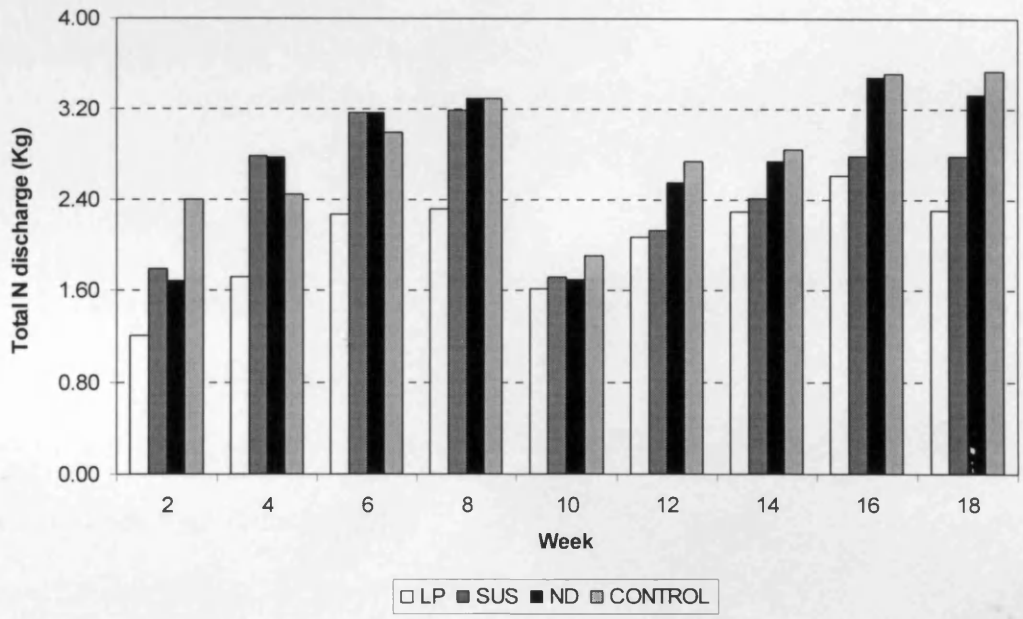


Figure 7.4 Total N discharge (Kg) estimated by mass balance in time periods of two weeks over the trial period from fish fed each of the different diet types.

Figure 7.5 shows calculated total particulate N discharge over the trail period in time periods of two weeks. Fish fed the LP diet had the lowest particulate N discharge of all groups from week 2 to week 10. From week 12 onwards, particulate N discharge was similar to fish fed the SUS diet. Fish fed the N and control diets had higher particulate N discharge rates of all the groups from week 12 onwards. Prior to this point, discharge rates for both groups were similar to fish fed the SUS diet. Total particulate N discharge rates for all groups were highest between weeks 6 and 8 and between weeks 16 and 18.

Total dissolved N discharge rates for all groups can be seen in Figure 7.6. Fish fed the LP diet had the lowest dissolved N discharge rates throughout the trial period. Fish fed the SUS diet had similar dissolved N discharge rates to fish fed the ND and control diets until week 10 apart from week 2 when fish fed the control diet had the highest discharge rates of all groups. Fish fed the ND and control diets had the highest dissolved N discharge rates of all groups from week 12 onwards. Maximum dissolved N discharge rates occurred during the same periods as for maximum particulate waste outputs and total feed input.

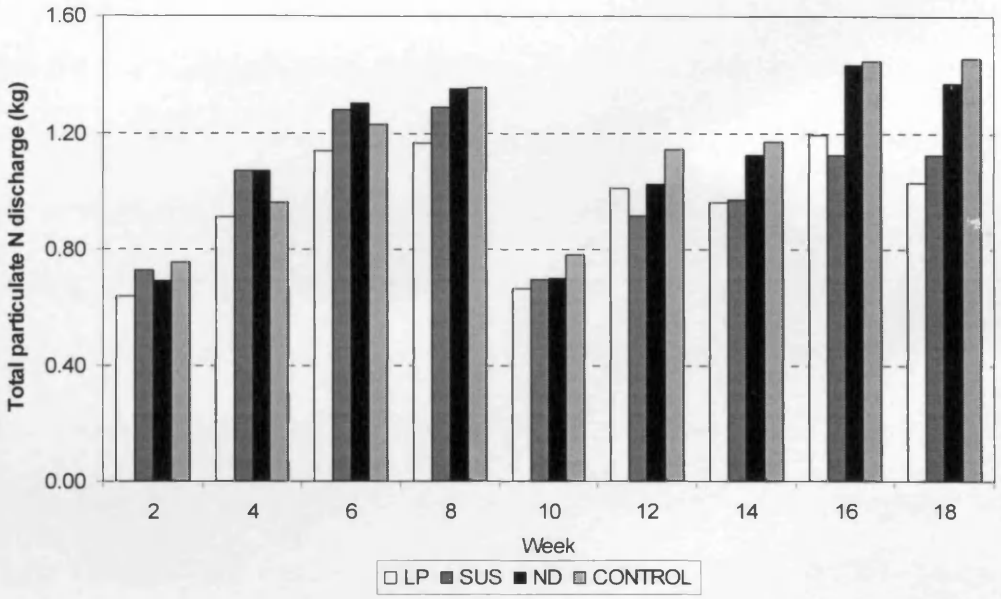


Figure 7.5 Total particulate N discharge (Kg) estimated by mass balance in time periods of two weeks over the trial period from fish fed each of the four different diet types.

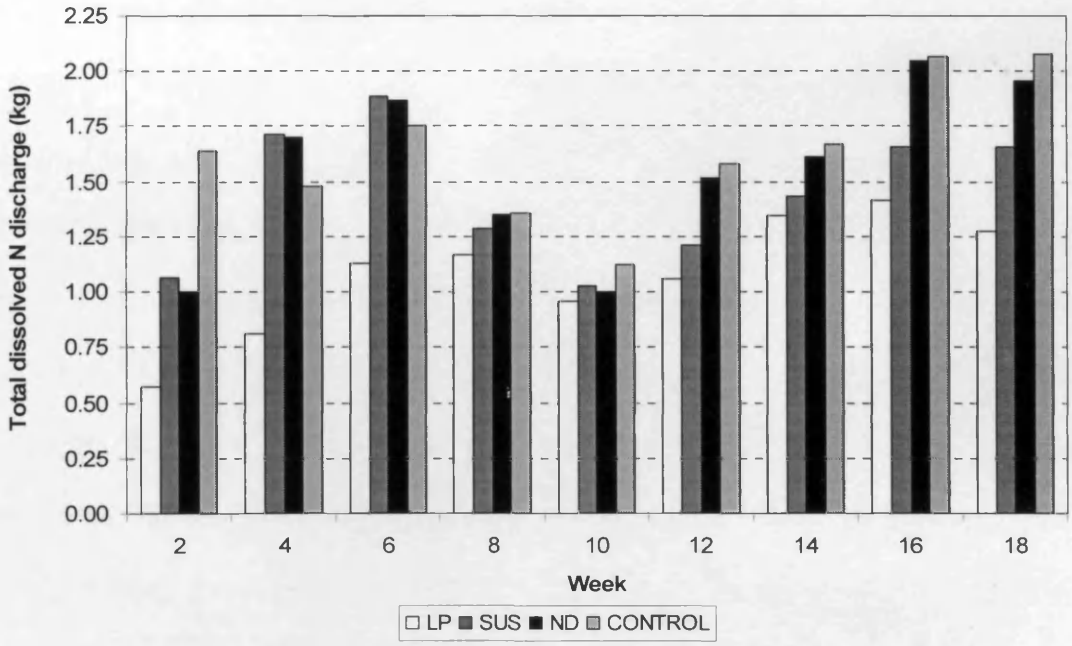


Figure 7.6 Total dissolved N discharge (kg) from all cages in time periods of two weeks throughout the duration of the trial from fish fed each of the four different diet types.

From the total dissolved N discharge rates obtained by the mass balance estimations, mean outputs were calculated in time periods of two weeks for each of the four diet types used in the trial (Figure 7.7). The LP diet had consistently lower dissolved N discharge rates, ranging from 0.19 kg at week 2 to 0.42 kg at week 16 compared to the other diets apart from week 10 were all values were similar. The dissolved N discharge rates from the other test diets were similar with no significant differences between them throughout the test period. The LP diet was significantly lower compared to the discharge rates from the SUS and ND diets at week 4 and significantly lower than all other diets at weeks 6 and 8 (Table 7.1). There were no significant differences at any other time period.

In the second simulation, the FCR values obtained for the four diets were used to estimate waste N outputs from a theoretical farm producing 1000 tonnes of salmon from smolt input to harvest (Table 7.1). For the LP diet, the N input in the feed was calculated to be partitioned as 49.8 % retained in fish growth, 16.4% lost as particulate waste and 33.8 % lost as dissolved waste. The predicted total amount of dissolved N released was 23.11 kg N T^{-1} fish produced. This represented 70 % of all waste produced. For the ND diet type, 53.2 % of the N input was retained as growth, 16% lost as particulate waste and 28.8 % lost as dissolved waste. The predicted total amount of dissolved waste was calculated to be 18.40 kg N T^{-1} fish produced which was 64.2 % of total waste produced.

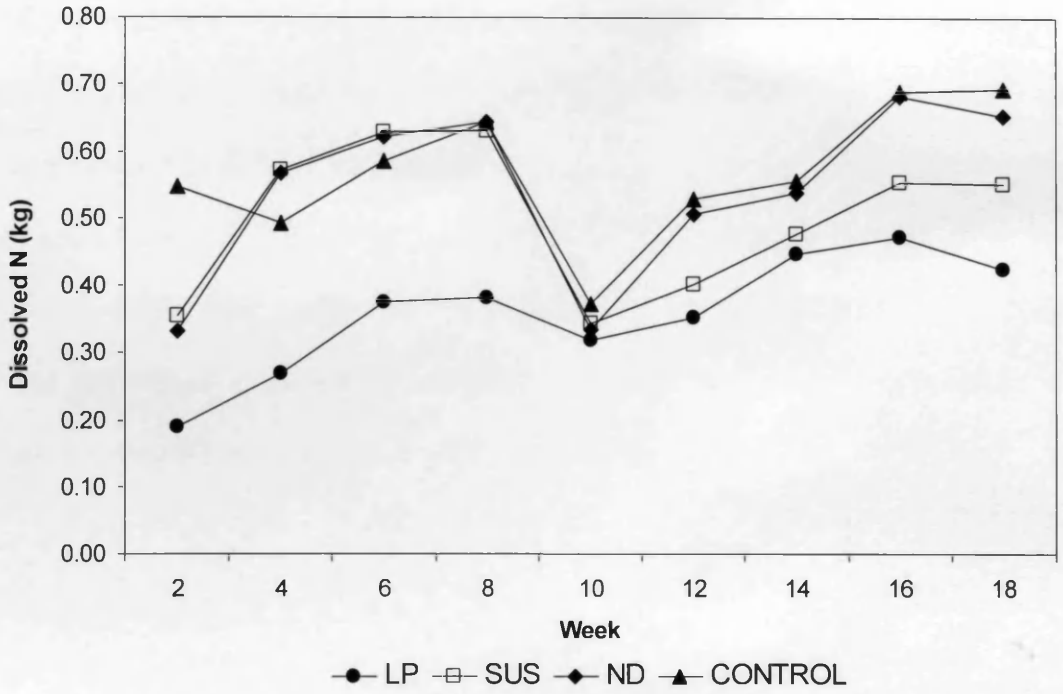


Figure 7.7 Mean dissolved N discharge rates in time periods of two weeks for the four different diet types.

Table 7.3 Results from analysis of variance (ANOVA) comparing mean dissolved N discharge rates from fish fed the different diet types. Where *P* is the significance level (d.f. = 3,8). Different superscripts on the same line, between diets, where found significantly different by Tukeys *post hoc* tests (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

	Diet types				ANOVA	
Week	**LP**	**SUS**	**ND**	**Control**	***F***	***P***
2	-	-	-	-	3.12	NS
4	a	b	b	ab	8.26	*
6	a	b	b	b	18.94	***
8	a	b	b	b	20.01	***
10	-	-	-	-	0.22	NS
12	-	-	-	-	2.44	NS
14	-	-	-	-	0.37	NS
16	-	-	-	-	2.08	NS
18	-	-	-	-	2.15	NS

For the SUS diet, 54.1 % of the total N input was retained as growth, with 16.6% and 27.3 % lost as particulate and dissolved waste respectively. The dissolved waste was calculated as 17.18 kg N T^{-1} fish produced which represented 62.0 % of all waste produced. The predicted amount of N retained as growth was 50.3 % of total N input for fish fed the control diet. 16.6 % was lost as particulate waste and 31.1 % lost as dissolved waste. The predicted total amount of dissolved N released was 21.03 kg N T^{-1} fish produced which represented 65 % of all waste produced (Table 7.1).

7.3.2 – Study 2

Feed input for each of the four groups can be seen in Figure 7.8. Feed input was highest during the second summer after the fish where transferred to sea. The maximum rate of fed input ranged from 172 tonnes in July to 175 tonnes in October per four week period. Feed input for each of the groups was similar throughout the trail period.

Monthly biomass gain for each of the four groups can be seen in Figure 7.9. All triplicate groups had similar increases in biomass throughout the trial period.

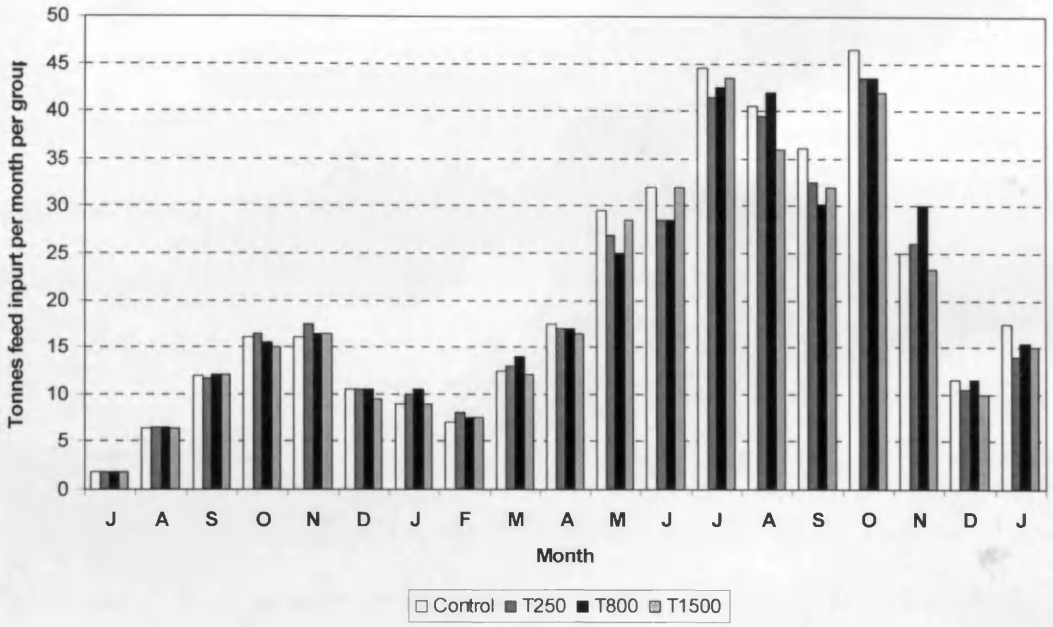


Figure 7.8 Actual feed input (kg) in time intervals of four weeks over the trial period for each of the four diets.

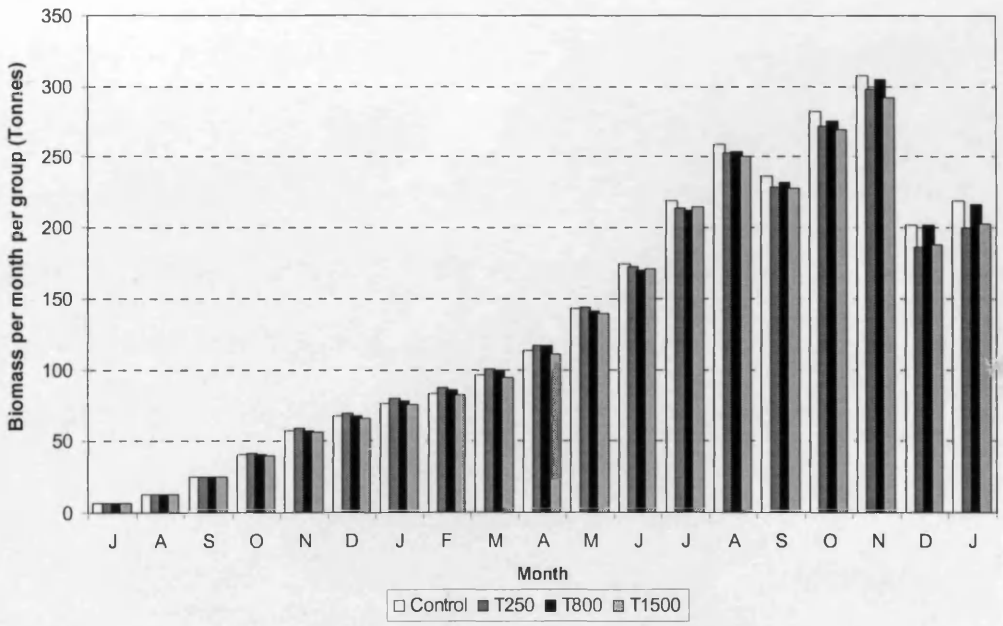


Figure 7.9 Actual monthly biomass gain over the trial period for each group of fish fed the different diet types.

Total monthly excretion of dissolved and particulate waste for each of the four groups can be seen in Figure 7.10. The maximum rate of total waste discharge occurred during July and October of the second year when feed input and biomass where at their maximum.

Figure 7.11 shows total particulate N discharge from each of the triplicate groups of fish over four week periods throughout the marine phase of production. Table 7.4 shows statistical differences for each four week period. There were no significant differences in particulate N discharge from each of the four groups between the start of the trial and November, 2003. In December of that year, fish fed the control diet excreted significantly more particulate matter compared to the T250 and T800 groups. There were no other significant differences between groups. There were no significant differences in particulate N discharge between January and April of the second year. However, between May and July, fish fed the control diet had higher particulate N discharge rates compared to the T250 and T800 groups. In August, the T1500 group had significantly less particulate N discharge compared to the control group. After this time until the end of January, 2005, there were no significant differences between the groups.

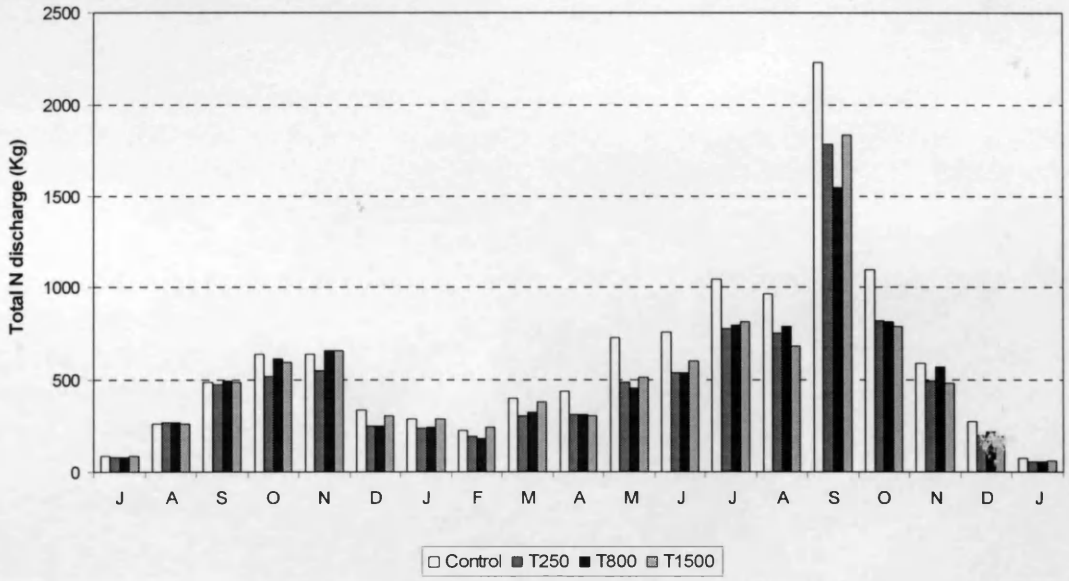


Figure 7.10 Total monthly N discharge (Kg) estimated by mass balance for each of the four groups of fish throughout the trial period.

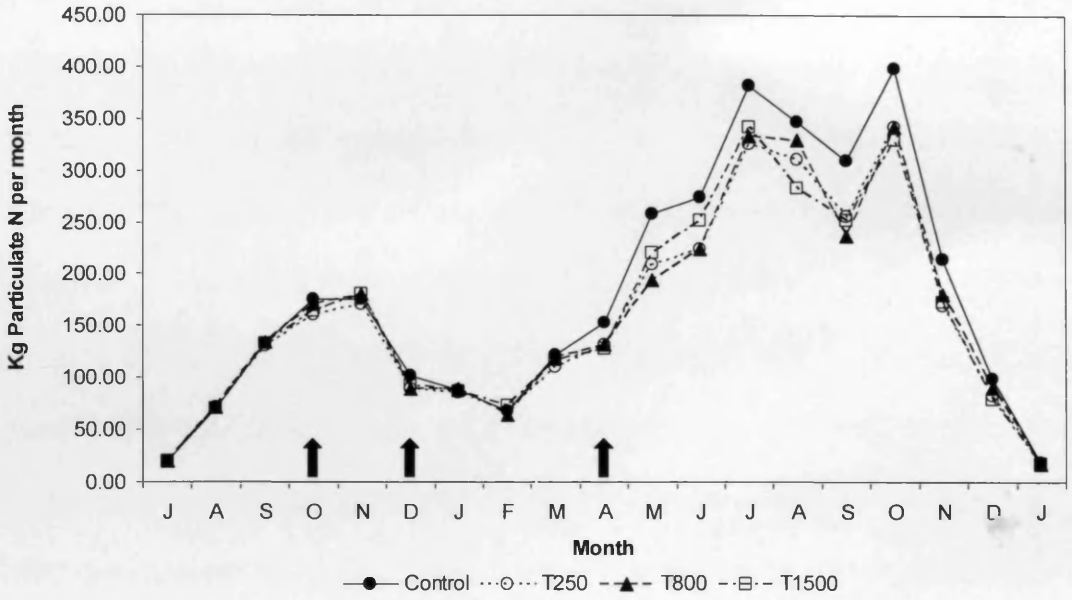


Figure 7.11 Modelled monthly particulate N excretion from groups of salmon fed diets containing different inclusion levels of protein. The arrows positioned at October, December and April indicate the introduction of low protein feeds to the C250, C800 and the C1500 groups respectively.

Table 7.4 Results from analysis of variance (ANOVA) comparing mean particulate N discharge rates from fish fed the different diet types. Where *P* is the significance level (d.f. =3, 8). Different superscripts on the same line, between diets, where found significantly different by Tukeys *post hoc* tests (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

	Diet types				ANOVA	
Month	**Control**	**C250**	**C800**	**C1500**	***F***	***P***
J	-	-	-	-	0.07	NS
A	-	-	-	-	0.99	NS
S	-	-	-	-	0.13	NS
O	-	-	-	-	1.23	NS
N	-	-	-	-	1.50	NS
D	a	b	b	ab	6.50	*
J	-	-	-	-	0.08	NS
F	-	-	-	-	0.52	NS
M	-	-	-	-	0.57	NS
A	-	-	-	-	3.69	NS
M	a	b	b	ab	8.48	**
J	a	b	b	ab	13.80	**
J	a	b	b	ab	6.39	*
A	a	ab	ab	b	4.44	*
S	-	-	-	-	3.47	NS
O	-	-	-	-	1.05	NS
N	-	-	-	-	0.05	NS
D	-	-	-	-	0.04	NS
J	-	-	-	-	0.01	NS

Figure 7.12 shows total dissolved N discharge from each of the triplicate groups of fish over four week periods throughout the marine phase of production. There is a clear decrease in the amount of dissolved N discharge on introduction of the low protein diets, immediately after they are introduced. Table 7.5 shows statistical differences for each four week period. There were no significant differences in dissolved N discharge during the period between July and September of the first year. From October in year one, the C250 group where fed a low protein feed which resulted in significantly lower dissolved N discharge from this point onwards (Table 7.5). In December of the first year, the C800 group were fed a low protein fed onwards. During this month the C250 and C800 group had significantly lower dissolved N discharge compared to the control and C1500 groups. There were no significant differences between groups in January even though both the C250 and C800 groups had lower dissolved N discharge. In February only the C800 group had significantly lower dissolved N discharge compared to the C1500 group. In March, both the C250 and C800 groups had significantly lower N discharge compared to the control group with only the C800 group being significantly lower compared to the C1500 group. In April the third group of fish (C1500) were fed a low protein feed from this point onwards. All the low protein fed groups had significantly lower dissolved N discharge compared to the control group. This trend continued up to December of the second year. In January, there were non significant differences between the groups.

