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OPTIMAL PROTEIN RATIO FOR THE GROWTH OF FARMED ARCTIC CHARR (*Salvelinus alpinus*)

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ABSTRACT

A study was carried out to determine the optimal protein and fat ratio for growth of farmed juvenile through to table sized Arctic charr (*Salvelinus alpinus*) in the northwest of Iceland in land based facilities between October 2007 and February 2008. The objective was to determine the effects of varying protein and oil ratios on the growth of juvenile to table sized Arctic charr. In order to attain this objective the specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), digestibility and chemical composition of the fed fish after every 4-5 weeks were determined. The fish were fed on six different dietary compositions which had an inclusion of celite marker at 1% and protein to fat compositions of 29% to 28.5%, 33% to 26.5%, 37% to 25.3%, 41% to 24%, 45% to 22.8% and 49% to 21.6% respectively. The diet comprised of capelin fishmeal, wheat, colour, ash and capelin fish oil.

The charr were also raised in two different conditions, one group was treated to saline water of 17o/oo salinity (start feed size: 250-/+40 g) and the other group was raised in fresh water (size: 230-/+10 g). Findings indicated that no significant difference occurred in growth while varying the protein and oil ratios. This is very important due to the fact that the study sought to find a way of reducing the protein and oil from fish thereby reducing production costs and increasing profits for the fish farmers while also taking into consideration the ethical implications of the use of trash fish to formulate fishmeal.

Key words: Arctic charr (*Salvelinus alpinus)*, feeding, fish meal and oil reduction in fish feed formulation, optimal protein ratio, growth.

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ACOAC - Official Methods of Analysis of the Association of Official Analytical Chemist
ADC - Apparent digestibility coefficient
ANOVA - Analysis of variance
FAO - Food Agriculture Organisation
FBW - Final body weight
FCR - Feed conversion ratio
FM - Fish meal
FO - Fish oil
K - Condition factor
MMT - Million metric tonnes
NRC - National Research Council
SGR - Specific growth rate
WWF - World Wide Fund
TIAA - The Icelandic Aquaculture Association
YSI - Yellow Springs Instruments

1 INTRODUCTION

1.1 Background

Currently the contribution of aquaculture to world fisheries production is on the increase with the FAO report (2005) indicating that the production was about 48 million tonnes (excluding aquatic plants). However it was also noted that capture fisheries had reached a stable state (FAO 2004) indicating that aquaculture needs to supply the surplus of the fish to the increasing world population in terms of a cheap protein source.

FAO (2006) estimates that in order to maintain the current level of per capita consumption, global aquaculture production will need to reach 80 million tonnes by 2050. Aquaculture has the potential to make a significant contribution to this increasing demand for aquatic food in most regions of the world; however, in order to achieve this, the sector (and aqua-farmers) will face great challenges (FAO 2006) including among others the high escalating feed costs which is usually a major part of production costs and supply of raw materials for the production of fish feeds may decrease.

According to FAO (2006), salmonids by far are the greatest contributors to total production in terms of aquaculture in the Atlantic and Pacific regions. However, the culture of carnivorous fish requires 40-60% protein in dietary feeds. Currently the costs of fish meal and fish oil are high because of high demand with competition between the fish feed industry other animal feed industries (WWF 2006).

According to Jesse (2006), Iceland is one of the largest producers of farmed Arctic charr in the world with about 2000 metric tonnes per year. Arctic charr (*Salvelinus alpinus*) is a circumpolar species found in the polar regions of North America and Europe. Arctic charr is a salmonid, and exists in both anadromous (seagoing) and non-anadromous (resident freshwater) forms.

Arctic charr is also a very valuable species in Iceland and may remain so for a long time given the suitable conditions required for its culture but is not a cheap source of protein as some other species. Therefore, reduction in feed costs in its production is vital as this will lead to improved earnings to the farmers and the country and will also lower the use of trash fish used to make the fish meal and oil, taking into consideration the ethical point of view in relation to consumers.

Compared to other aquaculture species such as salmon and trout, there is limited information concerning feed formulation development for Arctic charr, and much of the available data exists as grey literature (Jesse 2006). Also, there are no specific data concerning variation and or replacement of expensive fish meal protein and oil with cheaper sources such as vegetable protein meal and oil for Arctic charr protein dietary feeds, as they are fed a modified trout and salmon feed. As Arctic charr is primarily farmed in Iceland there is need to develop cheap feeds for this carnivorous fish by replacing expensive fish meal and oil with cheap plant protein and oil sources.