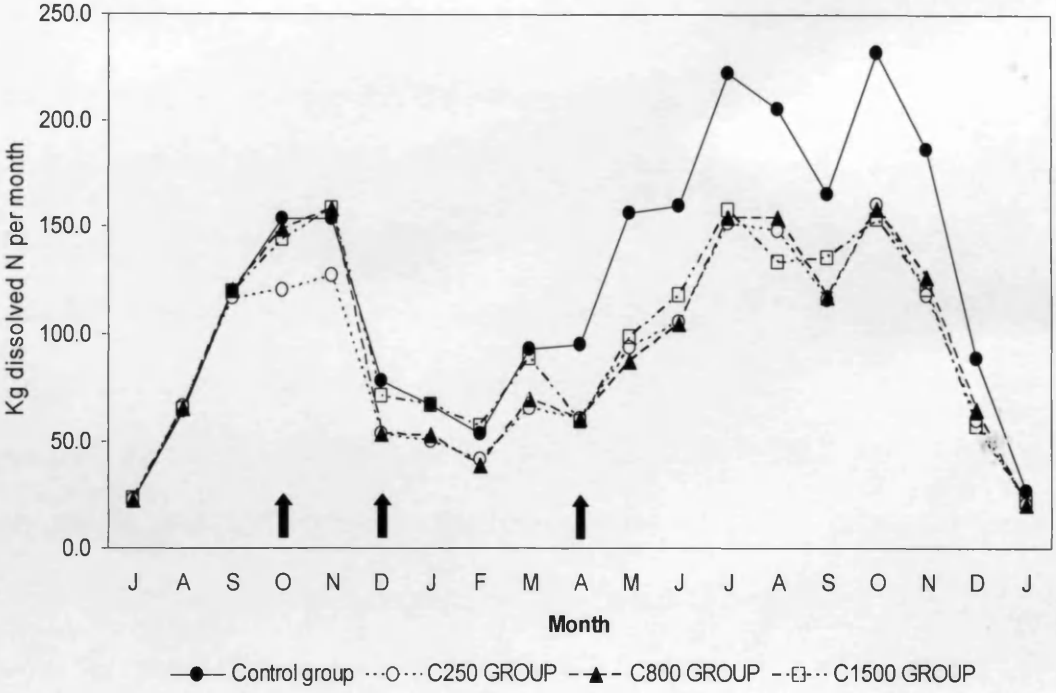


Figure 7.12 Modelled monthly dissolved N excretion from groups of salmon fed diets containing different inclusion levels of protein. The arrows positioned at October, December and April indicate the introduction of low protein feeds to the C250, C800 and the C1500 groups respectively.

Table 7.5 Results from analysis of variance (ANOVA) comparing mean dissolved N discharge rates from fish fed the different diet types. Where *P* is the significance level (d.f. =3, 8). Different superscripts on the same line, between diets, where found significantly different by Tukeys *post hoc* tests (NS = not significant; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$).

	Diet types				ANOVA	
Month	**Control**	**C250**	**C800**	**C1500**	***F***	***P***
J	-	-	-	-	0.09	NS
A	-	-	-	-	0.36	NS
S	-	-	-	-	0.35	NS
O	b	a	ab	ab	5.06	*
N	ab	a	b	b	5.28	*
D	b	a	a	b	51.64	***
J	-	-	-	-	4.96	NS
F	ab	ab	a	b	5.76	*
M	b	a	ac	bc	9.58	**
A	a	b	b	b	42.13	***
M	a	b	b	b	60.79	***
J	a	b	b	b	74.58	***
J	a	b	b	b	54.26	***
A	a	b	b	b	30.70	***
S	ac	b	b	c	85.48	***
O	a	b	b	b	7.29	*
N	a	b	b	b	48.39	***
D	a	b	b	b	27.62	**
J	-	-	-	-	4.53	NS

In this simulation, the overall mean FCR values obtained for each test diet coupled with information on N content of each diet, biomass gain and total feed input at the end of production were used to estimate total waste outputs for each group based on the model simulating 1000 tonnes of salmon from smolt input to harvest. The data is summarised in Table 7.2. For the control diet with a dietary N content of 6.43 % and overall FCR of 1.05, the N input in the feed was calculated to be utilized as 15 % in particulate waste, 50.4 % retained a fish growth and 34.5 % lost as dissolved waste. The total amount of dissolved waste released over the complete marine phase of production was 23.32 kg N tonne fish produced. For the C250 diet with a dietary N content of 5.99 % and overall FCR of 1.11, N input was partitioned as 33.85 and 15% for dissolved and particulate waste respectively. 51.1 % was retained as fish growth. Total amount of dissolved waste was 22.51 kg N tonne fish produced. The C800 and C1500 diets had similar N content as the C250 diet. For the C800 diet, 15 % of the N input was partitioned as particulate waste with 33.42 and 51.6 % partitioned as dissolved waste and retained as growth respectively. Total dissolved N released was 22.01 kg N tonne fish produced. For the C1500 diet, of the total N input, 15 % was partitioned as particulate waste and 32.56 % lost as dissolved N. 53.6 % was retained as fish growth with total dissolved N discharge over the full growth cycle being 21.07 kg N tonne fish produced.

7.4 – Discussion

The mass balance approach can prove useful by providing insights into why and where wastes occur but discrepancies exist. It takes no account of what happens to the wastes once it enters the environment (i.e. how much solids remain as solids or how much dissolves). Also there is little information on which form the wastes exist as (i.e. how much N wastes is organic and how much inorganic and what proportion of the latter is in the form of nitrate, nitrite or ammonia).

The mass balance model for nitrogen showed that between 62 and 70 % of the total N discharge was excreted as ammonia for fish fed the diets assessed in the first study and between 68 and 69 % of the total N discharge was excreted as ammonia for fish introduced to low protein diets (study 2). These results are lower than estimates in other studies which suggest that ammonia represents 70-90% of the total nitrogenous wastes from fish (Randall and Wright, 1987; Fivelstad *et al.* 1990; Kaushik and Cowey, 1991; Dosdat *et al.* 1996; Forseberg, 1996).

The results from the second simulation used to estimate waste N outputs from a theoretical farm producing 1000 tonnes of salmon from smolt input to harvest also showed that between 27 and 34 % of the estimated N intake was excreted as TAN by fish fed diets assessed in study 1 and between 33 and 35 % for diets assessed in study 2. These values are similar to values obtained by (Forsberg, 1996) on post-smolt Atlantic salmon which showed that about 35% of the estimated nitrogen intake was excreted as TAN. Taken over a full marine phase of production, the release of dissolved N is likely to be in the

range of 21 – 23 kg N tonne fish produced for fish fed low protein diets and between 17 and 23 Kg N tonne fish produced for fish fed different diet types. This is much less than GESAMP (1996) estimates (75 – 120 Kg N tonne fish produced) and less than estimates provided by Davies, (2000) (35 – 45 Kg N tonne fish produced). However, the estimated by Davies, (2000) were based on fish being fed high energy feeds with a higher dietary N content and a higher calculated FCR.

For the diets assessed in the first study, fish fed the LP diet type had the highest dissolved waste output compared to fish fed the other diet types (23.1 Kg N tonne fish produced) but had similar dissolved waste output compared to fish fed the low protein diets in study 2. The higher dissolved waste output may be attributed to the higher FCR obtained for this group and/or the higher N content of the diet which was determined using an elemental analyser. The N content was determined to be higher compared to the control diet even though the LP diet had less protein (31.5 %) compared to the control diet (35.6 %), thus it was assumed that this was a potential source of error. Due to a lower FCR, dissolved waste outputs for fish fed the ND diet were found to be lower compared to the other groups.

Fish fed the other three diet types (namely, the ND, SUS and control diets) had lower dissolved N discharge compared to waste outputs determined from fish introduced to low protein diets. This may be explained by the fact that the simulated waste outputs were determined from data generated over only three months whereas, the model outputs from fish fed low protein diets were derived

using real averaged farm records from full scale marine production. This would give a more accurate determination of actual waste output.

The compositions of the feed changes as fish grow. Smolt diets generally contain higher inclusion levels of protein compared to diets for larger fish. However, the fish assessed in study 2 were introduced to low protein diets throughout the growth period to assess the optimal time for introduction to low protein feeds without compromising on growth and performance (see chapter 3).

Introducing fish to low protein diets does not necessarily result in significantly poorer growth compared to commercial grower diets used in the industry at present. The FCR values used in this study ranged from 1.05 for fish fed the control diet to 1.1 for fish fed the C250 and C800 test diets. Cleary there is potential to maximise use of low protein diets, which are clearly more sustainable in terms of the amounts of marine fishmeal that would be required to achieve reasonable performance. This would represent a significant economic saving as well as reducing reliance on fishmeal.

The total amount of Atlantic salmon produced in Scotland in 2002 was 145,609 tonnes. The amount of feed required to be purchased by the Scottish aquaculture industry, based on an FCR of 1.3, would be 182, 292 tonnes. A reduction of even 2% in the amount of protein incorporated into fish feeds given protein inclusion levels of 30 – 45% would result in a reduction of 3645 tonnes of protein that would otherwise be used in the manufacture of fish feeds. However, introducing fish to low protein diets resulted in a reduction in the amount of protein used by approximately 9 % compared to feeding fish a

normal commercial ration. This represents an even greater reduction in the amount of protein required to be used in the manufacturing of fish feeds.

Studies have shown that variations in ammonia levels were only found during slack tides (Gowen *et al.* 1989; Aure and Stigebrandt, 1990; Weston, 1990). However, studies by Wallin and Håkanson (1991 a, b) on Swedish and Finnish coastal cage farms found strong correlations between fish farm loadings and dissolved nutrient levels. Thus, any measures that reduce the amount of dissolved wastes entering and their potential to impact on the environment would be beneficial if the industry is to continue expanding.

7.5 – Conclusions

The use of mass balance models can be used to provide estimates of dissolved and particulate wastes from intensively farmed fish. Comparisons with established estimates indicate that the use of low protein feeds result in comparatively lower rates of N released to receiving water bodies. As the industry strives to become more sustainable in its use of alternatives to marine fish meals and oils and coupled with the use of low protein diets, improvements in FCR values by approximately 0.05 per growth cycle by the Scottish industry (Davies, 2000) and if reductions in feed wastage and indigestibility can be achieved, the release rate of dissolved N would be further reduced compared to what can be achieved by the use of low protein diets.

Based on the results the hypothesis "Feeding Atlantic salmon different diet types does not result in differences in the rate of discharge of particulate and

dissolved wastes" may be rejected as fish fed the LP diet type in study one had a higher dissolved and particulate N discharge rate compared to fish fed the ND, SUS and control diets. In addition, results from the second study showed that fish fed low protein diets had significantly lower dissolved and particulate N discharge rates compared to fish fed the control feed.

Chapter 8 - Investigation into waste outputs from farmed Atlantic salmon (*Salmo salar* L.) using enclosed net pens

8.1 - Introduction

One of the main constraints of using a mass balance approach to estimate waste outputs from intensively farmed fish is the reliance on the use of several assumptions in order for the model to function. For example, most mass balance models assign a fixed value for the estimation of waste feed. Accurately determining the amount of waste feed is extremely difficult in large net pens and even in small-scale net pens may prove difficult without the aid of air up-lift systems. Further assumptions are often based on the percentage inclusion levels and the digestibility of the macronutrient components of the diets being assessed. In addition, the amount of a particular macronutrient retained in fish as somatic growth is also often assumed. For example, for carbon, the amount incorporated into body tissues is assumed at 14.3 % (Chen, 2000), whilst for nitrogen (N), the amount retained in fish is reported to be 3.4 % (8.1 % N as dry weight) (Ackefors and Enell, 1990). This value is often used in mass balance models to estimate nitrogen budgets. However, a study by (Ramseyer, 2000) using regression relationships has shown that for Atlantic salmon of 250 g, the amount of N retained in flesh is 3.0 % whilst for larger fish (4000 g), the amount has been calculated as 2.7 %. Clearly there is variation in the amount of N retained as somatic growth and both values are less than the value often used.

Although the assumptions used are often based on historical data and/or data supplied by feed companies for example, the fact remains that the models rely on these assumptions in order to determine waste outputs for any given diet.

Given that it has been shown in previous chapters (chapters 2 and 3) that it is now possible to reduce reliance on fish meals and fish oils and also reduce the amount of protein incorporated into diets fed to Atlantic salmon coupled with the need for determining waste outputs from intensively farmed fish species such as Atlantic salmon (as discussed in the previous chapter) due to the growing concern that waste outputs are impacting on the marine environment , It was decided to attempt to undertake a novel approach for collecting data on the amounts of dissolved and solid wastes discharged from farmed fish using enclosed net pens. Enclosed net pens or "closed containment units" have recently been developed by Future SEA Technologies (B.C. Canada) and have been used to intensively produce salmonids. They were designed to minimise the risk of escapees and to remove solid wastes thus reducing any potential environmental impacts. However, they have not been used to quantify waste outputs and have only been used commercially on a small scale.

The initial aim of the experiment was to examine the feasibility of enclosing a known biomass of Atlantic salmon in order to measure waste outputs. It was envisaged that replicate groups of fish would be transferred into the systems prior to the start of any feed trail thus not only allowing different diets and feeding strategies to be assessed it terms of growth and dietary performance but also allowing for accurate determination of wastes outputs.

A custom-made tarpaulin was designed to completely enclose a feed trail cage and once fish were enclosed, feed input would be recorded and waste feed and faeces collected. In addition, water samples would be taken to determine ammonia excretion rates. Using this data, it was hoped that it would be possible to accurately determine waste outputs for fish fed a particular diet type.

The ultimate aim of the study was to collect sufficient data in order to construct accurate mass balances of waste outputs from fish reared under commercial conditions and to compare waste outputs from fish fed different feed types and feeding strategies.

8.2 – Materials and methods

8.2.1 – Experimental design

The field trials described here were conducted at Gifas Research Station, Inndyr, Norway. Four custom-made impermeable collapsible tarpaulin systems were designed (Figure 8.1) which could be raised at any time to completely enclose a trial cage (5 x 5 x 5 m) to allow for the determination of waste outputs from fish as they were fed (manufactured by W & J Knox Ltd, Scotland). Each system had a centre hole fitted with an air up-lift system to allow for collection of faecal and feed waste and had a 2 m section of ordinary net attached to the top of the tarpaulin to allow for water exchange when the system was in the collapsed position.

During initial deployment, each system was shown to loose shape and rigidity during incoming and outgoing tides. Steel reinforcing rods were then inserted into loops that ran the length of the tarpaulin at the top, middle and bottom to help maintain rigidity and shape during the tidal cycle. Although distortion of the system was reduced, it was decided that a better solution was required. To this end, one tarpaulin system was modified by installing two steel pipes on two sides of the square cage 0.5 m below the vertical sides of the pen (Figure 8.1).

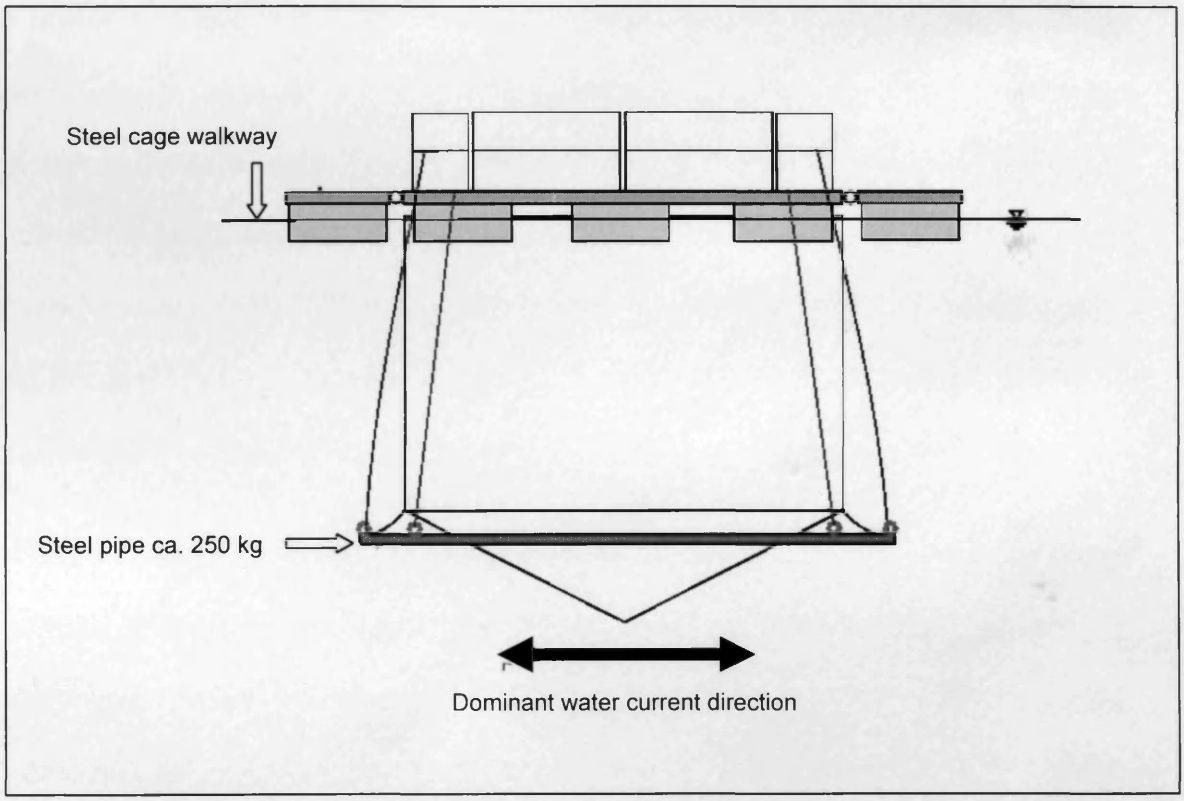


Figure8.1 Diagrammatic representation of the tarpaulin system used showing the steel pipes attached to prevent distortion.

The steel pipes were installed in the same direction as the dominant water current to minimize distortion and consequently volume reduction within the enclosed tarpaulin. The steel pipes were installed in such a way that it was not necessary to pull them up when the pen was removed for maintenance.

Further modifications were made by sewing on an ordinary cage net to the inside of the tarpaulin at the bottom of the sides (Figure 8.2) to further enhance water exchange when the system was in the collapsed position as well as providing additional security to prevent fish escaping. A pulley system was also designed and fitted to the tarpaulin in order to ease deployment and collapsing of the system when required. Figure 8.3 shows the tarpaulin system in the (a) collapsed position and (b) in the raised position.

The modified system was then deployed for a period of 20 weeks without fish to test its strength and reliability. During this time, no damage occurred and the deployed tarpaulin retained its shape throughout. The tarpaulin was then removed and cleaned and redeployed prior to the stocking of fish into the system.

8.2.2 – Preliminary studies

A series of studies was undertaken to assess the potential use of such as system. After the system was deployed in the collapsed position, 20 large Atlantic salmon with a mean weight of 2 kg were transferred into the cage. Daily feed intake was recorded to establish the amount of time required for fish to acclimatise to their new habitat and show a normal feeding response.

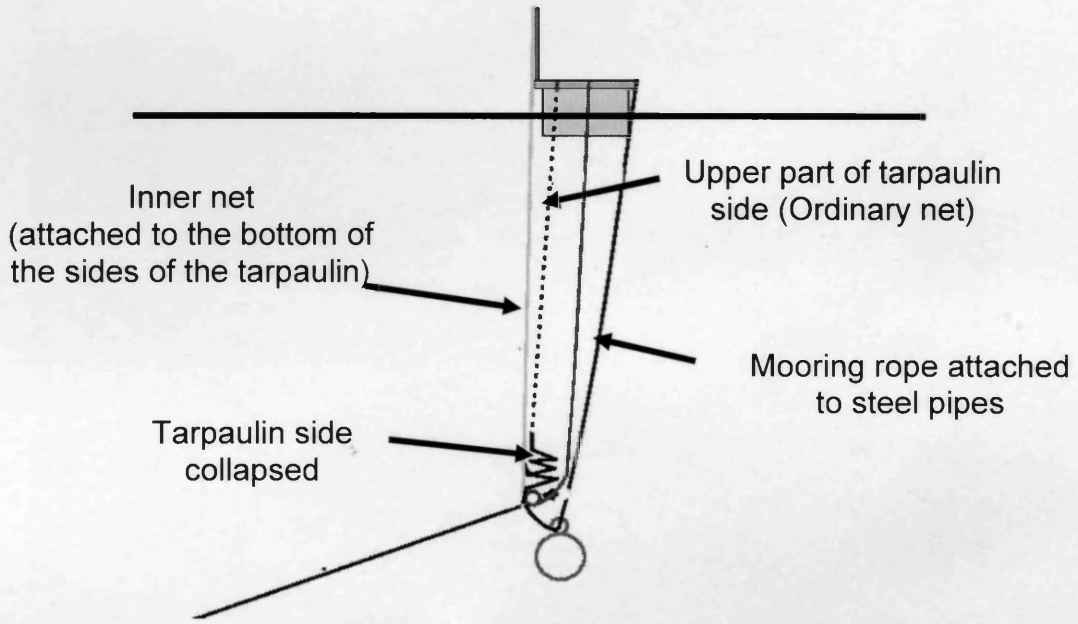


Figure 8.2 Diagrammatic representation of the tarpaulin system showing the pulley system for collapsing and raising the tarpaulin.

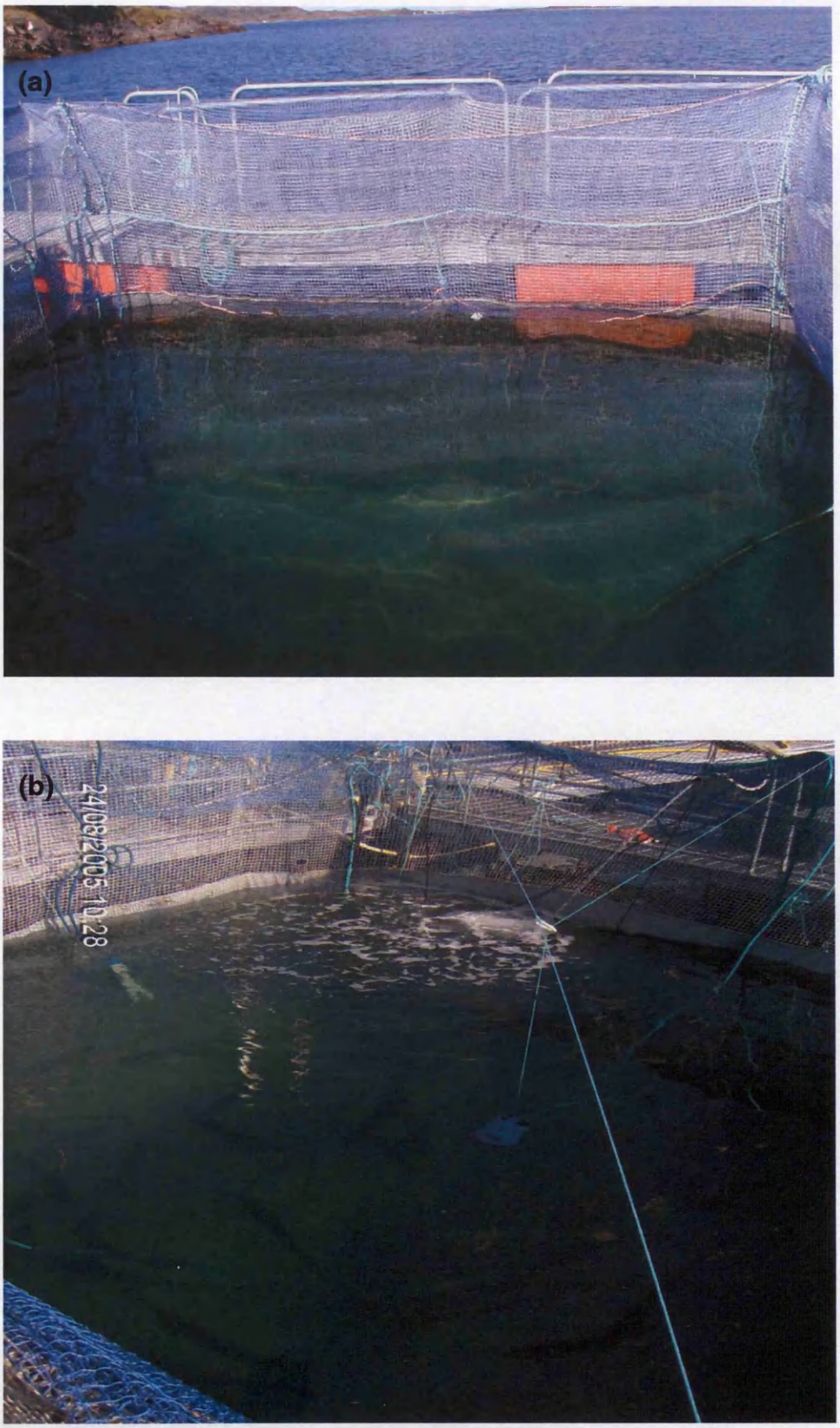


Figure 8.3 The tarpaulin system in the (a) collapsed position and (b) in the raised position.

Feeding behaviour was compared to a similar amount of fish located in a normal net pen. In addition, the system was enclosed for short time periods during each day then collapsed. It was show that the period of adaptation was approximately three days before the fish show a similar feeding response to fish in a normal net pen and appetite was not affecting by raising or lowering the tarpaulin sides after this time period. An initial study was then planned to collect data on waste outputs from a known biomass of fish and to measure variations in water quality throughout a period of time whilst the system was in the closed position.

8.2.2.1 – Study 1

An initial trail was initiated with a total of 164 Atlantic salmon with a mean weight of 485 g being transferred into the modified cage. Two separate studies were undertaken on the 26th (part 1) and 28th November (part 2), 2004.

8.2.2.1.1 – Part 1

Daily feed intake, temperature and salinity was recorded once fish had been transferred to the system and any mortalities recorded (Table 8.1). Once fish had a period of acclimatization to the system (seven days) and the feeding response was similar to fish fed in normal cages whilst the tarpaulin was raised, the study commenced. The fish were fed at 10 am and the cage was then enclosed with the tarpaulin.

Table 8.1 Daily feed input, salinity and temperature along with any mortality recorded once fish were transferred to the system.

Day	Feeding (g)			Dead Fish (g)		Salinity (5M)	Temperature (°C)		
	Start	End	Feed input (g)	No.	Weight	(psu)	1m	2m	3m
1	4470	3675	795	-	-	32.4	6.7	7.1	8.3
2	3675	3120	555	-	-	31.7	6.5	6.6	8.3
3	1709	936	773	-	-	33.6	7.8	8.1	7.9
4	936	764	172	-	-	33.0	7.0	7.3	8.0
5	764	460	304	-	-	32.3	6.7	7.6	7.8
6	0	0		-	-	33.2	6.9	7.3	7.6
7	1711	927	784	-	-	33.0	7.0	7.3	7.6

Table 8.2 Times of feeding fish during both studies showing feed input and waste pellets collected with the air up-lift system. Daily temperature and salinity are also shown.

	Start (g)	End (g)	Feed Input (g)	Waste pellets	Temperature (°C)	Salinity (psu)
1st Study						
1st Feeding	764	660	104	4	7.37	32.3
2nd Feeding	660	460	200	0	-	-
2nd Study						
1st Feeding	1711	1288	423	-	7.30	33.0
2nd Feeding	1288	927	361	-	-	-

No water exchange occurred during the experimental period. Fish were continually monitored for signs of stress. A second feed input was offered to the fish at 12:45 pm. The study continued until 3 pm. The amount of feed delivered to the fish for each meal was recorded (Table 8.2). Oxygen was available if required if fish showed signs of stress.

Water samples were taken every 15 minutes from a predetermined positions along a transect at three different depths (surface, 1, 2 and 3 m). Daily temperature was recorded at 1, 2 and 3 m depth and salinity recorded at 5 m depth at the edge of the cage block. It was planned to record water parameters (dissolved oxygen, pH and salinity) within the enclosed cage every 15 minutes but due to equipment failure this was not possible due to equipment failure. The water samples were then transferred to the laboratory and filtered through Whatman GF/C filter paper and then analysed for ammonia using the method used the indophenol blue method as described by Strickland and Parsons (1972). From each water sample; pH was measured using a pH probe (WTW pH 330i). Using the data obtained from the pH and ammonia analysis in conjunction with water temperature, the concentration of unionized ammonia was determined from each sample using a calibration graph (Stirling University) where the pKa was determined as:

$$pKa = ((-0.0325 * T) + 10.055)$$

Where:

T is degrees centigrade for each water sample.

Percent unionised ammonia (%UIA) was determined using the following equation:

$$\%UIA = 100 / (1+10)(pKa- pH)$$

Total unionised ammonia (μg L^{-1}) was determined using the equation:

$$\text{Total UIA } (\mu g\ L^{-1}) = TAN * (\%UIA / 100)$$

Where:

TAN is total ammonia in each sample (μg L^{-1}).

The system was also fitted with an air up-lift system to collect any waste feed and faeces. The lifted water runs through two filters (one for food and the other for faeces). The initial studies were only undertaken to show that it was possible to collect both waste feed and faeces from the system and no laboratory analysis was undertaken on the collected samples. When the experiment started, the up-lift system was activated and any faecal waste that has accumulated throughout the night was discarded.

8.2.2.1.2 – Part 2

A second experiment was conducted two days after the initial study. In this study, the cage was enclosed prior to feeding fish. The study started at 09:45 am with feeding commencing at 10 am and again at 1:30 pm (Table 8.2). All methods undertaken were the same as for study one.

8.2.2.2 – Study 2

A second set of studies were undertaken on 23rd (part 1) and 25th (part 2) August, 2005. A total of 42 Atlantic salmon with a mean weight of 2.7 kg were used in the study. The experiment commenced after five days post-transfer. Daily feed intake, temperature and salinity was recorded once fish had been transferred to the system and any mortalities recorded (Table 8.3).

8.2.2.2.1 – Part 1

For the first trial, the tarpaulin was raised at 12 pm and the fish were fed immediately after. Feeding was based on providing two meals per day and feeding to satiation. The study continued for 24 h and no water exchange occurred during the experimental period. Fish were continually monitored for signs of stress. Subsequent feed inputs were offered at 5 pm and 7am the following morning. The amount of feed delivered to the fish for each meal was recorded (Table 8.4). Oxygen was available if required. The fish were fed again at 3 pm the following day (intermediate feed) after the cessation of the trial.

8.2.2.2.2 – Part 2

For the second study, the fish were fed the same amount of feed based on the expected increase in initial biomass and relative feed index (FI) but the feed was offered in small hourly increments throughout daylight hours. The study commenced at 2pm and continued for 24 h with no water exchange occurring during the experimental period

Table 8.3 Daily feed input, salinity and temperature along with any mortality recorded once fish were transferred to the system.

Day	Feeding (g) Start	End	Feed input (g)	Dead Fish (g) No.	Weight	Salinity (5m) (psu)	Temperature (°C) 1m	2m	3m
1	-	-		-	-	31.2	12.6	12.5	12.,4
2	1509	1243	266	-	-	31.7	12.2	12.1	12.2
3	1409	1100		-	-	31.8	12.3	12.2	12.3
4	1100	951	309	-	-	32.6	12.1	12.0	12.2
5	823	738	85	-	-	32.1	12.2	12.2	12.1

Table 8.4 Times of feeding fish during both studies showing feed input and waste pellets collected with the air up-lift system.

	Start (g)	End (g)	Feed Input (g)	Waste pellets
Part 1				
1st Feeding	700	542	158	1
2nd Feeding	542	382	160	0
3rd Feeding	1080	765	315	2
Totals			633	3
Intermediate feed input			110	
	Start	**End**	**Feed Input (g)**	**Waste pellets**
Part 2	2pm	10pm	441	1
	6am	9am	196	1
Totals			637	2

The initial bulk weight of fish upon transfer to the system was 112.09 kg and feed input prior to the first study was 809 g. Using the assumption that the expected FCR would be 1.00, the initial biomass prior to study one was calculated as 112.89 kg. Feed input during study one was calculated as 633 g, thus the relative FI was:

$$0.633 / 112.89 \times 100\% = 0.561\ \%$$

The initial biomass for study two was calculated as:

$$112.89 + 0.633 + 0.11 = 113.64\ kg$$

Where:

0.11 is the amount of feed (kg) eaten for the intermediate meal.

Feed input for study two was calculated as:

$$0.561\% \times 113.64 = 637\ g$$

The total amount of feed was fed at 49 g increments from 2pm to 10 pm and from 6am to 9am the following day.

Water samples were taken every hour during both studies following the same sampling protocols as for the initial trail. Hourly temperature and salinity were recorded inside the system at surface, 1, 2 and 3 m depth using an automatic datalogger (Aaderaa 3660, Aanderaa Instruments, Nesttun, Norway).

It was not possible to record dissolved oxygen due to equipment failure. Water samples were transferred to the laboratory and analysed following the same protocols as for the initial study. From each water sample; pH was measured

using a pH probe (WTW pH 330i). Using the data obtained from the pH and ammonia analysis in conjunction with water temperature, the concentration of unionized ammonia was determined from each sample using a calibration graph. Ammonia concentration was calculated as μg l^{-1} or per kg of fish (wet weight) and calculated per kg feed fed to the fish during each sampling day (μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$,).

8.2.2.3 – Statistical analysis

Statistical significance of differences between ammonia outputs for both studies in August, 2005 was computed from one-way analysis of variance (ANOVA). using Minitab™ version 13 statistical software (Ryan and Joiner, 1994) The normality and homogeneity of the variance of all data sets was tested prior to parametric statistical analysis. Normality was tested by graphic examination of probability plots and the Anderson-Darling test. Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$) and results are presented as mean values ± standard error of the mean (SE). From the graphs plotted in the studies undertaken in August, 2005, the area under the curve was calculated using NCSS/PASS statistical software (Utah, USA) to allow for direct comparison of total and post-five hour ammonia excretion between feeding strategies. To allow for direct comparison, the first sample from each trail was subtracted from all subsequent samples. Regression analysis was used to determine the relationship between ammonia production

from fish fed two meals per day and from fish fed the same amount of food provided in small regular amounts.

8.3 – Results

8.3.1 – Study 1

The results for the ammonia analysis from the first study can be seen in Figure 8.4. There was a significant food related concentration peak of total ammonia (TAN) detected at 12.15 pm (33.26 μg L^{-1}) and a lesser one detected at 12.45 pm (26.99 μg L^{-1}). TAN detected in the enclosed system appeared to increase from 2 pm onwards until the end of the experiment. Total unionised ammonia (T_{UIA}) detected (Figure 8.5) over time followed the same trend as for TAN with the T_{UIA} concentration peaks of 0.58 and 0.49 μg L^{-1} representing 1.7 and 1.8 % of TAN detected. For the second study, TAN detected can be seen in Figure 8.6. There was a significant food related concentration peak of total ammonia (TAN) detected at 13.30 (35.35 μg L^{-1}). There was no clear increase in TAN detected with time. Total unionised ammonia (T_{UIA}) detected (Figure 8.7) showed that the T_{UIA} concentration peak of 0.51 μg L^{-1} represented 1.44 % of TAN. For pH, the first study showed that there was a slight increase detected with time (Figure 8.8) until 2 pm followed by a decrease till the end of the study period with values ranging from 8.04 to 8.10. For the second study (Figure 8.9), a clearer pattern was noted with a slight increase detected at the start followed by a decrease in pH from 10.15 am onwards with a mean pH detected at the end of the study period of 7.95.

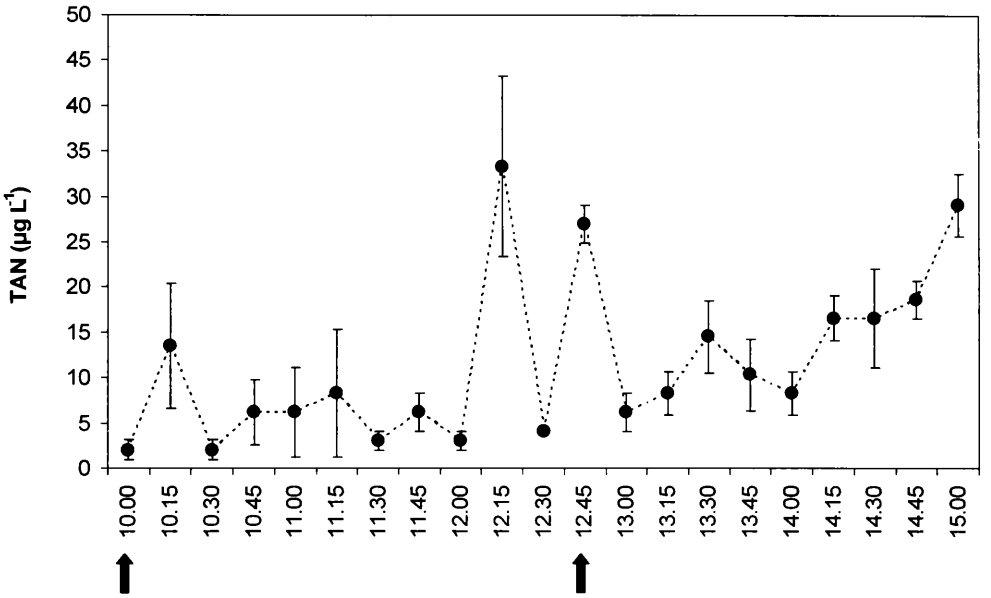


Figure 8.4 Total ammonia excretion (µg l^{-1}) detected during the first study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4). Arrows indicate time of feeding.

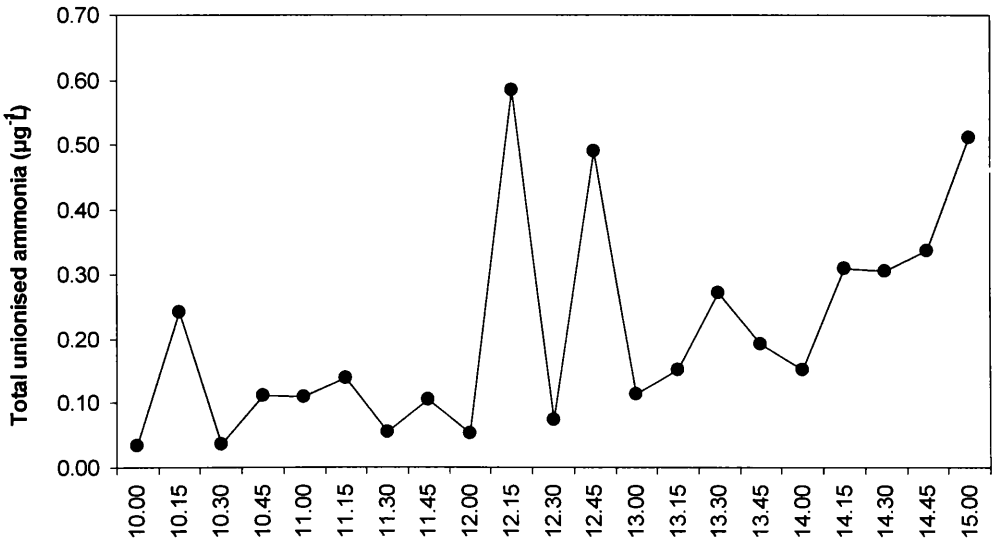


Figure 8.5 Total unionized ammonia (µg l^{-1}) detected during the first study. Data are shown as mean values from each time period.

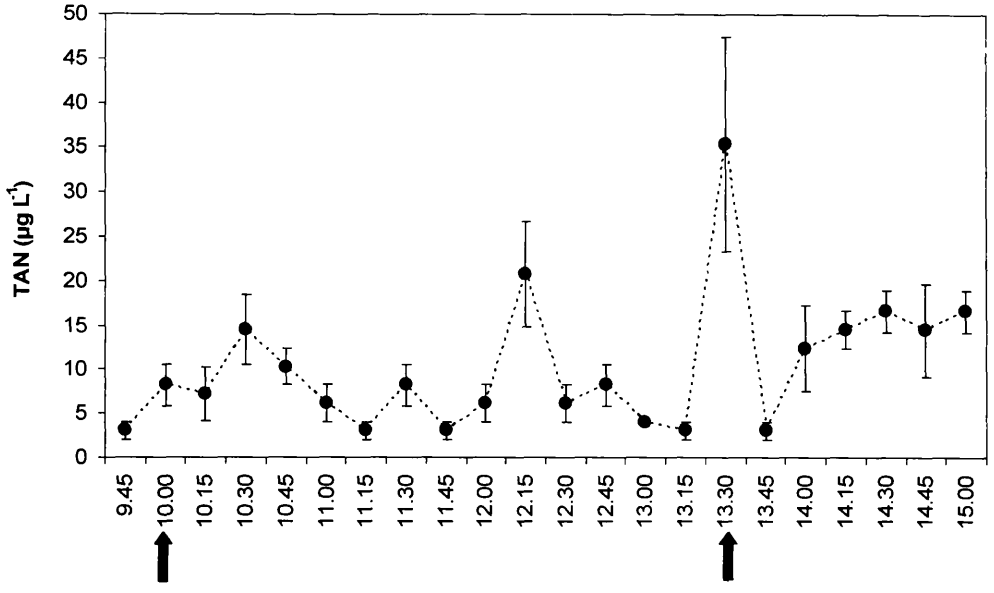


Figure 8.6 Total ammonia excretion (µg l^{-1}) detected during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4). Arrows indicate time of feeding.

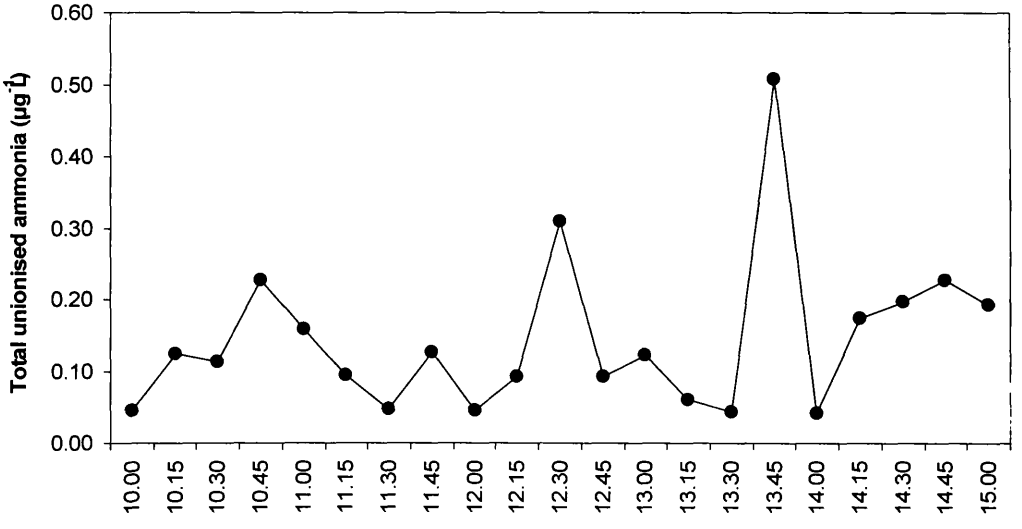


Figure 8.7 Total unionized ammonia (µg l^{-1}) detected during the second study. Data are shown as mean values from each time period.

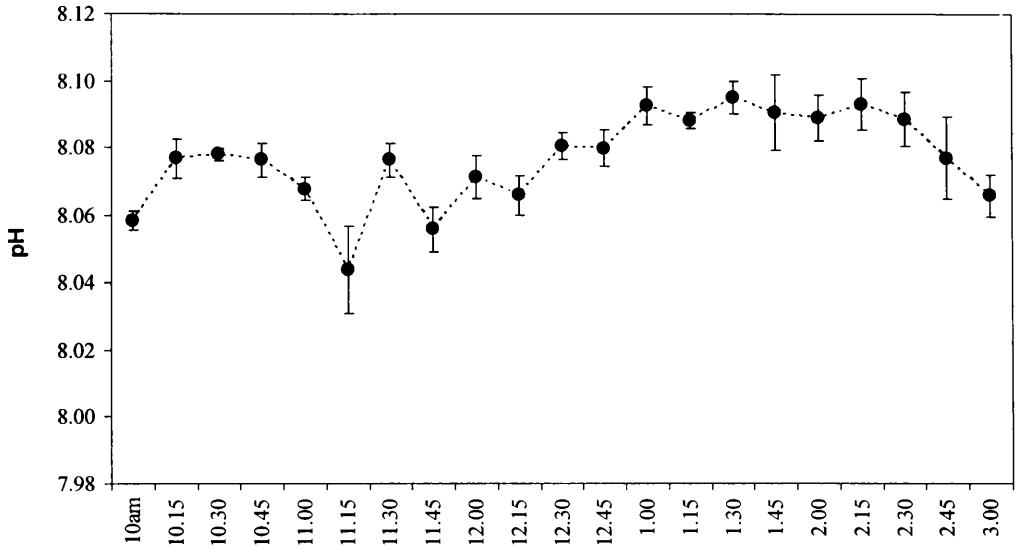


Figure 8.8 pH detected over time during part 1. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

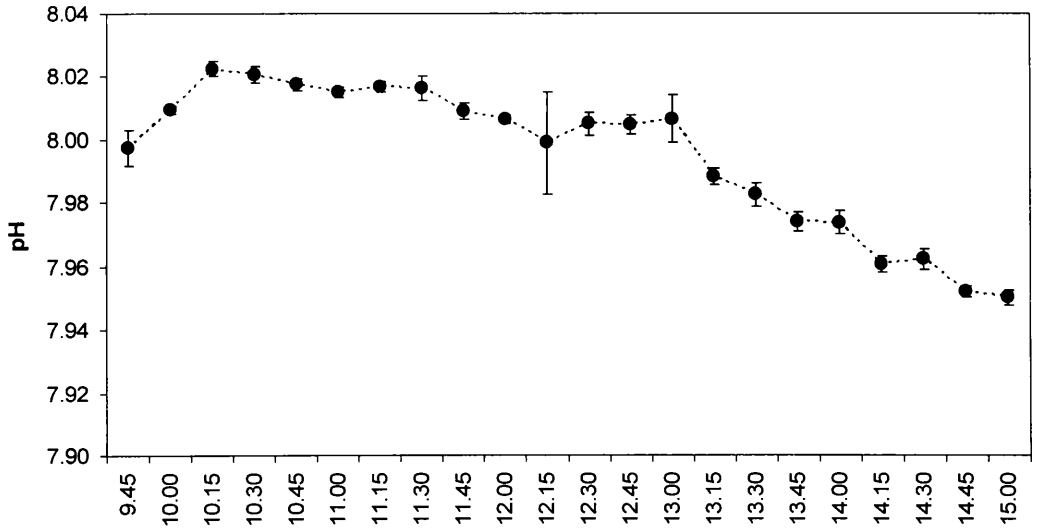


Figure 8.9 pH detected over time during part 2. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

8.3.2 – Study 2

The results for the ammonia analysis from the second study undertaken in August, 2005 can be seen in Figure 8.10. From the first trail, ammonia concentration increased over the 24 h period with two peaks detected at 9 h and again at 23 h of 131.71 and 296.05 μg L^{-1} respectively. The percentage of total ammonia in the unionised form (UIA) ranged from 0.54 to 3.06 μg L^{-1} (Figure 8.11). These values represented on average 1.6 % of total ammonia per sample.

Figure 8.13(a) shows variation in water temperature within the enclosed system. Temperature initially decreased from 12.4 °C at the start of the trial to 12.1 °C after 14 h. This was followed by an increased to 12.5 °C at the end of the trial. There was a decrease recorded of 12 3 °C after 23 h. The overall trend was for salinity to decline (Figure 8.13(b)) from 30.2 psu at the start of the trial to 29.5 psu at the end of the trail. A similar pattern was noted for pH (Figure 8.13(c)). pH declined from 8.04 at the start of the trail to 7.65 at the end of the trail period.

The results for the ammonia analysis from the second trail can be seen in Figure 8.13. Ammonia concentration increased over the 24 h period from 5.86 to 265.82 μg L^{-1}. There was a small concentration peak detected at 9 h of 106.71 μg L^{-1}. The percentage of total ammonia in the unionised form (UIA) ranged from 0.17 to 3.29 μg L^{-1} detected at 9 h (Figure 8.14). These values represented on average 2.2 % of total ammonia per sample.