The effect of plant protein ingredients in feeds for farmed fish on chemical composition of muscle shows contrasting results (De Francesco *et al.* 2007). Some studies have reported that increasing levels (from 10% to 30%) of plant ingredients in feed for European sea bass (Gouveia and Davies 2000) or gilthead sea bream (Pereira and Oliva-Teles 2002) does not affect the whole-body lipid content.

On the other hand, Robaina *et al.* (1998) observed a decrease in muscle total lipid content in sea bream fed a diet containing 30% of soy by-products and Kissil *et al.* (2000) reported a decrease in whole-body lipid content in sea bream fed 100% fish meal substitution diets based either on soybean or rapeseed protein concentrates.

In contrast with these results an increase of fat in fish fed diets containing increased levels of plant ingredients was observed in trout (Burel *et al.* 2000) and in sea bass (Kaushik *et al*. 2004).

In marine fish, Aoki *et al.* (1996) did not find any difference in flesh quality between adult red sea bream fed with or without fish meal as a dietary protein source. Kaushik *et al.* (1995) and De Francesco *et al.* (2004) showed that in rainbow trout fed diets containing plant ingredients the organoleptic characteristics were slightly affected by the dietary protein source.

Studies done by Gudmundsson and Petursdottir (1998) indicate that varying the protein/oil ratio had no effect on the growth of three sized fish groups of Arctic charr at two varying temperatures and fed on three varying diets. However, this left some gaps. For example, it was not explored what would happen if the variation was reduced further than the 34%. A cost benefit analysis done by Sigurgeirsson pers comm. showed that if the current 42% composition of protein is reduced to about 33% there would be a reduction from 100% to about 90% in feed costs implying a benefit for the farmers in terms profits and also a reduction in the use of trash fish in feed production which will have addressed the ethical point of view of using fish to produce fish as this practice will have been reduced. This study aims to further address the effect the varying the protein and oil ratio on the growth of Arctic charr.

1.2 Project statement

Given the lack of information on the effects of varying different dietary protein/oil ratios on the growth rate of Arctic charr, there was a need to evaluate if varying the protein and oil ratio would have an effect on the growth of Arctic charr of juvenile to market table size in both fresh and seawater environments. This was done in the bid to lower the costs of fish feed thereby increasing productivity for fish farmers. At lower feed costs profits for the famer will increase, as currently fish faming is still an unprofitable enterprise (per's com, Chairperson, The Icelandic Aquaculture Association (TIAA 2007).

1.3 Motivation/rationale for selecting this topic

Uganda earns substantial income from fisheries and in 2006 Nile perch constituted up to 96% of the fish exports to premium markets, 40% of the exports to region markets, and 30% of the fish consumed locally.

Fish in 2006 earned Uganda US$ 185-190 million with US$ 145 million from Nile perch exports to premium international markets, and US$ 40-50 million from regional fish trade. The level of investment in the fish processing and exporting industry currently stands at US$ 54 million although working at about 50% installed capacity due to shortage of Nile perch (Ogutu–Ohwayo and Kirema-Mukasa 2005) however currently most of the water bodies are overexploited and have been polluted and the future lies in commercialised aquaculture.

Aquaculture in the recent past has been extensive or semi intensive which requires little input and little management requirements. However, in the event of commercial aquaculture there is a need to formulate feeds which are from cheap protein sources like plants which are much cheaper than fish meal and oil more so in feeding carnivorous fish and no technology exists in my country on how to formulate feeds from cheap resources with little input. However, studies of the effect of varying protein ratios on the growth for a given species in culture should precede substitution of the feed protein.

Cheap formulated feeds is one major problem which fish farmers are faced with in East Africa even for currently cultured Nile tilapia in semi- intensive culture.

According to Liti *et al.* (2006), the challenges facing tilapia producers in Kenya today lie in the identification of cost-effective and efficient feeds for production of *Oreochromis niloticus* in fertilised ponds which is also true for Uganda and is the principle rationale for me to engage in this project hoping to contribute useful information to my country and possibly the region.