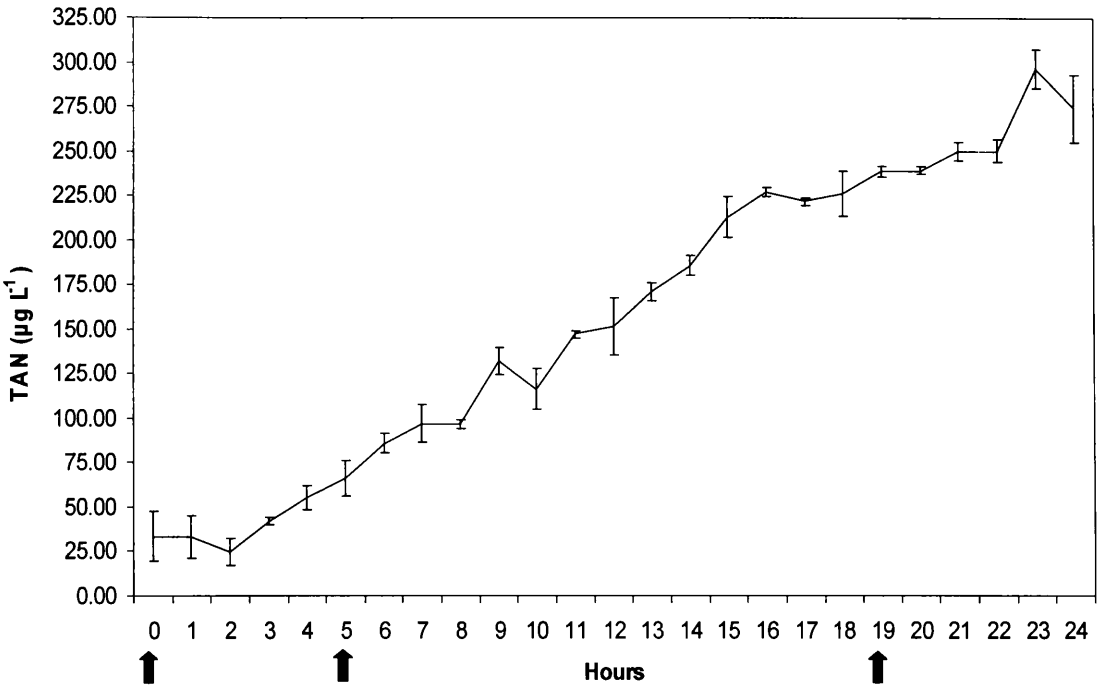


Figure 8.10 Total ammonia excretion (µg l-1) detected during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4). Arrows indicate time of feeding.

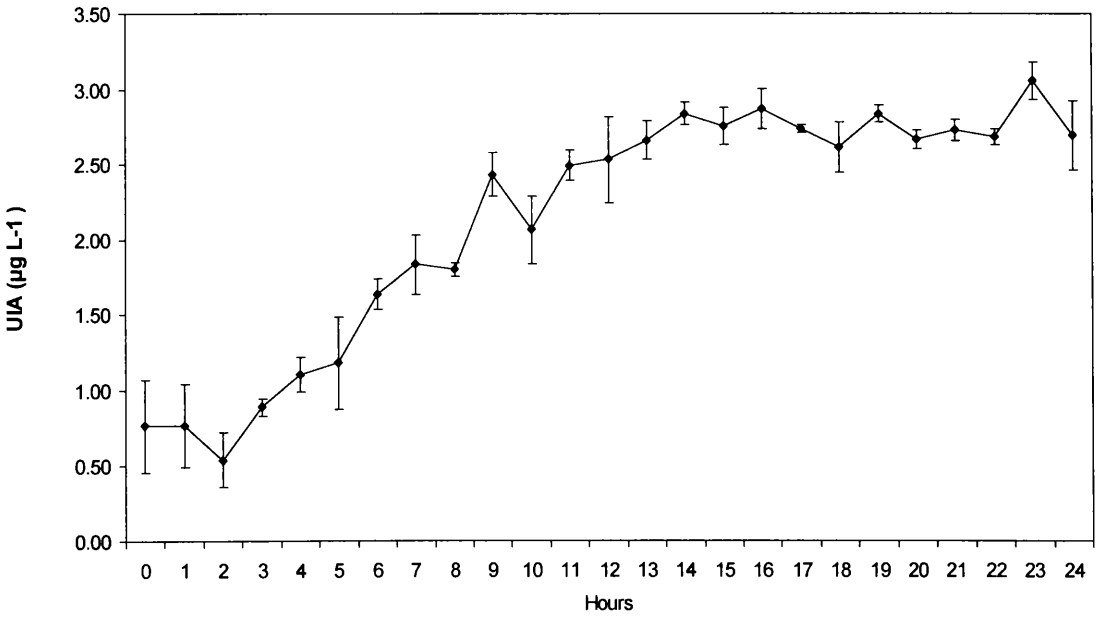


Figure 8.11 Total unionized ammonia (µg l^{-1}) detected during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

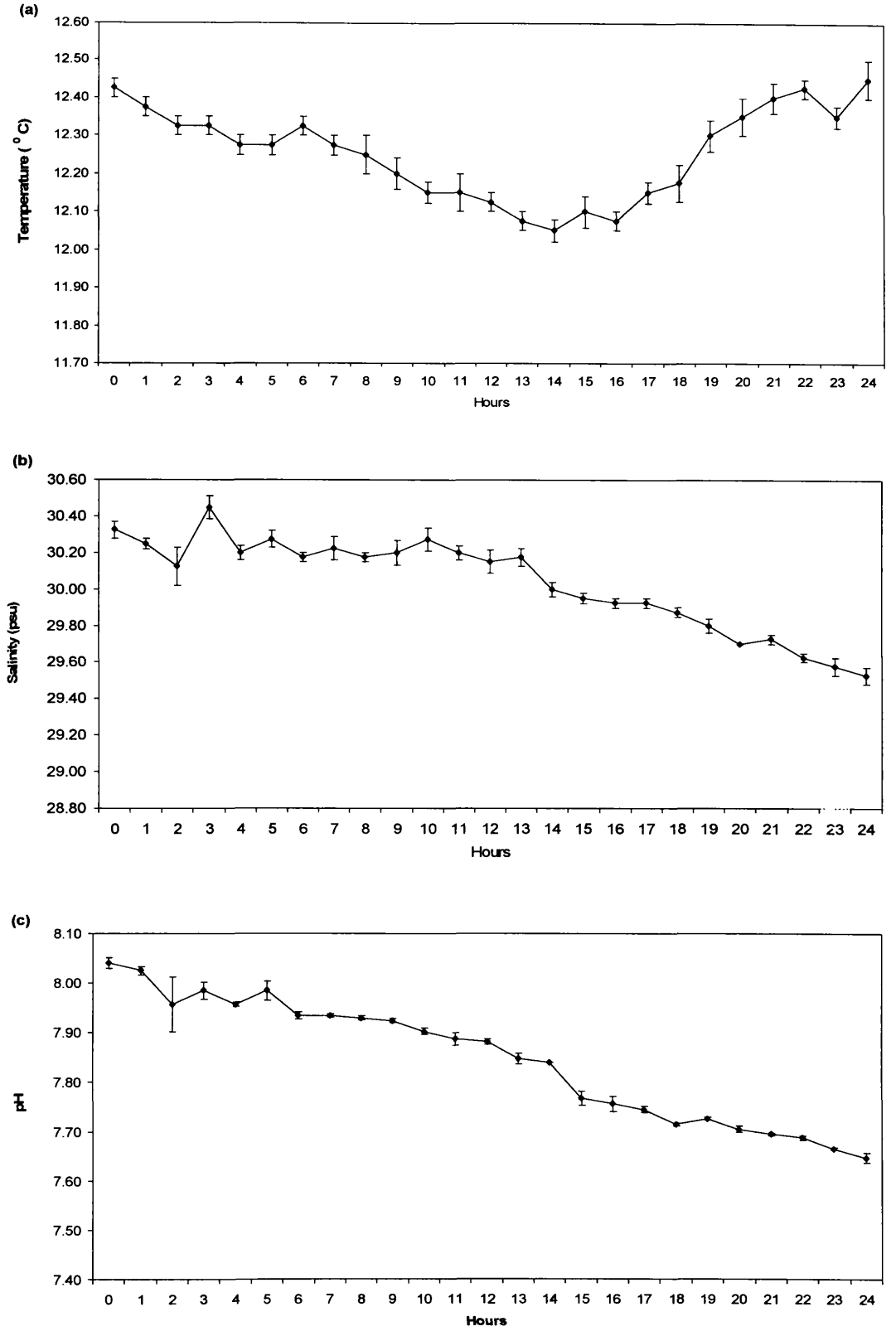


Figure 8.12 Temperature (a); Salinity (b) and pH (c) detected over time during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

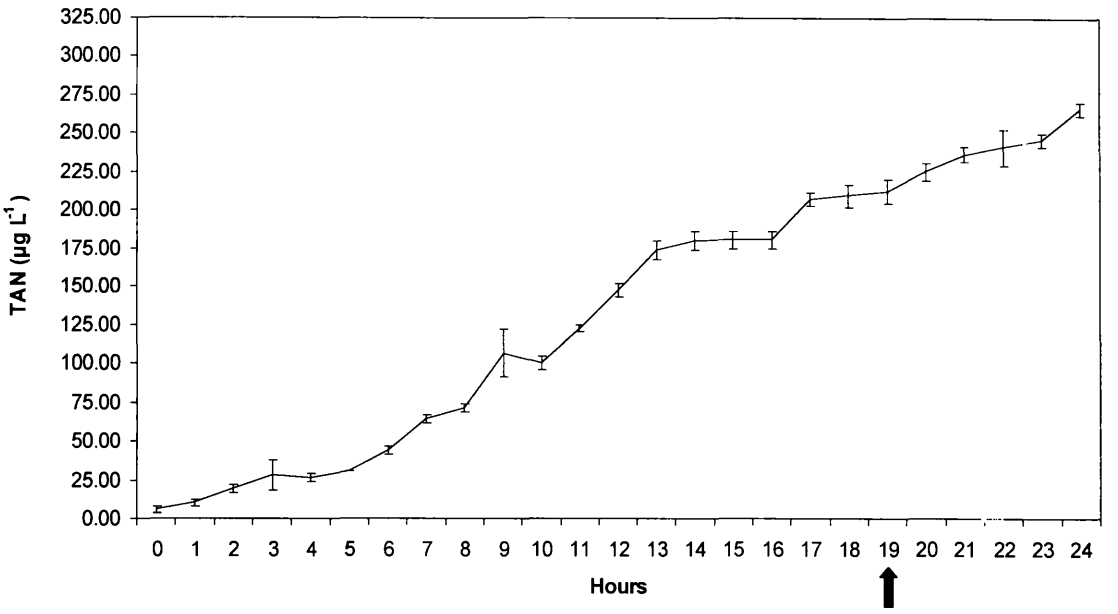


Figure 8.13 Total ammonia excretion (µg l^{-1}) detected during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4). Arrow indicate time of last feed input.

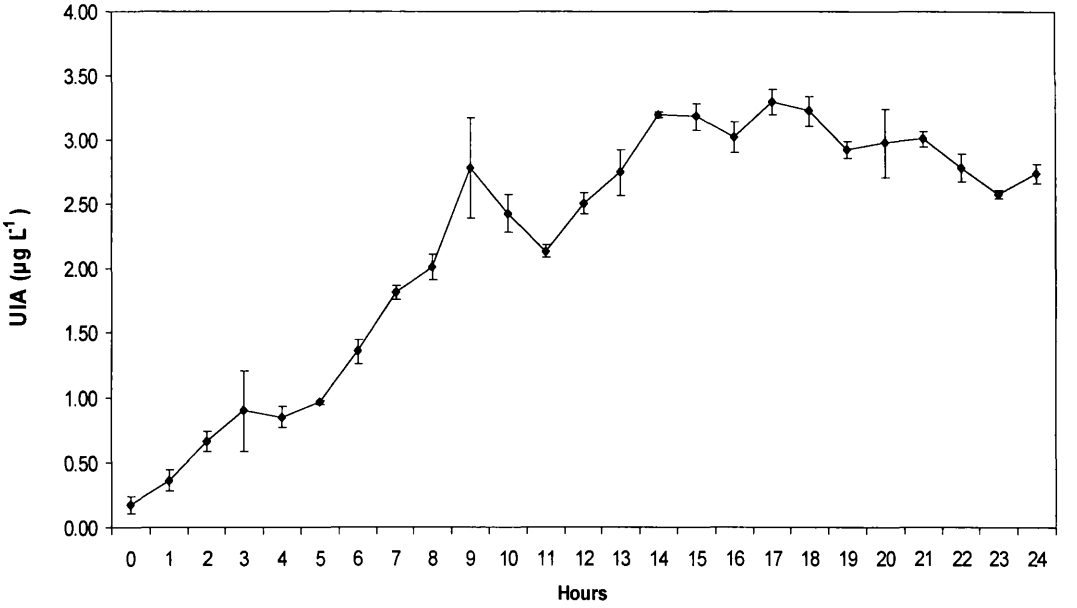


Figure 8.14 Total unionized ammonia (µg l^{-1}) detected during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

Figure 8.15(a) shows variation in water temperature within the enclosed system. Temperature initially decreased from 11.7 °C at the start of the trial to 11.3 °C after 15 h. This was followed by an increased to 11.7 °C at the end of the trial. Salinity fluctuated from 29.2 psu to 29.3 psu for the first 10 h; this was followed by a decline to 28.9 at 24 h (Figure 8.15(b)). pH declined with time from 8.16 recorded at the start of the trail to 7.69 after 24 h (Figure 8.15(c)).

Comparisons of ammonia concentrations (μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$) detected for the two trails can be seen in Figure 8.16. Ammonia concentrations detected for 13 and 14 h were significantly higher for fish from trail 2 ($F_{1,6}$ < 13.71; $p < 0.05$) and higher for fish for trail one at 16 h ($F_{1,6}$ 7.55; $p < 0.05$). There were no significant differences in ammonia concentration between trails for the rest of the time ($F_{1,6}$ 5.66; $p > 0.05$).

The calculated area under each curve was 52.01 and 44.14 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$ for trail one and two respectively. For the last five hours of both trails, the area under each curve was calculated as 14.73 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$ for the first trail and 13.37 μg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$ for the second trail.

When the data for both trails was combined, regression analysis for both trials (Figure 8.17) showed a strong linear relationship between both sets of data with an R^2 value > 0.978 indicating that there were no differences in ammonia excretion between feeding strategies.

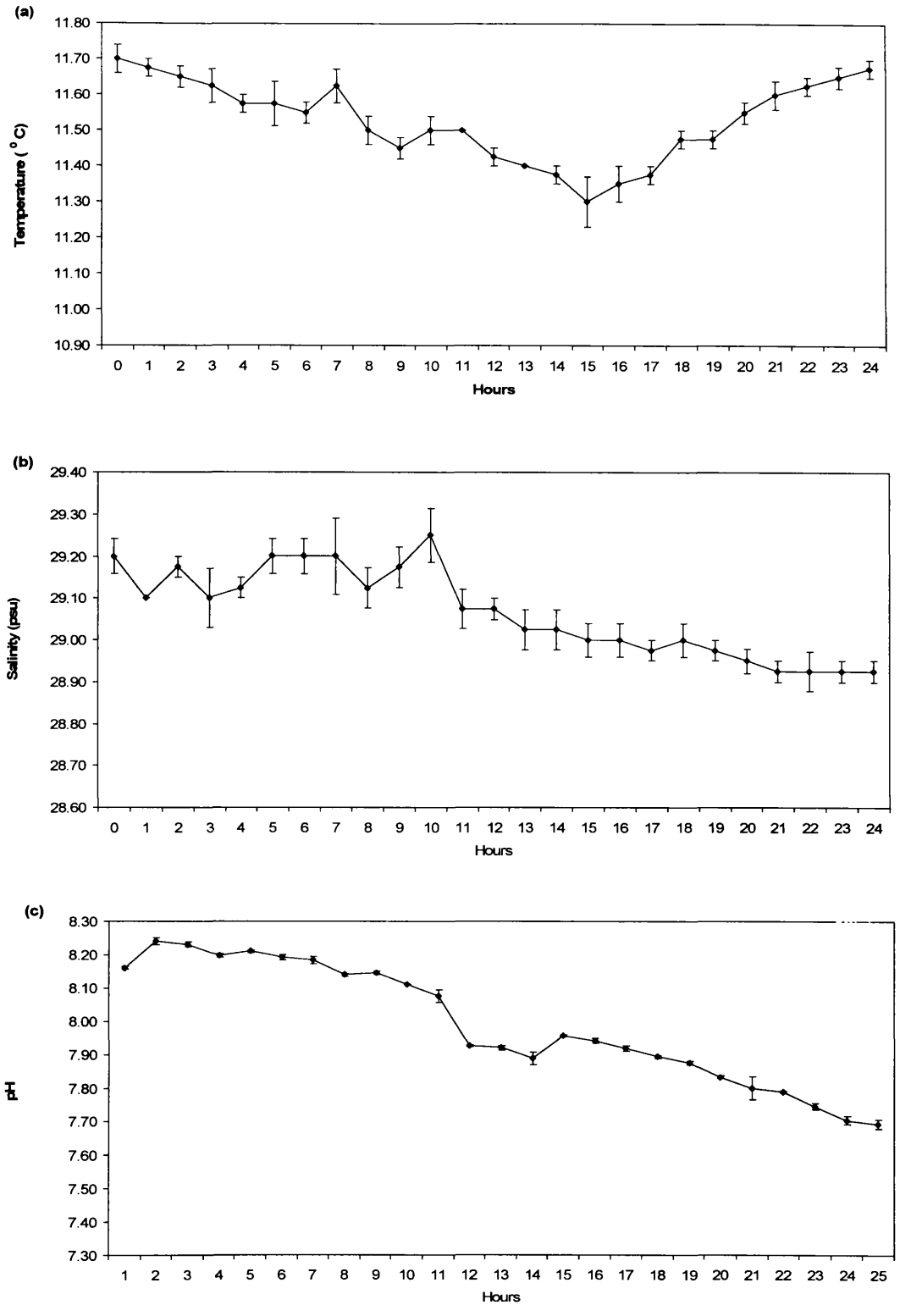


Figure 8.15 Temperature (a); Salinity (b) and pH (c) detected over time during the second study. Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

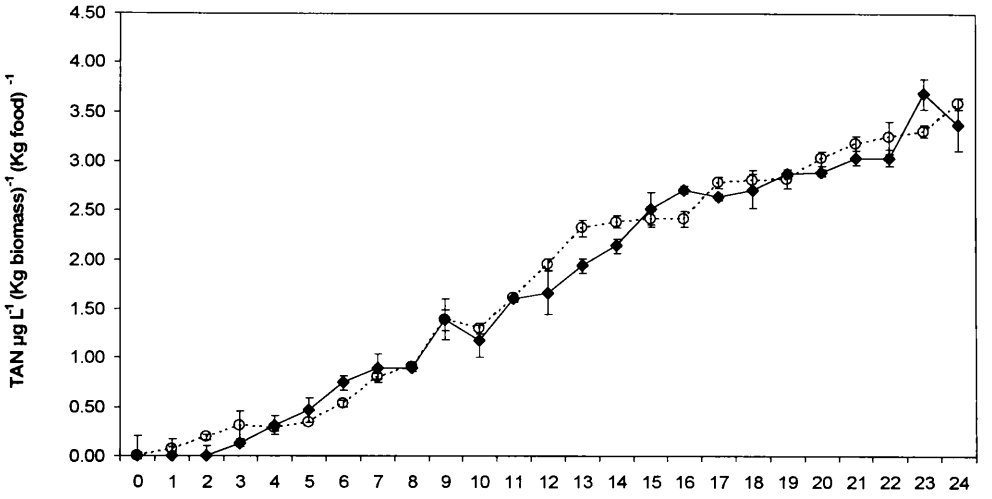


Figure 8.16Total ammonia excretion (µg l^{-1} (kg biomass)$^{-1}$ (kg food)$^{-1}$) detected for trail one (◆) and trail two (O). Data are shown as mean values from each time period with 95% confidence intervals (n = 4).

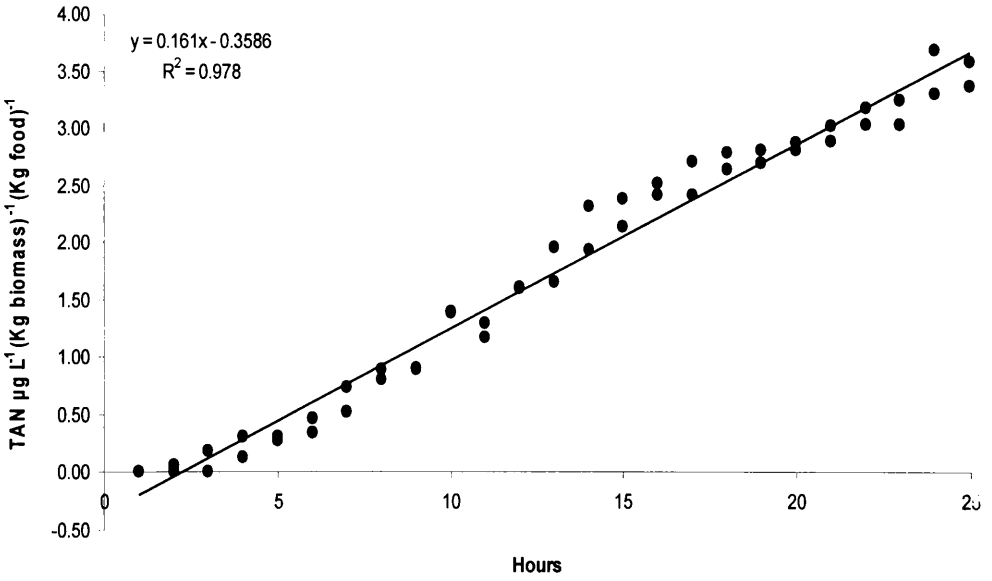


Figure8.17 Linear regression analyses of combined data from trail one and trail two.

8.4- Discussion

The initial studies have shown the feasibility of such a system to quantify waste outputs from intensively farmed Atlantic salmon. It has been shown that fish can be enclosed in such a system for prolonged periods of time without the need for oxygen or water exchange. No significant signs of stress were noted during both studies. Even after 18 h, fish in the second study were noted to be feeding normally. The amount of unionised ammonia detected was lower than levels suggested by Fivelstad *et al* (1995) who showed that no significant effects were observed at levels between 9 and 17 μg l^{-1}.

Ammonia excretion rates are directly related to the dietary protein intake in all teleosts (Rychly and Marina, 1977; Kaushik and Cowey, 1991). Studies have shown that by reducing the amount of dietary protein results in reduction in ammonia excretion (Rychly ad Marina, 1977; Cho *et al.* 1991; Cho *et al.* 1994). The variations in ammonia excretion noted in the first study show clear peaks in concentration and in the second study during trail one, small peaks were also noted several hours post-feeding. It is known that peak ammonia excretion occurred within 3-5 hours after a meal (Rychly and Marina, 1977) and is a direct result of protein catabolism. The variation in ammonia excretion noted in the first study is also similar to variations in ammonia excretion by rainbow trout in a study by (Rychly and Marina, 1977). There were no significant differences in ammonia excretion between different feeding strategies during study two. This may indicate that fish fed a similar amount of feed using two distinctly different feeding strategies has no effect on ammonia excretion. However, further validation is required as no replication was possible for these studies.

It is known that water temperature affects feed intake, rate of food transit through the digestive tract as well as affecting endogenous N excretion (Kaushik and Cowey, 1991). However, there were no significant differences in ammonia excretion from fish fed the two different feeding strategies during study two. It has been show that under well fed conditions, N catabolic waste expressed as a proportion of N intake are little affected by water temperature and it is only adaptation to sudden rises in temperature that result in greater N loses (Kaushik and Cowey, 1991).

There was a decrease in pH recorded for both trails but no significant differences in ammonia excretion detected for study two.. The decrease in pH was due to CO_2 excretion being converted to carbonic acid in the gill epithelium of the fish (Randall and Wright, 1987). The authors suggest that under pH conditions of between 6.6 to 7.8 that there would be no significant effects on ammonia excretion. Thus the changing pH conditions noted during both trails would result in no significant effects on ammonia excretion.

There are several uses that such a system could be utilised for. For example, the determination of ammonia excretion patterns from intensively farmed fish such as Atlantic salmon and Atlantic cod could be achieved as the initial studies have shown that food related concentration peaks of ammonia were detected several hours post-feeding. As mentioned in chapter 4, quantification of ammonia excretion from fish in net pens has proven to be difficult and although clear ammonia excretion patterns were detected from 22 m polar circle cages, the results could not be used to quantify actual levels of excretion due to ammonia excretion being subjected to dilution factors. The use of such as

system removes the variability of dilution and thus allows for more accurate determination of actual ammonia excretion patterns from fish fed different diet types. The system can also be used to directly compare waste outputs from fish subjected to different feeding strategies such as feeding to satiation and under satiation or feeding groups of fish different amounts of meals. This would allow for determination of the optimal feeding strategy to utilise both maximise performance and reduction in waste outputs from intensively farmed fish.

The advantage of using such a system compared to land-based tank trails is that the fish are being reared under realistic environmental marine conditions and are subjected to changes in water quality parameters such as temperature which can have a direct effect on protein catabolism. In addition, assessment of waste outputs can also be done in conjunction with assessment of growth and dietary performance under realistic commercial conditions.

The ultimate aim of the study was to accurately determine all the data required to conduct a mass balance model with the use of laboratory analysis of the feed and fish along with data collected in the field. Thus the data collected will allow for calculating an accurate mass balance for any diet type. This information can then be used to assess the potential environmental impacts of any modified diet fed to intensively farmed fish. However as only faecal sampling and not analysis was undertaken for both studies, no mass balance model outputs were generated. In addition, macronutrient content of flesh would also be required in order to construct mass balances.

8.5 - Conclusions

There is clearly a need for accurate determination of actual waste outputs from intensively farmed fish species such as Atlantic salmon and cod. It has been demonstrated that there is potential for the use of such a system to collect data on waste outputs. In addition, as almost all intensively farmed Atlantic salmon are reared in the marine environment in sea cages, the use of such a system allows for the determination of wastes from fish reared in the marine environment thus taking into account seasonal and daily fluctuations in environmental parameters such as temperature and salinity, as well as studying the effects of feeding fish modified diets and how the fish respond to these diets.

Unfortunately, there was not enough time to fully elucidate the use of such a system and further work is required in order to fully maximise the potential use of this method to quantify waste outputs.

Chapter 9 - Physical characteristics of four experimental diets used during a small-scale feed trial on Atlantic salmon (*Salmo salar* L.) and environmental implications based on waste dispersion modelling.

9.1 Introduction

The potential to cause detrimental effects on the Scottish marine environment has lead to the regulation of the aquaculture industry by several government departments and related organisations (Gillibrand and Turell, 1997). These include the Scottish Environmental Protection Agency (SEPA) and the Crown Estates (CE). Before a new farm is established or an increase in production has been applied for, the potential impact on the surrounding environment is assessed. The assessment is aided by the use of hydrodynamic and benthic models to predict the dispersion of organic particles from marine fish farms and the effects of waste effluents on receiving water bodies.

Waste dispersion models generally have two components. Firstly, mass balance principles are used to quantify wastes and secondly, settlement and disposal algorithms are used to characterise dispersal (Gowen and Bradbury, 1987; Gowen, 1994; Chen *et al.* 1999).

The model DEPOMOD (Cromey *et al.* 2000; Cromey *er al.* 2002) has been developed to predict deposition of organic and chemical wastes and predict impacts on the benthic community. It has been incorporated into regulatory processes directly as part of the discharge consent.

Geographical Information Systems (GIS) can be applied to model waste dispersal and environmental capacity. One such model has been incorporated into GIS (Perez *et al.* 2002) and further developed by Brooker, (2002). The reason for this is its capacity for fast image generation and manipulation, flexibility to run alternative scenarios, statistical analysis of the image and

generation of sophisticated output, which helps visual interpretation of results (Pérez *et al.* 2002).

Waste dispersion models used to predict the dispersion of organic particles from intensively farmed Atlantic salmon (*Salmo salar*) use data sets of feed settling velocities which take account of pellet size, environmental conditions (temperature and salinity) and feed formulation (Chen, 2000). However, recent developments by feed manufactures on the use of alternatives to fish meal and fish oils in diets for farmed Atlantic salmon may affect the physical characteristics and settling velocities of such diets by altering the macronutrient components. If such diets are to be used commercially, there is a need to determine whether changing the macronutrient components will affect the physical characteristics such as settling velocity before being incorporated into waste dispersion models.

The physical property of feed pellets is important for a number of reasons. Transportation and handling in both the factory and on the farm require pellets that have certain integrity without the production of fines due to attrition stress. In addition, pellets of high physical quality must have properties which give a high nutritional quality in terms of higher feed intake and improved nutritional quality (Thomas and van der Poel. 1996)

The aim of this study was to determine and compare the physical characteristics and settling velocities of four different diets that could be used to feed Atlantic salmon so that the data may be incorporated into models for more accurate prediction of dispersion of wastes and use.

In addition, the data obtained was used to obtain theoretical model outputs produced using a GIS based solid waste dispersion model under development at the Institute of Aquaculture, University of Stirling (Perez *et al.* 2002; Brooker, 2002; Corner *et al.* in press).

The main hypotheses that have been tested were:

H_o^1: Different environmental conditions and different feed ingredients have no influence on the physical characteristics of modified diets fed to Atlantic salmon.

H_o^2: There are no significant differences between diet types in theoretical waste outputs using a GIS based solid waste dispersion model.

9.2 Material and methods

Four diets (assessed for growth performance in chapter 2) were used in this study. The diets were a nutrient dense diet, a low protein diet, a partial vegetable oil substitution diet and a control diet (labelled ND, LP, SUS and C respectively). All pellets used were 11mm. Table 2.1 shows the dietary composition of the experimental diets.

9.2.1 - Hardness and Friability

The equipment used to determine pellet hardness and friability was designed and built according to feed industry specifications. The methodologies used followed those described for industry by (Thomas and van der Poel. 1996).

To test hardness, 20 pellets from each diet, which were within the size range of the test equipment, where placed individually in a pellet crusher (Kahl pellet hardness tester, 4-11 mm) and the pressure at which they disintegrated recorded. An attrition mill was used to determine friability (Pfost, 1963 in Thomas and van der Poel, 1996). Samples of 25 g from each diet were placed in the attrition mill box and the box revolved for 10 minutes at 50 rpm. Samples were then sieved (mesh size 2 mm) and the proportion of the original sample weight that passed through the sieve recorded. Each diet was tested in triplicate.

9.2.2– Determination of the water stability of each diet type.

Comparison of water stability of each diet type was facilitated by determining the weight loss of pellets from each diet immersed in sea water over time under four different environmental conditions (5 ^{0}C at 25 psu; 5^{0}C at 33 psu; 15^{0}C at 25 psu and 15^{0}C at 33 psu) to approximate environmental conditions of cage culture in Scotland during winter and summer respectively.

Three groups of five pellets from each diet were weighed and placed in glass beakers containing 100 ml of sea water and left to stand with occasional gentle shaking for 20 seconds, 2, 5, 10 and 20 minutes respectively. Each pellet type was studied in triplicate over each environmental regime.

After each immersion time, the pellets were then passed through a 2mm sieve and the collected material retained on the sieve was placed on a pre-weighed foil tray and placed in the oven (Gallenkamp OVE 300) at 85^{0}C for 24 h to dry

and then reweighed (Mettler, model AJ 100; precision 0.1 mg). The remaining dry weight was expressed as a percentage of the original dry weight according to the calculation from the moisture (M %) determination following:

$$M\ (\%) = (a - b)/a \times 100\ \%$$

Where:

a = initial dry weight of sample (g),

b = final weight after 24 h drying (g).

9.2.3- Determination of the leaching rates of each diet type.

Leaching rates were determined for each diet by initially weighing five dry pellets from each diet and drying them in the oven (Gallenkamp OVE 300) at 85^0C for 24 h. After drying and weighing, all groups of pellets used in the analysis of weight loss over time were then carefully ground and homogenised to a fine consistency using a mortar and pestle. Samples were then retained in airtight containers and analysed in triplicate for total C and N as a percentage of total dry weight using a Perkin Elmer 2400 Elemental Analyser.

9.2.4- Determination of settling velocity for each diet type.

A 1.25 m length of 10 cm diameter clear Perspex tube was used to determine the settling velocity of each diet using seawater as the test medium. The tube was marked every 10 cm and secured in a vertical position with a support

stand. The settling rates of each diet were determined at temperatures of 5^0C and 15^0C and at two salinities (25 psu and 35 psu respectively). Thirty pellets from each diet were taken at random and individual weights were recorded. Maximum lengths and diameter of each pellet were recorded using digital callipers (digiMax, model m2000; precision 0.1 mm). The settling velocity of each pellet was determined from the time taken for each pellet to descend between two marks 100 cm apart, the first of which was 5 cm below the surface to negate effects of surface tension. (Robison, 1981). A supply of seawater was stored at each temperature and the water in the Perspex tube changed prior to testing each diet type. Pellets coming into contact with the sides of the apparatus or those with air bubbles entrained on their surface were excluded from the experiment.

9.2.5- Solid waste dispersion modelling

A mass balance model for carbon was used to determine waste outputs for each diet (Figure 9.1). The basis of the model assumed that the major source of carbon to the fish was derived from the diet. That is, they receive a negligible proportion of their nutrition from natural sources. It was assumed that a proportion of the feed would be lost from the system as uneaten feed pellets. This was set at 5 % to reflect current farming practices in Scotland (Gowen and Bradbury, 1987; Davies, 2000; Brooks *et al* 2002). The model was based on 1000 tonnes of salmon being produced each year from real-time farm records from a producer in Scotland. The expected fish production was multiplied by the FCR obtained for each diet during the trial period.

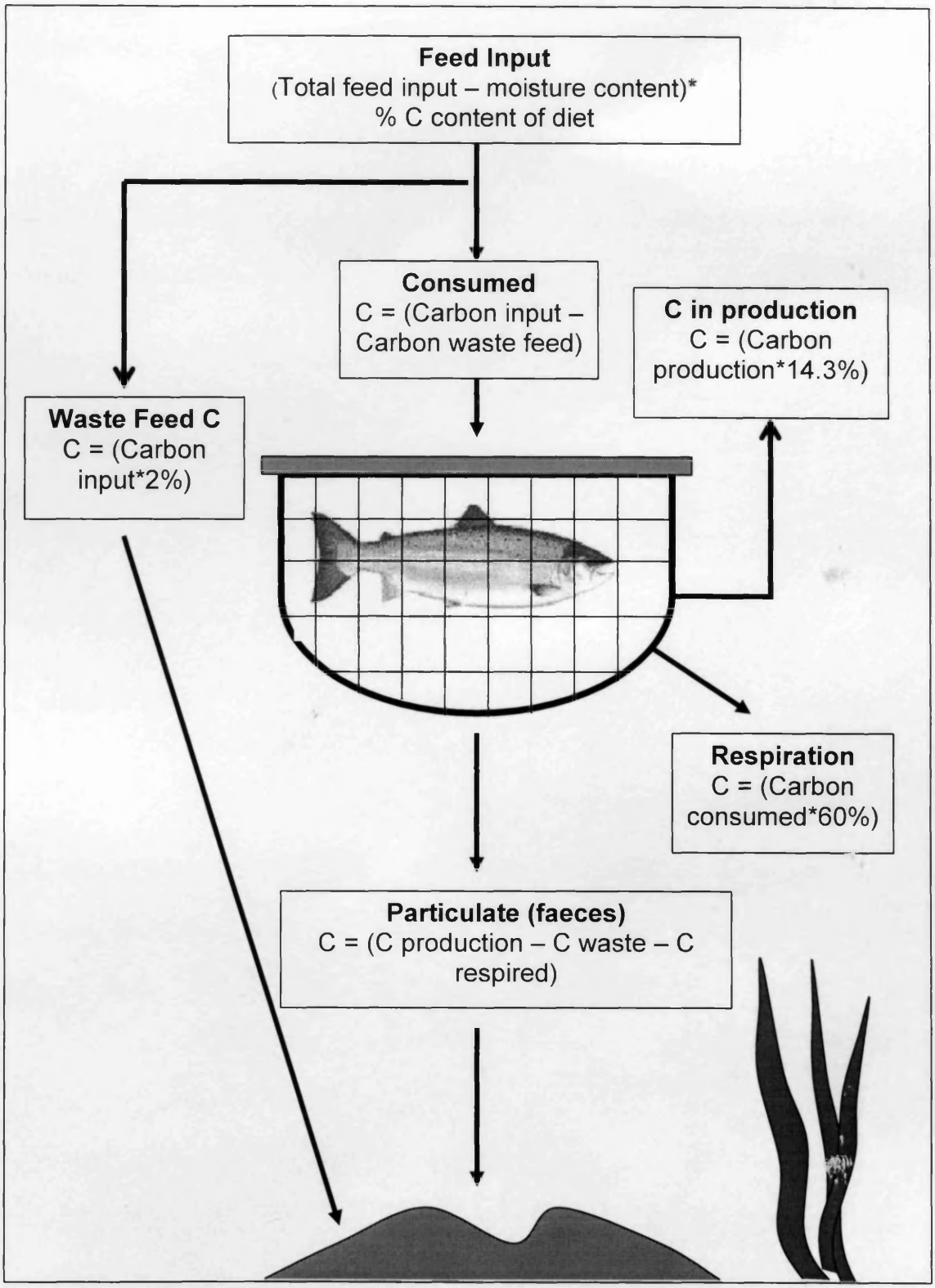


Figure 9.1 Theoretical mass balance for Carbon used to calculate waste outputs from the different diet types.

C input was calculated as follows:

$$[C\ input = (FCR \times \%\ C\ in\ feed)]$$

Where % C in feed was calculated as a percentage of total dry weight using a Perkin Elmer 2400 Elemental Analyser.

The amount of C consumed was calculated as follows:

$$C_{consumed} = (C_{input} - C_{waste})$$

Where:

C consumed is total C ingested by fish,

C waste is uneaten food (assumed at 2% as discussed in section 9.2.5 above).

It is assumed that of the C consumed, 60% is respired as carbon dioxide CO_2 (Gowen and Ezzi, 1992) and that 14.3 % is incorporated into body tissues (Chen, 2000). The C in faecal material is calculated as follows:

$$C_{faecal} = (C_{consumed} - C_{respired} - C_{production})$$

Where $C_{production}$ was total biomass at harvest x 14.3 %.

9.2.5.1- Spatial modelling

Theoretical model outputs were produced using a GIS based solid waste dispersion model under development at the Institute of Aquaculture, University of Stirling. This solid particle-tracking model has gone through a series of developments from complex spreadsheet models to a fully integrated GIS coastal zone model (Brooker, 2002; Perez, 2002).

Dispersion modelling of algorithms and hydrographic data input was integrated into the IDRISI GIS software (Clark Labs, Worcester MA, USA) using the Borland Delphi development tool and IDRISI API (Applications Programming Interface) which provides a GUI (Graphical User Interface) and allows communication between the model application and the GIS software. The current version of the model calculates positions of the cages within a digital map of the coastline, and then estimates waste dispersion from the site using fish production data, measured hydrographic data, variable bathymetry, variable settling velocities and random point source. The use of variable settling velocities of the waste particles accounts for the variation in size and shape of the feed and faecal pellets. To account for the assumption that a waste particle can originate from any point in a cage the starting point within the cage for each waste particle is chosen at random. The model can also approximate further dispersion of waste on the seabed due to cage movements by tidal currents. The model output is a two dimensional spatial gradient map representing carbon waste settlement on the seabed over a defined period, which can then be analysed using the extensive suite of tools available within the GIS software.

Modelling of the experimental diets was based on an existing Scottish cage site consisting of 12 polar circles, giving a realistic hypothetical comparison of the new feeds. A hydrographic dataset of 15 days duration (20 minute intervals) was recorded at the site using two Valeport BFM 106 Self-recording current meters, placed 3 m from the seabed and approximately 3 m from the surface at the lowest tide level during the deployment. The calibrated impeller measured currents speeds with an accuracy of +/- 0.005 m/s at < 0.1 m/s current speed and startup speed of 0.02 m/s. Current direction was measured by alignment of the current meter with the main direction of current flow and recoding on a flux gate compass (+/- 0.1 degree). Cage movement data was available to use in the model as a series of measurements of cage movements about an origin over a complete tidal cycle (Corner, 2004).

A theoretical production of 1000T y^{-1} was assumed and each diet was modelled producing outputs of waste feed, waste faeces and total carbon waste deposited over one year. For each model output the maximum deposition rate (kg C m^2 y^{-1}), total deposition (T C y^{-1}), deposition area or footprint (km^2) and mean deposition rate (kg m^2 y^{-1}) were calculated to allow comparison of each diet.

9.2.6- Statistical Analysis

The normality and homogeneity of the variance of all data sets was checked prior to parametric statistical analysis. Normality was checked by graphic examination of probability plots and the Anderson-Darling test.

Statistical significance of differences among the treatments were computed from one-way or two-way analysis of variance (ANOVA) using MINITAB^{TM} statistics package, version 13 (Ryan and Joiner, 1994).

All percentage or proportional data were normalised by arcsine transformation prior to statistical analysis (Zar, 1984). The nutrient content of each diet were analysed by one-way ANOVA using immersion times as factors (0, 20 s and 2, 5, 10 and 20 min). Nutrient leaching data were analysed using ANOVA by a general linear model (GLM) approach to determine the variation between all experimental factors, i.e. the leaching rate associated with each experimental diet and immersion time (0, 20 sand 2, 5, 10 and 20 min). Significant differences between treatments were determined by Tukey's multiple range test ($p < 0.05$).

9.3 Results

9.3.1 – Hardness and friability

Results from pellet hardness (pressure; kg cm^{-2}) are given in Figure 9.2. Analysis of variance comparing hardness of each experimental diet shows that there were significant differences ($F_{3,76} = 16.4$, $p < 0.05$) between the ND diet and the other test diets as well as between the SUS diet and the LP and control diets ($F_{3,76} = 16.4$, $p < 0.005$). The softest pellets were from the control diet (mean hardness value 4.67 kg force cm^{-2}) whilst the hardest pellets were from the ND diet (6.24 kg force cm^{-2}). Hardness of the LP and SUS diets was 5.46 and 6.13 kg force cm^{-2} respectively.

Results from the friability of the experimental diets (Figure 9.3) showed that there were significant differences between the ND and control diets and between the LP and SUS and control diets ($F_{3,8}$ 13.02: $p < 0.05$). Pellets from the control diet were found to be more durable and less friable with a percentage friability of 0.51%. Pellets from the LP diet were found to be the most friable (3.93 %) compared to the other diets. Pellets from the SUS diet and the ND diet had 2.04% and 3.17% friability respectively.

9.3.2 – Water stability of each diet type

Figure 9.4 shows percentage loss in weigh of pellets samples plotted against immersion time for each experimental diet under (a) 5^0 C -25 psu and (b) 5^0 C – 33 psu. And Figure 9.5 shows percentage weight loss of pellets plotted against time for each diet under (a) 15^0 C – 25 psu and (b) 15^0 C - 33 psu. There were significant differences in percentage weight loss of pellets over time ($F_{5,275}$ 86.59: $p < 0.05$) and for each environmental condition ($F_{3,275}$ 4.82: $p < 0.05$). In addition, there were significant differences in weight loss between diet types ($F_{4,275}$ 12.6: $p < 0.05$).Following immersion at 5^0 C at both salinities, all diet types showed similar water stability with time with good water stability after 20 min immersion in sea water, with less than 6 % loss of integrity.

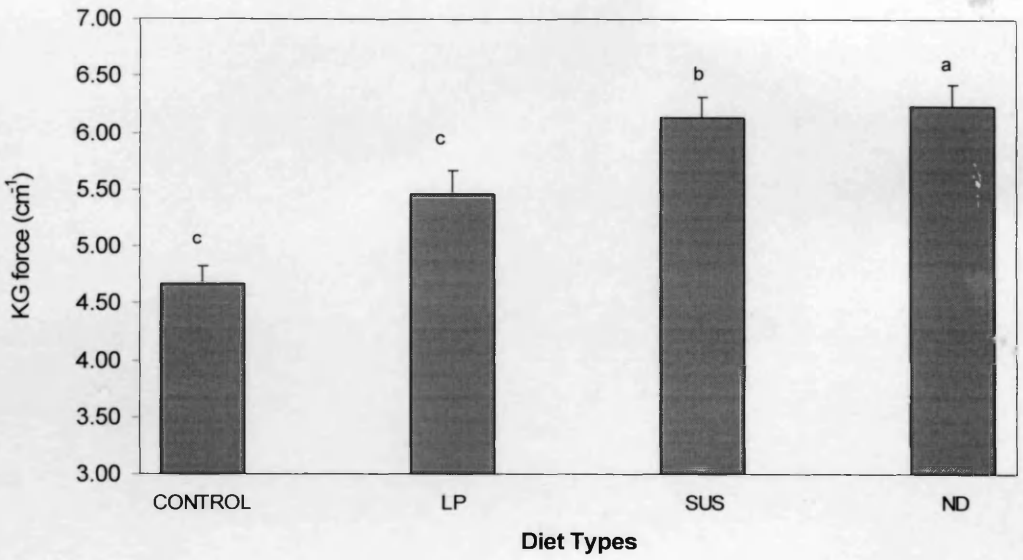


Figure 9.2 Mean hardness values expressed in terms of kg force cm^{-2} pellet, for each experimental diet. Values represent means ± S.E. Mean values marked with different superscripts where found significantly different by Tukeys *post hoc* test.

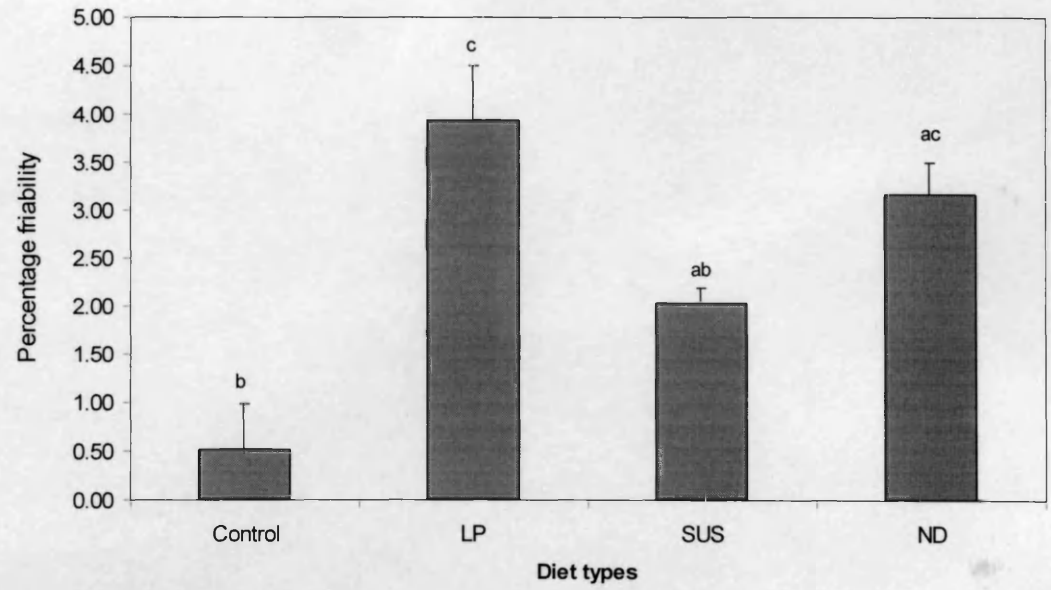


Figure 9.3 Friability (%n = 3) of each experimental diet. Values represent means ± S.E. Mean values marked with different superscripts where found significantly different by Tukeys *post hoc* test.

A different pattern was noted following immersion at the higher temperature at both salinities. There were significant differences in percentage weight loss following immersion over time ($F_{5,134}$ 55.91: $p < 0.05$) and between diet types ($F_{3,134}$ 16.92: $p < 0.05$). Both the ND and LP pellets showed poorer water stability following immersion after two min compared to the other two diets which showed a similar pattern of weight loss compared to immersion at the lower temperature. There was less than 5 % loss of integrity for both the control and SUS diets after 20 min immersion but the ND and LP diets had between 10 and 14 % loss of integrity after 20 min immersion.

There were no significant difference in the pattern of weight loss between diets at both 15^0 C – 25 psu and 15^0 C – 33 psu ($F_{1, 134}$ 1.67: $p > 0.05$).

9.3.3 – Settling velocities of each diet type

The results from the settling velocity experiment can be seen in Figure 9.6. There were significant differences in settling velocities between diets for each environmental condition (Table 9.1). At 5^0 C – 25 psu and 5^0 C – 33 psu, pellets from the control diet had a greater settling velocity (14.37and 13.12 cm s^{-1}) compared to the LP, SUS and ND diets, whereas, pellets from the LP diet had the slowest settling velocity (9.10 and 9.23 cm s^{-1}) which was significantly slower compared both the SUS (12.97 and 12.49 cm s^{-1}) and ND diet (12.59 and 11.10 cm s^{-1} respectively).

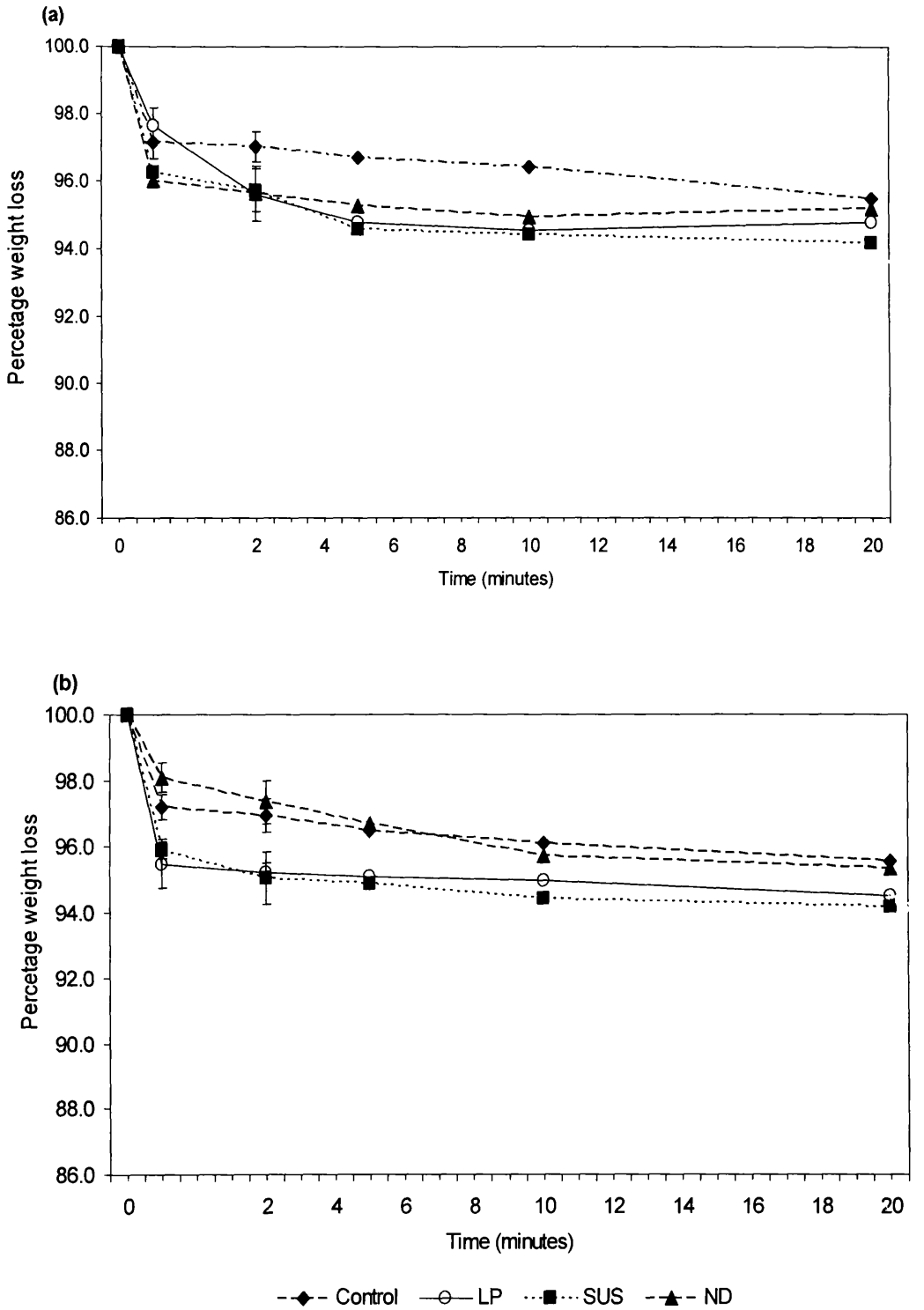


Figure 9.4 Mean water stability (%) of each experimental diet immersed up to 20 min in sea water at (a) 5^0 C at25 psu and (b) 5^0 C at 33 psu.

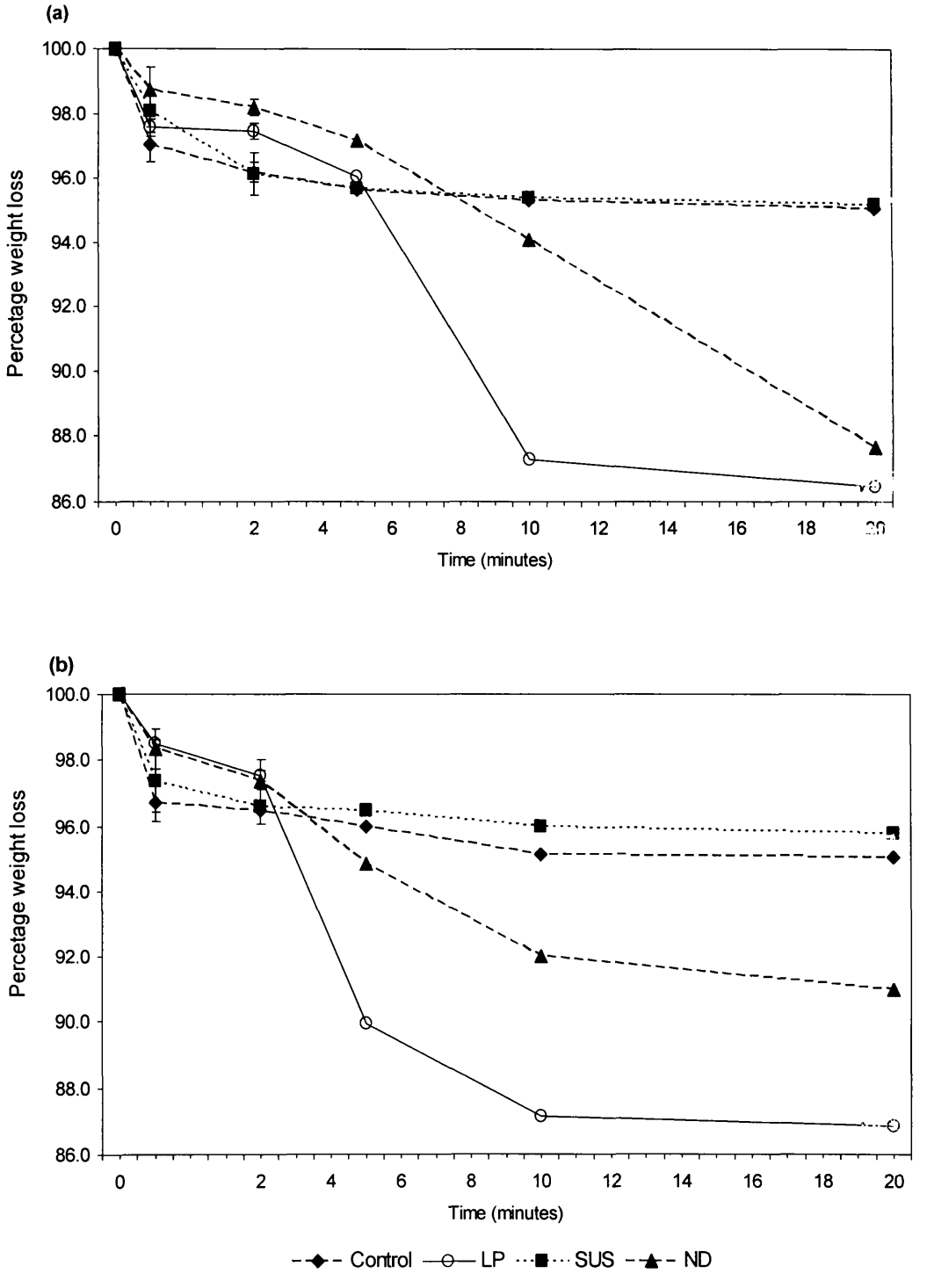


Figure 9.5 Mean water stability (%) of each experimental diet immersed up to 20 min in sea water at (a) 15^0 C at 25 psu and (b) 15^0 C at 33 psu.

At 15^0 C – 25 psu, again pellets from the control diet had the greatest settling velocity (13.40 cm s^{-1}) which was significantly faster compared to the LP and ND diets (9.37 and 8.20 cm s^{-1} respectively). Pellets from the SUS and ND diets also had significantly greater settling velocities compared to the LP diet. At 15^0 C – 33 psu, the trend for the control diet pellets to have a significantly greater settling velocity (13.53 cm s^{-1}) compared to the other diets continued. Pellets from the SUS and ND diets also had greater settling velocities (12.56 and 11.25 cm s^{-1} respectively) compared to the LP diet (8.20 cm s^{-1}).

At 5^0 C – 25 psu, both the control ($F_{3, 76}$ 9.92; $p < 0.05$) and ND ($F_{3, 76}$ 13.76; $p < 0.05$) had the fastest settling velocities compared to the other environmental treatments. There was no significant differences in settling velocity over each environmental condition for the SUS diet ($F_{3, 76}$ 2.33: $p > 0.05$) whereas pellets from the LP diet had the lowest settling velocity at 15^0 C – 33 psu compared to the other treatments ($F_{3,76}$ 6.57; $p < 0.05$).

Table 9.1 Results from analysis of variance (ANOVA) comparing mean settling velocities of each diet type. Where *P* is the significance level (d.f. =3, 76). Different superscripts on the same line, between diets where found significantly different by Tukeys *post hoc* test ($^{***}P \leq 0.001$).

	DIETS					
	Control	**LP**	**SUS**	**ND**	***F***	***P***
5^0 C at 25 psu	a	b	c	c	109.57	***
5^0 C at 33 psu	a	b	c	d	154.76	***
15^0 C at 25 psu	a	b	ac	d	156.50	***
15^0 C at 33 psu	a	b	c	d	117.65	***

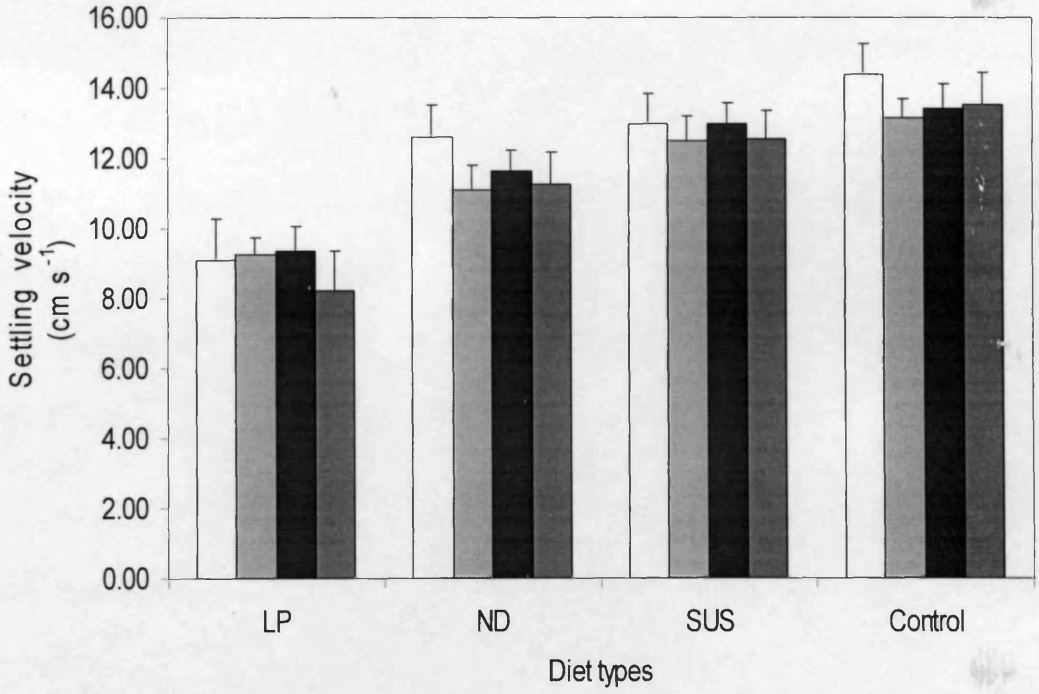


Figure 9.6 Settling velocities (cm s^{-1}) of each diet type under a range of environmental conditions. (□) 5^0 C at 25 psu; (■) 5^0 C at 33 psu; (■) 15^0 C at 25 psu and (■) 15^0 C at 33 psu. Error bars represent standard deviation.

9.3.4 – Nutrient leaching rates

The amount of nutrient leached from pellets fed to salmon can be expressed as the difference in total nutrient content of the pellet before and after immersion in sea water. For analysis of pellet C content over time, there were no significant differences in C content after immersion at the four different environmental conditions ($F_{5.12} < 3.00$: $p > 0.05$). However, there was a trend for pellet C content to decline with time for each environmental condition. At 5^0 C - 25 psu (Figure 9.7), the C content of the control diet decreased by 1.1 %, whereas, the C content of the LP, SUS and ND diets decreased by 0.7, 1.9 and 1.4 % respectively. At 5^0 C - 33 psu (Figure 9.8) the C content of the control, LP, SUS and ND diets decreased by 0.4, 0.9, 1.1 and 1.4 % respectively. At the higher temperature of 15^0 C - 25 psu (Figure 9.9), the C content of the control, LP SUS and ND diets decreased by 0.2, 1.6, 1.9 and 0.3 % respectively. At 15^0 C - 33 psu (Figure 9.10), the C content of the control, LP, SUS and ND diets decreased by 2.9, 1.1, 1.6 and 0.5 % respectively.

For analysis of pellet N content over time there were no significant differences in N content ($F_{5.12} < 3.2$; $p > 0.05$) after immersion up to 20 min at 5^0 C – 25 psu (Figure 9.11) even though there was a trend for pellet N content to decline with time. The control and LP diets had the greatest decline with 5.9 and 4.4 % respectively after 20 min immersion. Whereas, the pellet N content of the SUS and ND diets declined by only 2.2 and 0.5 % respectively after 20 min immersion.

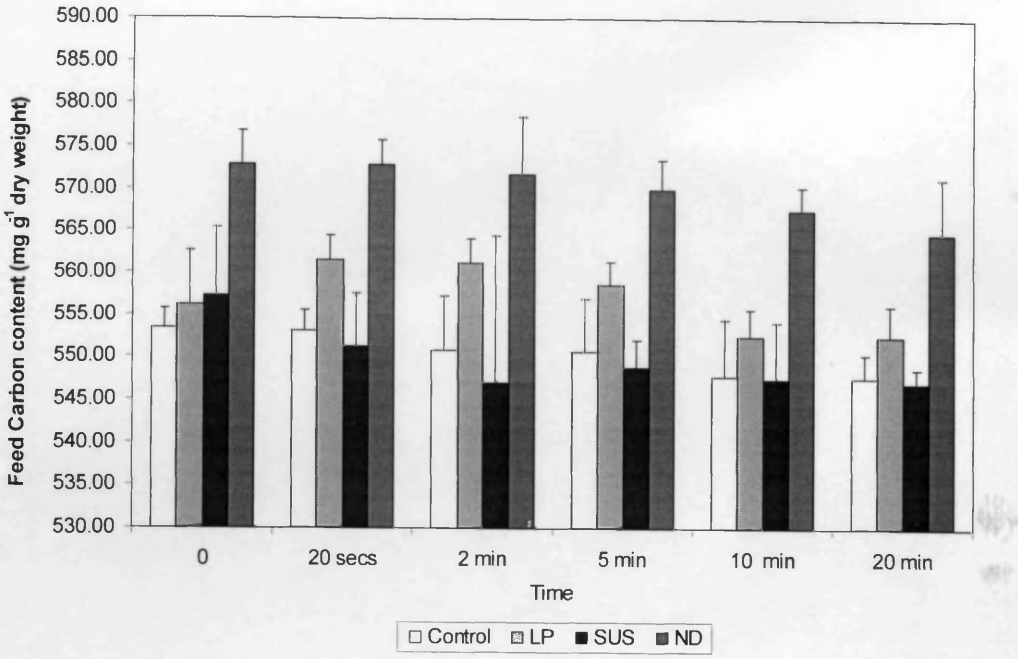


Figure 9.7 Mean concentration of feed carbon content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (5^0 C at 25 psu) for up to 20 min

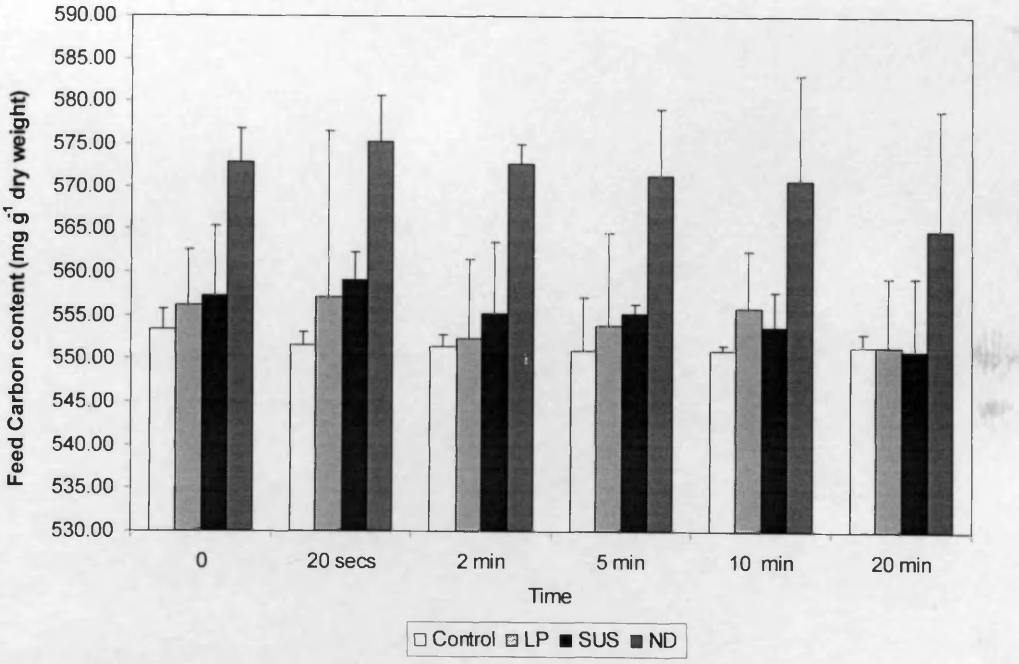


Figure 9.8 Mean concentration of feed carbon content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (5^0 C at 33 psu) for up to 20 min.

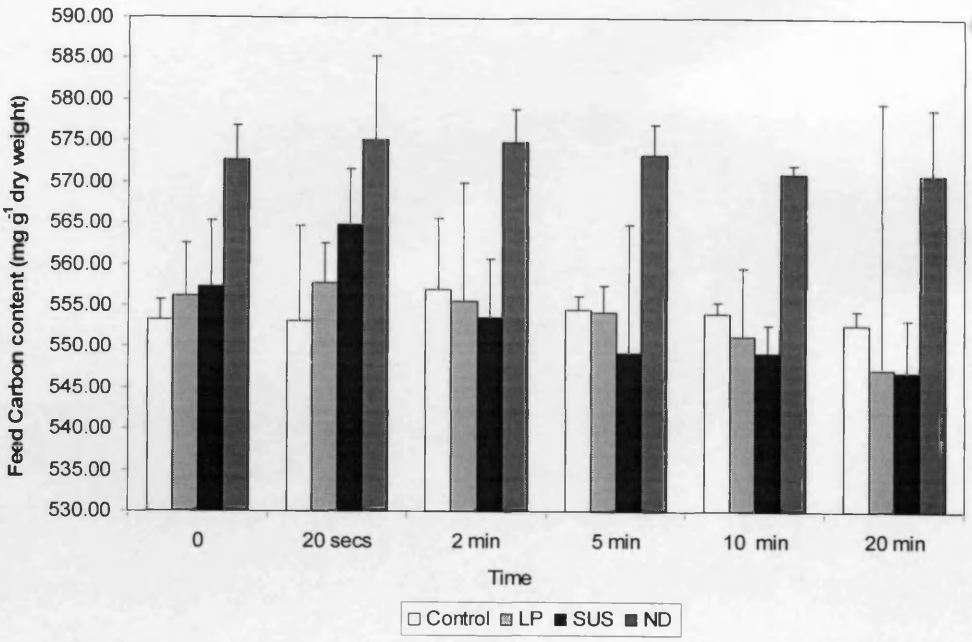


Figure 9.9 Mean concentration of feed carbon content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (15^0 C at 25 psu) for up to 20 min.

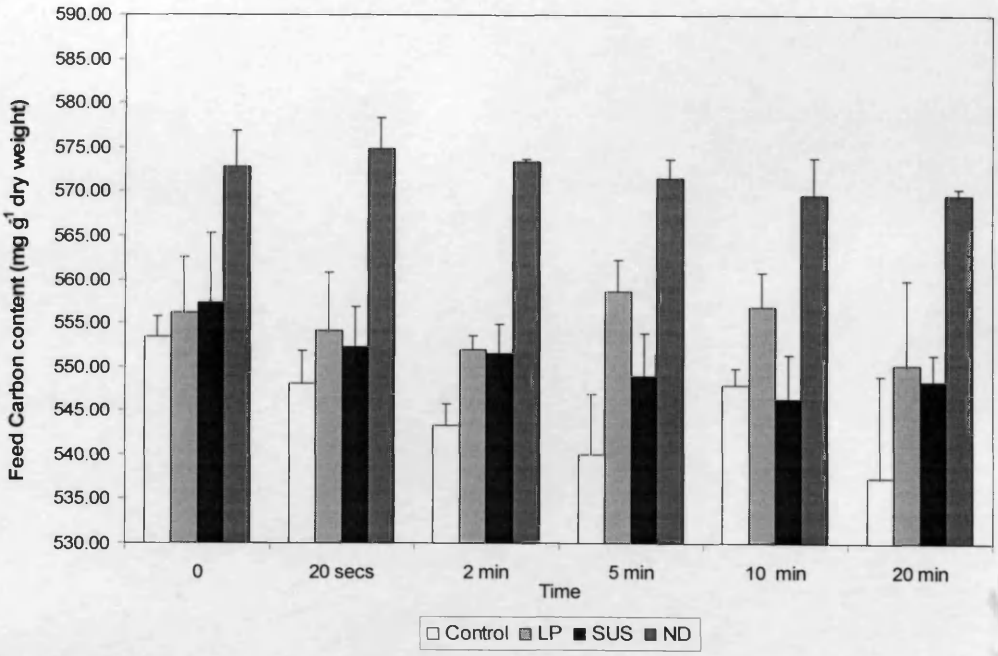


Figure 9.10 Mean concentration of feed carbon content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (15^0 C at 33 psu) for up to 20 min

At 5^0 C – 33 psu (Figure 9.12), there were no significant differences in pellet N content after 20 min immersion for the control, SUS and ND diets ($F_{5.12} < 3.09$: $p > 0.05$) with each diet losing 4.7, 4.6 and 5.5 % N content respectively after 20 min. However, pellet N content of the LP diet was found to be significantly lower after 5 min immersion ($F_{5.12}$ 5.29: $p < 0.05$) wit the N content decreasing from 62.7 to 55.97 mg g^{-1} dry weight after 20 min immersion, this represented an overall loss of 10.7 %.

At 15^0 C – 25 psu (Figure 9.13), there were no significant loss in pellet N content after 20 min immersion for the LP, SUS and ND diets ($_{5.12} < 3.06$; $p > 0.05$). Pellets from the three diets lost 8.5, 7.9 and 4.6 % N content respectively after 20 min. There was a significant loss of pellet N content after 20 min for the control diet ($F_{5.12}$ 5.05; $p < 0.05$). This represented an 11.2 % loss.

At 15^0 C – 33 psu (Figure 9.14), pellets from the LP diet showed a significant loss in N content after 5 min immersion ($F_{5.12}$ 9.82: $p < 0.05$). After 20 min, the N content had decreased by 9.7 %. There were no significant loss in pellet N content after 20 min immersion for the control, SUS and ND diets ($F_{5,12} < 2.61$: $p > 0.05$) even though there was a trend for pellet N content to decline with time. After 20 min immersion, pellet N content had decreased by 9.7, 3.7 and 6.8 % respectively.

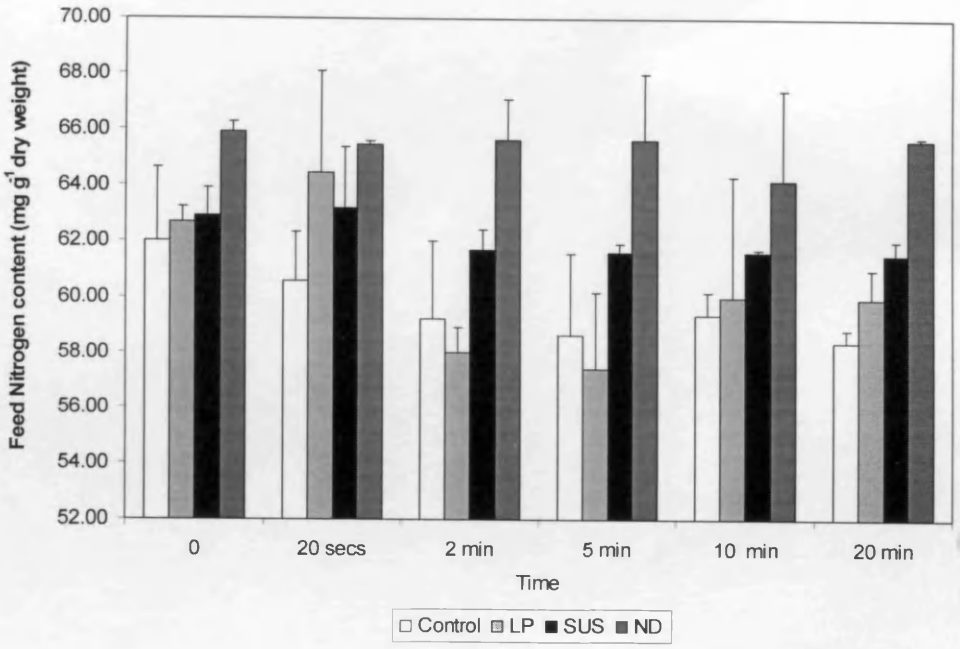


Figure 9.11 Mean concentration of feed nitrogen content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (5^0 C at 25 psu) for up to 20 min.

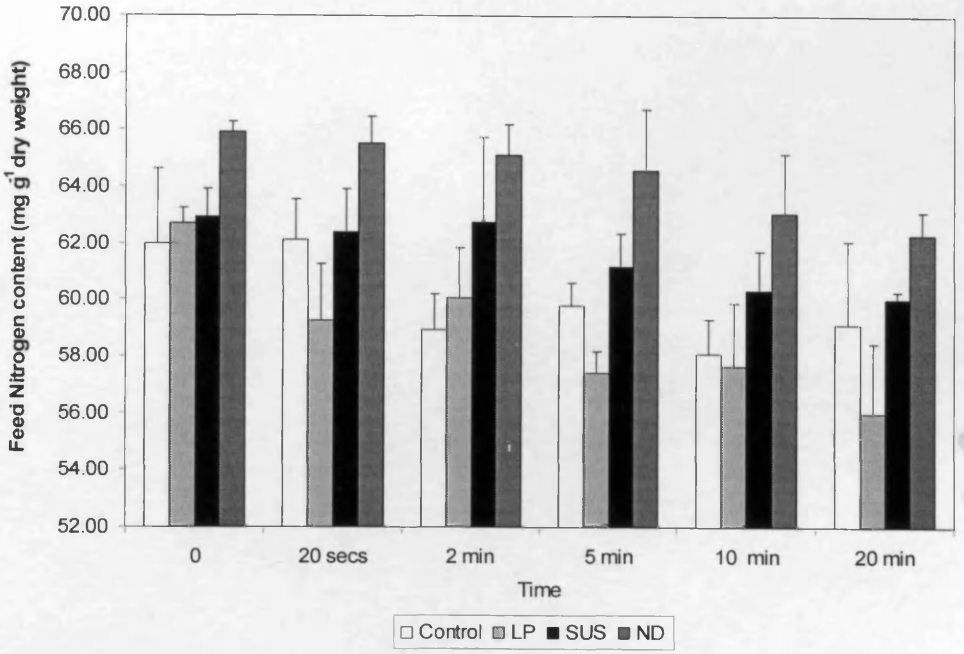


Figure 9.12 Mean concentration of feed nitrogen content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (5^0 C at 33 psu) for up to 20 min.

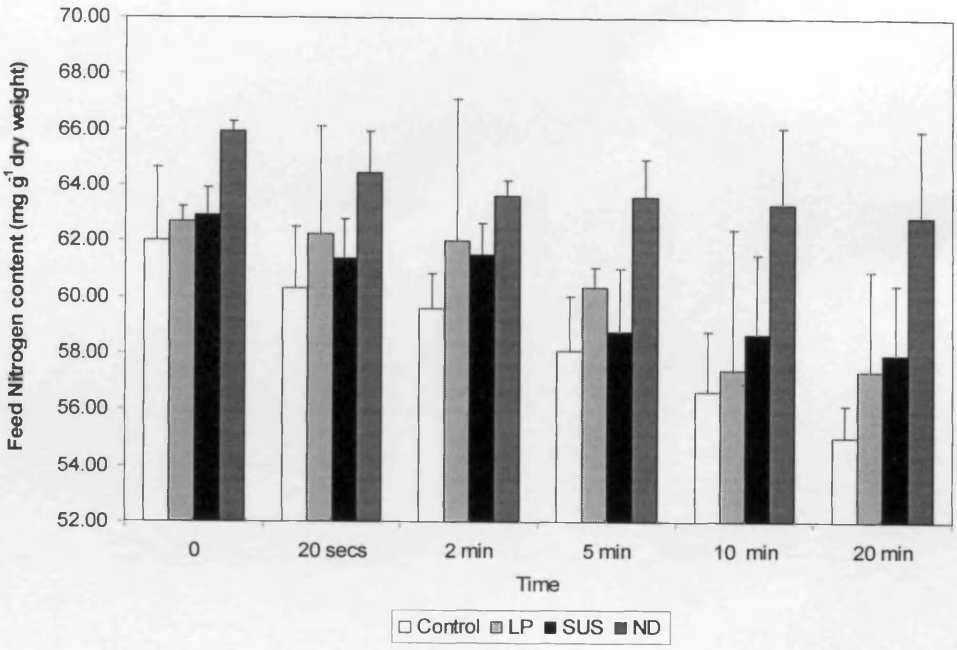


Figure 9.13 Mean concentration of feed nitrogen content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (15^0 C at 25 psu) for up to 20 min.

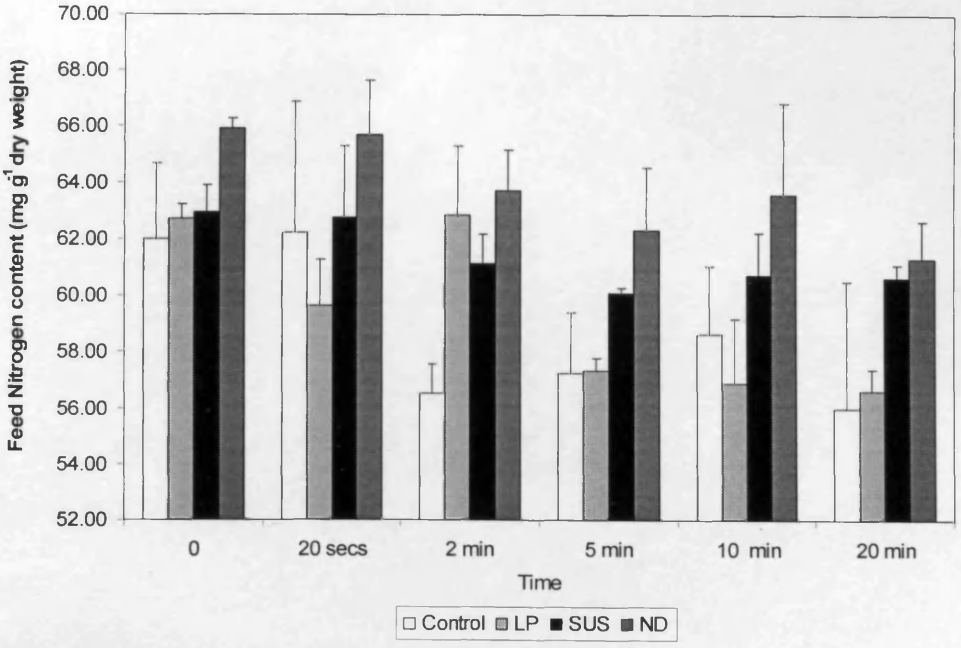


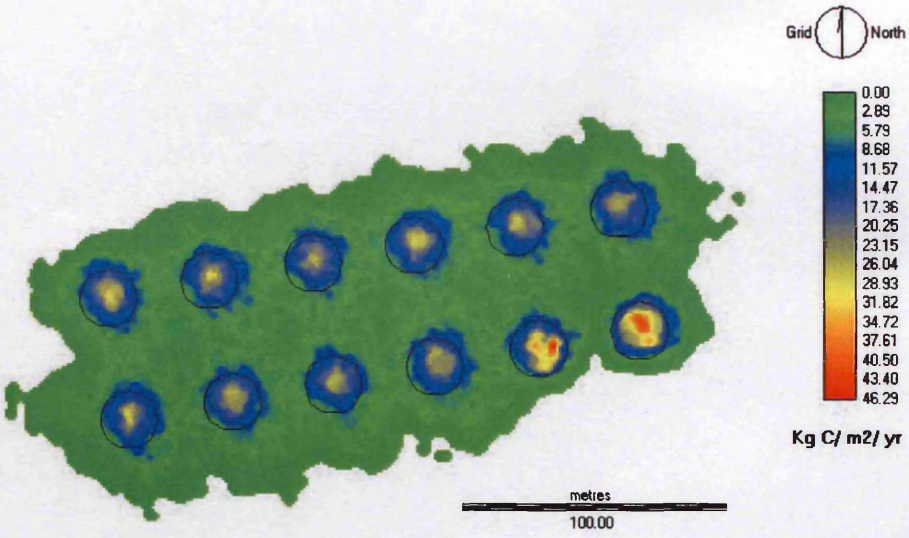
Figure 9.14 Mean concentration of feed nitrogen content (mg g^{-1} dry weight; mean ± 2 S.D.) of four experimental Atlantic salmon diets after immersion in sea water (15^0 C at 33 psu) for up to 20 min.

9.3.5 – Solid waste dispersion modelling

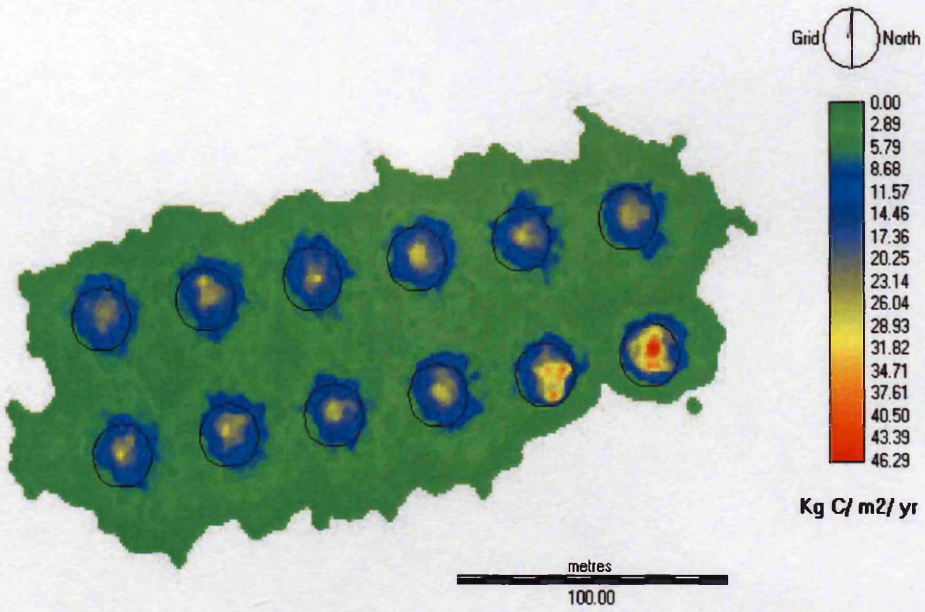
Figure 9.15 shows the total carbon waste output for the four diets. The general pattern of waste distribution was very similar with each of the diets, although the quantities of waste on the seabed were different. The maximum deposition was found under the cage centres, with deposition rates declining significantly for each diet as distance from the cage edges increased ($F_{2,72} < 274.95$; $p < 0.05$). Under the south-easterly end cage for all diets waste was distributed less and the concentration was higher due to the model taking into account the close proximity to the shore and the lower depth under that cage.

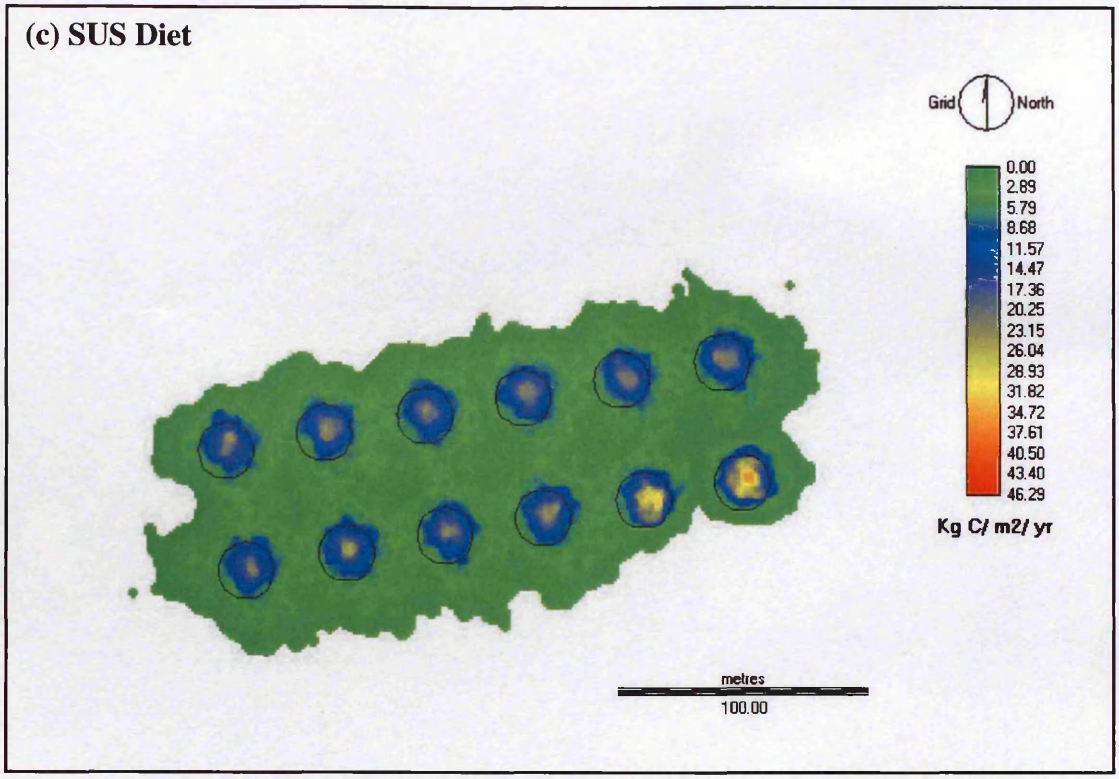
Table 9.10 shows a comparison of the total deposition and mean rate of deposition within the 25m and 50m AZE areas as defined by SEPA, as well as the area inside the 365g C m^{-2} yr^{-1} contour for each diet. The SUS diet had the lowest total deposition within the 25m and 50m AZE areas of 115.481 T C^{-1} and 0.434 T C^{-1} respectively, as it had a lower FCR than the low protein and control diets and a lower carbon content than the nutrient dense diet. Similarly, the mean rates of deposition were also lowest for the SUS diet, being 3.559 Kg C m^{-2} yr^{-1} and 0.019 Kg C m^{-2} yr^{-1} for the 25m and 50m AZE areas respectively. The nutrient dense diet also had a considerably lower deposition rates of 3.683 Kg C m^{-2} yr^{-1} and 0.020 Kg C m^{-2} yr^{-1} as it had a lower FCR than the low protein and control diets, the FCR value being the most sensitive parameter determining the quantity of waste on the seabed. The area inside the 365g C m^{-2} yr^{-1} contour was lowest for the nutrient dense diet (23,873m^2) as it had a high settling velocity which confined the waste food into a smaller area. The area inside the 365g C m^{-2} yr^{-1} was similarly low for the SUS diet at 23,976 m^2, the control area being 24,388m^2.

(a) Control Diet



(b) LP Diet





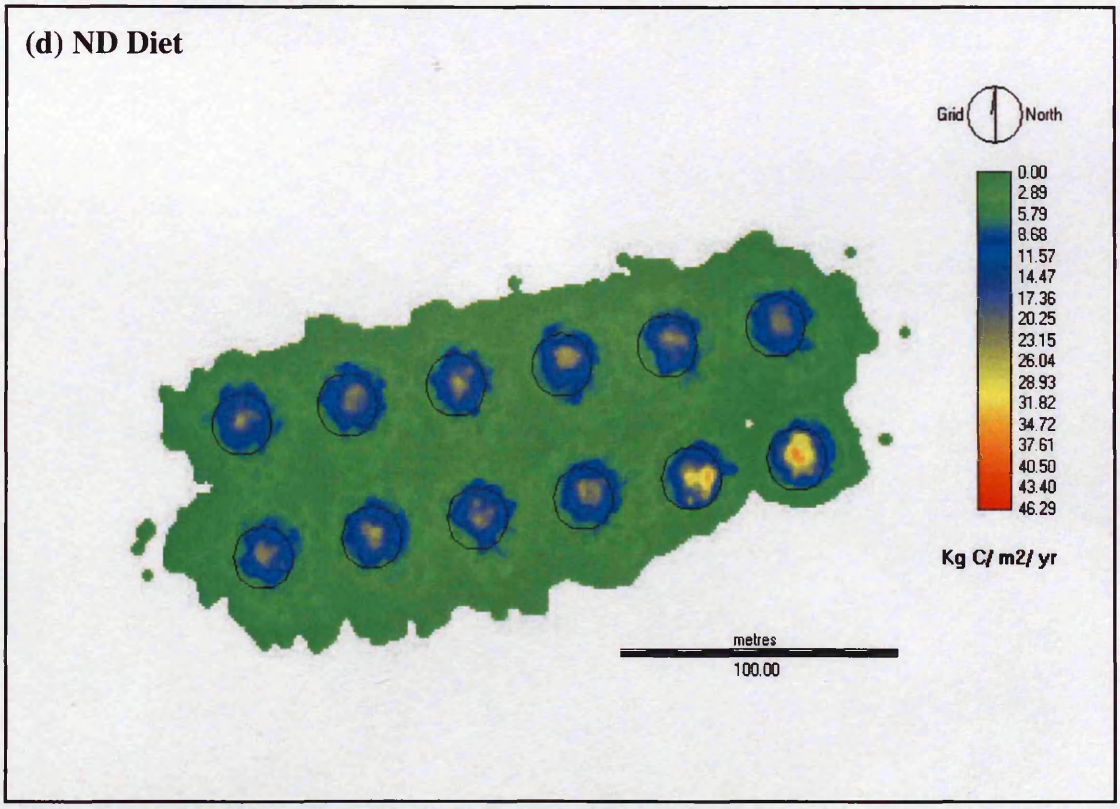


Figure 9.15 Total waste carbon distributions over one year for each diet type. (a) Control; (b) LP; (c) SUS and (d) ND diet.

Table 9.2Total deposition, mean deposition rate and calculated area inside 365 g C m^{-2} contour for each of the four different diet types.

	Total deposition (T C yr^{-1})		Mean deposition rate (Kg C m^{-2} yr^{-1})		Area inside 365g C m^{-2} y^{-1} contour (m^2)
Diet type	25m AZE	50m AZE	25m AZE	50m AZE	
Control	139.052	0.591	4.286	0.027	24,388
LP	139.322	0.597	4.294	0.027	24,500
SUS	115.481	0.434	3.559	0.019	23,976
ND	119.499	0.455	3.683	0.020	23,873

9.4 – Discussion

The results from this study have shown that there were significant differences in the physical characteristics between the four Atlantic salmon diets assessed. Although a study by (Wood, 1987) found relationships between pellet hardness and friability, there were no such relationships found in this study, they are two distinct measures of physical qualities of feed pellets. Relationships between the two parameters are generally only found when the feed ingredients are the same (Thomas and van der Poel. 1996). Pellets from the control diet were found to be less friable and softer compared to the other diets whereas pellets from the ND diet were found to have similar hardness to pellets from the SUS diet but were more friable compared to pellets from the SUS and LP diets. Studies by Robholm and Apelt, 1985 (in Thomas and van der Poel, 1996) showed that small diameter pellets were found to be more susceptible to breakage than larger diameter pellets. As the pellets assessed in this study were of similar diameter, the differences in friability and hardness must be attributed to the variations in feed formulation between each diet.

The provision of essential nutrients is the primary purpose of any animal ration (Fagbenro and Jauncey, 1995). The loss of nutrients due to leaching is an important consideration in aquaculture feeds. Over 94 % of the initial dry weight of pellets from each of the four diets was recovered after immersion in seawater at 5 ^{0}C for up to 20 min regardless of salinity and over 95 % recovered for pellets fro the control and SUS diets at 15 ^{0}C at both salinities. However, pellets from the ND and LP diets lost between 10 and 14 % of initial dry weight at the

higher temperature and at both salinities. The loss of dry weight of pellets at 5 ^{0}C at both salinities may partially be attributed to leaching of both C and N from the pellets over time. However, significantly more N was found to be leaching from the LP diet at 5 ^{0}C – 33 psu (10.7 %) compared to the other diet types and yet results suggests that percentage weight loss was similar for all pellet types for this treatment. This may indicate a potential source of error during the analysis of these samples.

The loss of dry weight at 15 ^{0}C for pellets from both the control and SUS diets was similar to the loss of dry weight at the lower temperature (< 5.0 %). However, pellets from both the ND and LP diets lost more dry weight than the other diet types. Again, some of this dry weight loss may be attributed to leaching of both N and C from the diets over time but both the percentage loss of N and C from these diets are similar to some of the values obtained for pellets from the other diets for the different treatments. Both the ND and LP diets have the highest oil inclusion levels of all the diets and it may be that more of this oil is leaching at the higher temperature. It has been shown that the use of binders is important in the manufacture of experimental diets used in aquaculture in order to improve water stability, aid prehension thus increasing feed efficiency and reducing wastage (Fagbenr and Jauncey, 1995). Even though the types of binders used in these diets are unknown, the differences in physical stability of these pellets may reflect the respective viscosity of the binders used in these diets.

There is very little data on the settling velocities of aquaculture feeds. (Gowen and Bradbury, 1987) quote settling velocities of pellets ranging from 9 to 15 cm s^{-1} (no pellet sizes given). (Elberizon, 1998) showed settling velocities of freshwater salmonid pellets ranging from 5 to 12 cm s^{-1} for 2mm and 8mm pellet sizes respectively whereas a study by Chen *et al.* (1999) showed settling velocities of unsoaked pellets in seawater ranging from 5.6 to 13.9 cm s^{-1} for 2mm and 10 mm pellet sizes respectively. These results are similar to settling velocities found here under marine conditions for all diet types apart for pellets from the LP diet which had a lower mean settling velocity of 8.98 cm s^{-1} over all environmental conditions.

The LP diet has higher oil content (40.6 %) compared to both the SUS and control diets (34.6 and 37.7 % respectively). This may explain its lower settling velocity compared to these two diets. However, the ND diet had higher oil content (45.6 %) than the LP diet yet the settling velocity for pellets from this diet were significantly higher than pellets from the LP diet. However, the LP diet had an additional oil source (20%) in the form of oil/legume seed oil incorporated into the diet and this may have resulted in the lower settling velocities recorded for pellets from this diet.

The density of seawater decreases by approximately 0.2 % between 10 and 20 ^{0}C (Kalle, 1971). Thus, it was expected that food pellets would sink more rapidly at 15 ^{0}C than at 5 ^{0}C. However, there were no significant differences in sinking rates for each pellet type between treatments. Pellets from the control and ND diet sank faster at 5 ^{0}C than at 15 ^{0}C whilst pellets from the LP diet had the lowest settling velocity recorded at 15 ^{0} C – 33 psu. Perhaps the difference

in temperature was not great enough to result in significant changes in the density of seawater and thus would not affect the sinking rates at the different treatments. Estimates of the time taken for the pellets to fall through 30 m water depth (typical depth of most fish farm sites in Scotland) at 5 ^{0} C -33 psu and 15 ^{0}C – 33 psu were calculated. For pellets from the control diet, it would take between 3 min. 42 s and 3 min. 48 s to fall this distance. For pellets from the LP diet it would take between 5 min. 24 s and 6 min. 6s to fall this distance. For the SUS diet, it would take between 3 min. 54 s and 4 min and for the ND diet it would take between 4 min. 24 s and 4 min. 30 s to fall a similar distance. The time taken for both the control and SUS pellets to fall 30 m is similar to values obtained by (Chen *et al.* 1999) for pellets of similar size falling in seawater of 10 ^{o}C at 33 psu, whilst the time taken for ND diet pellets was slightly longer and much longer for LP diet pellets.

Of the four diets the SUS diet had the lowest total deposition, lowest mean deposition and a smaller footprint area inside the 365g C m^{-2} yr^{-1} contour than the control and low protein diets, indicating that this diet would have a lower environmental impact than the others. The nutrient dense diet had lower total deposition and lower mean deposition than the low protein and the control diets and the smallest footprint area inside the 365g C m-2 yr^{-1} contour of the four diets, as it has a low FCR and less food needs to be used for the growing cycle. 365g is the level of carbon estimated to cause a significant impact in the sediments (Beveridge, 2004). However, the low protein diet produced more waste and a larger waste footprint area on the seabed indicating greater environmental impacts than the control diet, as although there is less fish meal and fish oil in the diet, more food is required throughout the growing cycle. It is

important to note that the control diet is a typical commercial diet which has been developed with low environmental impacts in mind, so the fact that the SUS and nutrient dense diets showed lower environmental impacts for solid wastes in the model is very significant.

In SEPA's regulations for solid waste impacts from fish farms a detrimental impact on the seabed within the 25m AZE is considered to be acceptable, although this would trigger increased benthic monitoring. As a level of 365g C m-2 yr^{-1} has been estimated to cause a significant impact on the sediments, it is useful to calculate the difference in area between the 25m AZE and the area inside the 365g C m-2 yr^{-1} contour. For all four diets the area inside the 365g C m-2 yr^{-1} contour was less than the area of the 25m AZE indicating that the significantly impacted sediments would be contained within the 25m AZE, with the nutrient dense and SUS diets giving the smallest impacted areas.

9.5 – Conclusions

Changing the macronutrient inclusion level and source results in differences in the physical characteristics of extruded Atlantic salmon pelleted feeds. Substitution diets such as the SUS diet and the high-energy diet (ND diet) assessed here have similar settling velocities and are harder compared to a standard commercial diet whilst compared to currently available commercial diets such as the control diet used here, they both produced lower environmental impacts when modelled for solid waste impacts on the seabed. A

reduction of environmental impacts is always desirable, not only for environmental preservation, but also to allow fish farmers to farm more fish at a particular site and still remain within environmental regulations. Pelleted LP diets have slower sinking rates but generate more waste and a larger waste footprint area on the seabed indicating greater environmental impacts than the control diet. Although there is less fish meal and fish oil in the LP diet, the higher FCR value calculated for this diet (see Chapter2) indicate that more food is required to produce a comparable biomass to fish fed both the ND and SUS diet types. In addition, pellets from both the LP and ND diets appear to be less water stable at higher water temperatures, thus any waste feed from these diets may generate greater negative impacts on the seabed from leaching compared to pellets from the other diets types.

The data collected here should be used in conjunction with other data for a range of feeds and environmental conditions to employ a "look-up-table" approach to differentiating between diets when modelling waste dispersion.

Based on the results the hypothesis "Different environmental conditions and different feed ingredients had no influence on the physical characteristics of modified diets fed to Atlantic salmon" may be rejected as there were significant differences in hardness, friability, settling velocities and leaching rates between diets. In addition, based on the results, the hypothesis "There are no significant differences between diet types in theoretical waste outputs using a GIS based solid waste dispersion model" may also be rejected as there were differences in deposition rates between diet types.

Chapter 10 – General Discussion

10.1 – Introduction

The potential for intensive aquaculture to have a negative impact on the marine environment has lead to increasing concern and criticism from both the public sector and scientific community. Recent criticism has centred on the use of fish meal and fish oil in aquaculture diets. (Naylor *et al.* 2000) reports that aquaculture is a contributing factor to the collapse of world fishery stocks and that the expansion of aquaculture will result in ever increasing amounts of small pelagic fish being caught for use in aquaculture feeds to expand the total supply of commercially valuable fish. Concern also exists regarding the potential for dissolved wastes from intensive cage aquaculture to impact on the marine environment. This has lead to considerable concern and speculation regarding the effects that these nutrients may cause on receiving water bodies (Gowen and Bradbury, 1987; Aure and Stigebrandt, 1990; Silvert and Sowles, 1996).

These issues were investigated in a series of studies designed to assess the potential for dissolved wastes to impact on the marine environment and to investigate the potential for intensive cage aquaculture to become more sustainable by reducing its reliance on marine fish meal and fish oil by using alternative terrestrial sources.

10.2 – Growth performance and sustainability

The objectives of the present study have been met in that the potential for using different diet formulations containing different inclusion levels and sources of dietary protein and oil fed to large Atlantic salmon was determined. In addition, the amounts of marine fish meal and fish oil required to produce 1 Kg intensively farmed Atlantic salmon fed different diet types was also determined.

It has been shown that it is possible to utilise alternative terrestrial sources of protein and oil without compromising growth and performance in large Atlantic salmon. Fish fed the SUS diet containing partial replacement of fishmeal with corn gluten and fish oil with rapeseed oil were shown to have better growth and similar carcass quality to fish fed a standard commercial ration. It has been shown that partial replacement of fish oil with rapeseed oil does not affect growth with inclusion levels up to 50 % (Bell *et al.* 2003). The potential for using high energy diets such as the ND diet used in this study has been explored in several studies (Kaushik and Cowey, 1991; Cho *et al.* 1994; Alsted *et al.* 1995). These diets have been shown to improve growth and feed utilisation as well as reducing the production of solid and nitrogenous wastes (Cho *et al.* 1991; Kaushik and Cowey, 1991). However, in this study there was only a small improvement in growth with fish fed this diet having a similar FCR to fish fed the SUS diet type. It is worth bearing in mind that the duration of this study was only 139 days and in order to fully evaluate the potential of such diets, further large-scale trails over a full marine phase of production would be required.

The use of low protein diets has been shown to a great potential for use within the industry. Fish introduced to low protein diets from approximately 330 g were shown to have similar growth and performance to fish fed a standard commercial ration. The advent of modern extruded diets has allowed the reduction in the protein content of diets for salmonids by replacing the reduced protein content with the addition of more fat (Johnsen and Wandsvik, 1991; Hillestad and Johnsen, 1994). This has resulted in enhanced N retention efficiency, better growth and feed utilisation and reduced N excretion for salmonids (Azevedo *et al.* 2002).

In terms of sustainability, diet types such as the SUS diet were shown to be more sustainable compared to diets used within the industry at present. The results showed that lower conversions of wild fish to farmed fish were achieved compared to conversions achieved with standard commercial rations used in the industry at present. The use of high energy diets whilst enhancing growth and performance are clearly unsustainable in terms of the amount of fish meal and fish oil incorporated into the diet. The conversions achieved using low protein diets were similar to those of a standard commercial ration based on fish oil inclusion levels but were similar to those of the SUS diet based on fish meal inclusion levels. Cleary, there is the potential for reducing these conversions by replacing the fish oil with rapeseed oil in low protein diets to give conversions that would be more sustainable than diets used in the industry at present.

Thus, as well as being more sustainable, farmers would achieve similar performance in terms of growth. In addition, reducing the amount of marine fish

meal incorporated into the diets fed to Atlantic salmon should reduce the cost of producing such diets. This potential saving could then be passed onto farmers as an encouragement to use such diets. Clearly, a more detailed economic study would be required to assess the economic potential of using such diets. However, this is beyond the scope of this present study.

One area of concern is the industries attempt to increase the intensive cultivation of Atlantic cod in northern Europe. Unfortunately, diets fed to farmed cod have inclusion levels of protein in the form of fish meal of around 50 % (Biomar, *pers comm.*). Given that it can take up to 24 months for fish to achieve harvest size, the use of such diets would place greater strain on the use of fish meal resources if production is to increase as expected. The potential increase in demand for fish meal could also be potentially met by the use of by-catch from wild capture fisheries (Howgate, 1995). The amount of by-catch killed and discarded each year is estimated to be between 18 and 40 million tonnes (FAO, 2000). This is comparable to the total amount of fish currently being harvested for fish meal production (30 million tonnes) (Tidwell and Allan, 2001). Norway, Canada and Iceland have all introduced a ban on the at-sea discarding of certain commercial species and a proportion of the by-catch is used by fish feed manufacturers.

10.3 – Ammonia excretion and the environment

The objectives of the present study have been met in that ammonia excretion rates and patterns from intensively farmed Atlantic salmon fed different diet

types were determined and assessment of the potential ecological risk between discharges of dissolved ammonia from intensively farmed rainbow trout and Atlantic salmon and uptake of these excess nutrients by phytoplankton communities was undertaken.

In assessment of ammonia excretion patterns, it has been clearly shown that fish fed low protein diets appear to excrete less ammonia than fish fed a standard commercial ration. However, the study was undertaken in open cage systems using submersible pumps and any ammonia detected was subjected to a dilution factor. Clearly, although such studies can highlight patterns of excretion, they cannot be used to quantify dissolved waste outputs under commercial farming conditions.

The potential for dissolved wastes to impact on the marine environment was investigated in two mesocosm studies. It was clearly shown that enclosures that received the highest concentration of ammonia in the form of NH_4Cl had the greatest effect on phytoplankton cell numbers. In the first study, the amount of fertilisation was based on data collected during the assessment of excretion patterns from intensively farmed rainbow trout. In the second study, the addition of NH_4Cl was based on excretion patterns from fish fed either a low protein or nutrient dense diet.

For both studies, the enclosures that received the highest concentration of ammonia had the highest increase in phytoplankton cell numbers. This clearly demonstrates that there is the potential for dissolved wastes to impact on marine pelagic environments and that any reduction in protein content in the diets fed to intensively farmed salmonids would reduce the potential risk. The

addition of NH_4Cl was based on food-related concentration peaks of ammonia detected from fish in open cages and as discussed above, any ammonia detected was subjected to a dilution factor. It may be that the concentration peaks detected are in reality much higher and therefore the risk to pelagic systems is much greater.

It has been shown that using low protein diets commercially is feasible without affecting growth performance. The use of such diets would also reduce the actual amounts of dissolved wastes and thus, reduce the potential risk to marine environments. In addition, such diets could be used in low energy systems where water exchange and currents are low in order to reduce the potential for dissolved wastes to directly affect phytoplankton populations and cell numbers.

The expected increase in production of farmed Atlantic cod may have greater potential to impact on marine pelagic environments. Dissolved waste discharge may be greater from these fish when compared to farmed salmon due to then being fed higher protein diets.

10.4 – Quantifying waste outputs and modelling

The need to quantify waste outputs as discussed above has lead to the extensive use of mass balance models. These models have been used to predict waste outputs for all major species farmed intensively in northern

Europe (Ackefors and Enell, 1990; Seymour and Bergheim, 1991; Cho, 1993; Cho and Bureau, 1998; Davies, 2000; Davies and Slaski, 2003).

A report for the Scottish Executive by (Gillibrand *et al.* 2002) used models to provide guidance to the industry and regulatory bodies on the environmental suitability of coastal areas for fish farming. The models predict the relative levels of nutrient enhancement and percentage areas of seabed degraded by organic carbon deposition for several sea lochs. The results are used to construct a combined index and on the basis of this index, areas are designated as Category 1, 2 or 3, where Category 1 areas are considered to be the most environmentally sensitive to further fish farming development due to high predicted levels of nutrient enhancement and / or benthic impact. However the nutrient enhancement model uses a mass balance model to predict the amount of dissolved wastes derived from 1000 tonnes of salmon based on records form a major produced in Scotland. The model output showed that for a diet containing 7.2 % N, with feed waste set at 5 %, digestibility at 90 % and an overall FCR of 1.17, the predicted amount of dissolved wastes was 35.6 Kg^{-1} NT^{-1} Production. In these studies, it has been shown that changing the inclusion level of dietary protein results in less dissolved wastes being excreted compared to the levels cited above with levels ranging from 21 -23 Kg^{-1} NT^{-1} Production. Clearly if such models are to be used to assess carrying capacities of sea lochs in Scotland then changes in diets fed to intensively farmed salmon must be taken into account if such diets were to be used commercially.

The use of enclosed net pens has been shown to be a good method in the quantification of dissolved wastes and in addition has the potential for

quantification of solid wastes. This allows for accurate predictions of waste outputs using a mass balance approach for any given biomass of fish. The use of such a system allows for accurate determination of amounts of waste feed and faeces as well as determination of digestibility and nutrient composition of fish by direct analysis. Used in conjunction with a method to categorise risk such as the one discussed above, allows for the potential for assessing the carrying capacity of sea lochs based on fish production and feeding different diet types. In addition, quantifying dissolved wastes also allows for more accurate analysis of the potential for these wastes to impact on plankton communities. Enclosed net pens also allow for direct comparison of waste outputs from fish fed different diet types and feeding strategies.

It has also been shown that changing the macronutrient composition of diets directly affects its physical characteristics. For example, the low protein diet assessed in chapter 2 was shown to have a significantly lower settling velocity compared to the other diets assessed. This has obvious implications for modelling waste outputs and the physical characteristics of diets must be taken into account when predicting waste outputs.

10.5 – Conclusions

The diets used in this study demonstrated the range of different diets that could be provided by feed manufactures to salmon farmers. Each experimental diet used in this present study was manufactured to reflect the potential to alter the level of or change the source of feed components based on current industry knowledge.

Feeding low protein to Atlantic salmon from 330 g throughout a full marine phase of production does not result in differences in growth and performance nor are there negative effects on growth when feeding fish diets containing partial substitution of fish meal and fish oil.

These results clearly demonstrate that the salmon farming industry can be more environmentally sustainable by reducing reliance on finite sources of fish meal and fish oil as well as reducing the potential for dissolved wastes to impact on the marine environment by using such diets.

10.6 – Future work

In order to determine the potential risk for dissolved wastes to impact on the marine environment, more research is required. Quantifying actual dissolved waste outputs is a priority in order to allow for more accurate determination of the effects of these wastes on plankton communities. In addition, further mesocosm experiments are required to fully elucidate any potential effects. The use of the enclosed net pens developed during these studies allows for the possibility to do so. The enclosed net pens also allow for the investigation of

waste outputs from fish fed experimental diets. Thus, as well as assessing performance of diets in terms of growth, potential environmental impacts may also be assessed.

More research is required on waste outputs from other intensively farmed marine species such as Atlantic cod and halibut. Cod in particular are fed high protein diets and may have a greater potential to impact on marine pelagic environments than salmon due to higher ammonia excretion.

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Appendix 1

Groups and numbers of phytoplankton identified on each sampling day (Chapter 6)

Study 1

Class	Sub-Class	Order	Family	Bags	Day 1	5	8	11	17	26
Bacillariophyceae	**Centricae**		*Skeletonema*				**Diatoms**			
			Chaetoceros	**C1**	4000	12000	16000	12000	16000	6000
			Rhizozolenia	**C2**	8500	10500	14500	16500	18500	6000
			Thalassiosira							
			Leptocylindrus	**LN1**	6000	4000	18000	16000	24000	10000
			Ceratulina	**LN2**	6500	8500	20500	22500	24500	12000
			Eucampia							
			Coscinodiscus	**HN1**	6000	10000	20500	24000	26000	14000
	Penatae		*Navicula*	**HN2**	6000	10500	18500	28500	28500	10000
			Fragilaria							
			Asterionella							
							Dinoflagellates			
Phytomastigophora		**Dinoflagellida**	*Dinophyis*	**C1**	2000	2000	2000	4000	6000	4000
			Peridinium	**C2**	2000	4000	2000	4000	4000	2000

	Gymnodinium							
		LN1	2000	2000	4000	2000	2000	2000
		LN2	0	2000	4000	4000	2000	2000
		HN1	2000	2000	4000	6000		2000
		HN2	2000	4000	6000	6000		2000
Chlorophycaea	*Nanochlorus*	**Flagellates**						
	Chlamydomona	**C1**	4000	6000	8000	10000	10000	2000
Chrysophyceae	*Isochrysis*	**C2**	6000	10000	12000	10000	10000	2000
	Pyramimonas							
	Dinobryon	**LN1**	2000	4000	8000	10000	4000	4000
Cryptophyceae	*Rhodomonas*	**LN2**	0	6000	6000	8000	6000	6000
	Cryptomonas							
		HN1	2000	2000	4000	6000	4000	4000
		HN2	4000	4000	6000	6000	6000	4000

Study 2

Class	Sub-Class	Order	Family	Bags	Day 1	5	8	12	16	21
					Diatoms					
Bacillariophyceae	**Centricae**		*Skeletonema*							
			Chaetoceros	**C1**	8000	10000	8000	8000	14000	6000
			Rhizozolenia	**C2**	10000	0	6000	16000	12000	6000
			Thalassiosira							
			Leptocylindrus	**LP1**	0	6000	10000	18000	24000	10000
			Ceratulina	**LP2**	4000	8000	24000	10000	16000	4000
			Eucampia							
			Coscinodiscus	**ND1**	6000	16000	10000	16000	28888	8000
	Penatae		*Navicula*	**ND2**	4000	2000	14000	10000	20000	22000
			Fragilaria							
			Asterionella							
					Dinoflagellates					
Phytomastigophora		**Dinoflagellida**	*Dinophyis*	**C1**	2000	0	4000	2000	2000	2000
			Peridinium	**C2**	0	0	0	2000	2000	0
			Gymnodinium							
				LP1	0	0	0	0	2000	0
				LP2	2000	0	0	0	2000	0
				ND1	0	4000	0	4000	0	0

		ND2	0	0	0	4000	0	0
Chlorophycaea	*Nanochlorus*	**Flagellates**						
	Chlamydomona	**C1**	0	0	0	2000	4000	0
Chrysophyceae	*Isochrysis*	**C2**	0	0	0	2000	4000	0
	Pyramimonas							
	Dinobryon	**LP1**	0	2000	2000	10000	0	2000
Cryptophyceae	*Rhodomonas*	**LP2**	0	4000	2000	6000	0	4000
	Cryptomonas							
		ND1	0	0	0	6000	4000	2000
		ND2	2000	0	0	4000	2000	4000

Appendix 2

Groups and numbers of zooplankton identified on each sampling day (Chapter 6)

Study 1

Phylum	Class	Order	Family	Bags	Day 1	5	8	11	17	26
Arthropoda	Copepoda	Calanoids	*Acartiidae*	**Total Copepods**						
			Temoridae	**C1**	11	13	26	19	19	6
			Centropagidae	**C2**	10	14	18	24	23	4
			Pseudocalanidae							
			Paracalanidae	**LN1**	11	17	26	27	26	7
			Unidentified Calanus	**LN2**	13	19	24	27	23	12
		Harpacticoida	Microstella sp							
			Euterpina sp	**HN1**	11	23	21	35	27	5
				HN2	13	23	27	41	28	18
				Total (Other taxa)						
		Cyclopoida		**C1**	1	4	4	6	6	6
	Ostracoda	Myodocopa		**C2**	0	2	5	8	3	1
	Branchipoda	Diplostraca								
	Malacostraca	Isopoda		**LN1**	3	3	7	0	0	6

Sacromastigophora		Foraminiferida		**LN2**	2	2	4	3	4	2
Cnidaria	Hydrazoa	Leptomedusaae	Obelia sp							
Rotifera				**HN1**	0	6	3	4	2	9
Mollusca	Gastropoda	Thecosomata	Spiratella sp	**HN2**	1	2	8	3	6	4
Choradata	Larvacea									
Annelida	Polychaeta		*Larvae*							
Mollusca	Bivalvae		Velioger larvae							
Echinodermata			larvae							
Bryozoa										
Athropoda	Malacostraca	Decopda	Zoeae larvae							
EGGS										

Study 2

Phylum	Class	Order	Family	Bags	Day 1	5	8	12	16	21
				Total Copepods						
Arthropoda	Copepoda	Calanoids	*Acartiidae*							
			Temoridae	**C1**	3	2	11	17	9	15
			Centropagidae	**C2**	3	1	11	16	15	10
			Pseudocalanidae							
			Paracalanidae	**LN1**	11	6	10	11	23	14
			Unidentified Calanus	**LN2**	13	4	19	13	20	14
		Harpacticoida	Microstella sp							
			Euterpina sp	**HN1**	11	0	2	20	18	26
				HN2	13	0	10	17	21	24
				Total (Other taxa)						
		Cyclopoida		**C1**	1	1	7	1	5	8
	Ostracoda	Myodocopa		**C2**	2	3	6	5	1	11
	Branchipoda	Diplostraca								
	Malacostraca	Isopoda		**LN1**	3	3	6	5	4	6
Sacromastigophora		Foraminiferida		**LN2**	0	4	2	4	6	11
Cnidaria	Hydrazoa	Leptomedusaae	Obelia sp							
Rotifera				**HN1**	1	2	3	2	5	1

Mollusca	Gastropoda	Thecosomata	Spiratella sp	**HN2**	1	1	5	1	6	1
Choradata	Larvacea									
Annelida	Polychaeta		*Larvae*							
Mollusca	Bivalvae		Velioger larvae							
Echinodermata			larvae							
Bryozoa										
Athropoda	Malacostraca	Decopda	Zoeae larvae							
EGGS										

Appendix 3

Example of the spread sheet model used to calculate waste outputs

INPUT VALUES	TEST250	TEST800	CONTR	TEST800	TEST1500	TEST250	DIETS CONTR	TEST1500	TEST250	TEST1500	TEST800	CONTR	Monthly Totals
Cage number	**301**	**302**	**303**	**304**	**305**	**306**	**307**	**308**	**309**	**310**	**311**	**312**	
Ingoing													
Number	23201.00	22726.00	22901.00	22481.00	23155.00	23017.00	23002.00	23005.00	22980.00	22796.00	24068.00	22937.00	
weight (g)	100.16	101.18	100.92	99.37	98.94	96.97	101.05	100.79	102.12	100.86	97.98	97.27	
biomass (kg)	2323.77	2299.37	2311.26	2234.02	2291.03	2231.97	2324.35	2318.72	2346.79	2299.27	2358.11	2231.11	**27.57**
Mortalities													
Number	30	25	38	66	23	20	24	41	29	59	34	95	
weight (kg)	3.79	3.24	5.14	9.04	2.96	3.18	2.83	6.03	4.37	8.82	4.39	12.32	
mean weight (g)	126.33	129.60	135.26	136.97	128.70	159.00	117.92	147.07	150.69	149.49	129.12	129.68	
Outgoing													
Number	23171.00	22701.00	22863.00	22415.00	23132.00	22997.00	22978.00	22964.00	22951.00	22737.00	24034.00	22842.00	
weight (g)	192.91	194.75	195.56	192.50	188.62	188.80	190.03	194.33	199.01	196.18	191.97	189.95	
biomass (kg)	4469.98	4421.13	4471.12	4314.98	4363.07	4341.79	4366.52	4462.69	4567.42	4460.45	4613.72	4338.79	
biomass (T)	4.47	4.42	4.47	4.31	4.36	4.34	4.37	4.46	4.57	4.46	4.61	4.34	**53.19**
Feed Input (kg)	2150.00	2125.00	2165.00	2090.00	2075.00	2113.00	2045.00	2150.00	2225.00	2170.00	2260.00	2120.00	**25688.00**
Feed Input (T)	2.15	2.13	2.17	2.09	2.08	2.11	2.05	2.15	2.23	2.17	2.26	2.12	**25.69**
FCR	0.75	0.71	0.72	0.73	0.61	0.67	0.68	0.64	0.70	0.76	0.66	0.67	
N content of diet (%)	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38	7.38	
Digestibility (%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	
Feed Waste (%)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
Mortalities (%)	0.13	0.11	0.17	0.29	0.10	0.09	0.10	0.18	0.13	0.26	0.14	0.41	**0.18**
OUTPUT VALUES													
N input	**158.67**	**156.83**	**159.78**	**154.24**	**153.14**	**155.94**	**150.92**	**158.67**	**164.21**	**160.15**	**166.79**	**156.46**	
waste feed N (kg)	**7.93**	**7.84**	**7.99**	**7.71**	**7.66**	**7.80**	**7.55**	**7.93**	**8.21**	**8.01**	**8.34**	**7.82**	
Total N consumed (kg)	**150.74**	**148.98**	**151.79**	**146.53**	**145.48**	**148.14**	**143.37**	**150.74**	**155.99**	**152.14**	**158.45**	**148.63**	
Faeces (kg)	**15.87**	**15.68**	**15.98**	**15.42**	**15.31**	**15.59**	**15.09**	**15.87**	**16.42**	**16.01**	**16.68**	**15.65**	
N retained in fish (3.4%)	**69.57**	**68.74**	**70.04**	**67.35**	**67.05**	**68.33**	**66.03**	**69.49**	**72.10**	**70.08**	**73.29**	**68.26**	
Particulate discharge	23.80	23.52	23.97	23.14	22.97	23.39	22.64	23.80	24.63	24.02	25.02	23.47	284.37
Total dissolved N	65.30	64.56	65.78	63.75	63.12	64.21	62.25	65.37	67.47	66.04	68.48	64.73	781.06
Total N discharge	88.98	87.99	89.59	86.63	86.00	87.53	84.80	89.02	91.99	89.83	93.37	87.83	1063.56
% particulate	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	
% retained in growth	45.99	46.00	45.96	45.87	46.00	46.00	46.01	45.94	45.98	45.88	45.98	45.80	
% dissolved waste	41.15	41.17	41.17	41.33	41.22	41.18	41.25	41.20	41.09	41.24	41.06	41.37	