1.4 Expected outcome from the project

- Identification of the optimal protein ratio in replaced protein/oil feed sources for juvenile Arctic charr growth.
- Enhancement of my knowledge and skills in fish nutrition studies.
- Dissemination of information to my fisheries and aquaculture students on how cheap feeds can be formulated which would justify the possibility of starting commercial fish farming.
- Technology transfer to the current animal feed industry especially in fish feed formulation for carnivorous fish in the event that Nile perch culture technology developments come into place.
- Resources/references to look for and establish the effect of various feed formulations will be put into place.

1.5 Objective

The main aim of my study was to:

- Determine the optimum protein diet ratio required in rearing juvenile Arctic charr.

1.5.1 Goals

- To determine the effect of varying protein and oil ratios on the growth of Arctic charr through determination of the feed conversion ratio(FCR), specific growth rate (SGR), and condition factor (K), digestibility and chemical composition of the fish.

- To compare the growth and digestibility/chemical composition of different protein ratios in feed of Arctic charr in fresh water and saline water within treatment groups.

1.5.1.1 Research questions/null hypothesis

The research questions in this study were:

- Does variation of protein and oil ratios have an effect on the growth of Arctic charr?
- Does the environment in which Arctic charr are raised have an effect within treatment groups on growth?

Null hypothesis:

- Varying protein and oil ratios in administered feed does not affect growth in Arctic charr.
- Varying protein and oil ratios in administered feed does not affect the feed conversion ratio (FCR) in Arctic charr.
- Varying protein and oil ratios in administered feed does not affect the condition factor (K) in Arctic charr.
- Varying protein and oil ratios in administered feed does not affect digestibility in Arctic charr.

2 REVIEW OF RELATED LITERATURE

2.1 Growth of Arctic charr

Growth of charr is rapid during the early fresh water rearing stages, and quite good rates of growth can be maintained at low water temperatures (Jobling 1983). Growth may, however, be sub-maximal if charr are reared in systems designed for other salmonids, and problems may arise when charr are held at low stocking densities. Growth performance and food conversion can be improved by forcing the fish to swim at moderate speeds by exposing them to water currents. Arctic charr are known to be territorial in behaviour in their natural existence. Therefore, the dominant will protect a given territory of a school of fish. Recent studies have demonstrated that Arctic charr exhibit higher growth rates when held at high stocking densities. It has been argued that these increased growth rates are a result of decreased social interactions at the increased densities (Brown *et al.* 1992). In higher densities of fish the hierarchy tendency is broken with the growth and better feed conversion. This is because they have a schooling behaviour tendency whereby they are always swimming around and therefore the energy is spent in locomotion at the expense of growth. The optimum current is equivalent to 1-2 fish lengths/second. Fish are lining up but not struggling against the current.

Seawater growth performance has been reported as being highly variable, and this probably results from seasonal changes in the hypo-osmoregulatory ability of the charr, combined with the use of inappropriate rearing techniques during the on-growing phase in seawater. In nature, Arctic charr live in seawater for a few weeks during summer and move to fresh water creating osmotic problems especially in the cold winters due to salt tolerance and temperature differences. This is because osmoregulation is very costly to maintain especially in marine fish where they must constantly expel various solutes, such as sodium and chloride ions, against an osmotic gradient, a great deal of energy is required. Therefore, anything that one can do to lower the osmotic gradient will benefit the fish in terms of energy expenditure. The simplest way of doing this is to lower the salinity of the water as much as possible, particularly for a fish in distress (i.e. diseased). This alone can sometimes be enough to ease their burden. Of course any such change must be extremely gradual and must not get to the point where the fish is in obvious stress.

Rates of growth of Arctic charr were as high as those reported for other salmonid species reared under similar conditions. Preliminary results suggested that growth rates of charr may be lower in salt water than in fresh water.

2.1.1 Effects of temperature on growth

According to Jobling (1983), when Arctic charr is reared for a period of 6 months at a temperature of 10°C Arctic charr increased in weight from 18 g to approximately 135 g. Specific growth rates decreased as the fish increased in size and the relationship between size and growth rate could be described by the equation

$$\text{Log}_e\ G_W = 1{,}722 - 0{,}325\ \text{Log}_e\ W$$

Where Gw is specific growth rate and *W* is fish weight in grams. Temperature effects upon growth were examined using previously published data. Below the optimum growth temperature, the growth rate of a fish of a given size could be predicted using the equation:

$$\text{Log}_e\ G_W = \text{Log}_e\ [7, 5\ (0, 0219 + 0, 0727T)] - 0{,}325\ \text{Log}_e\ W$$ (Jobling 1983)

Where T is the rearing temperature.

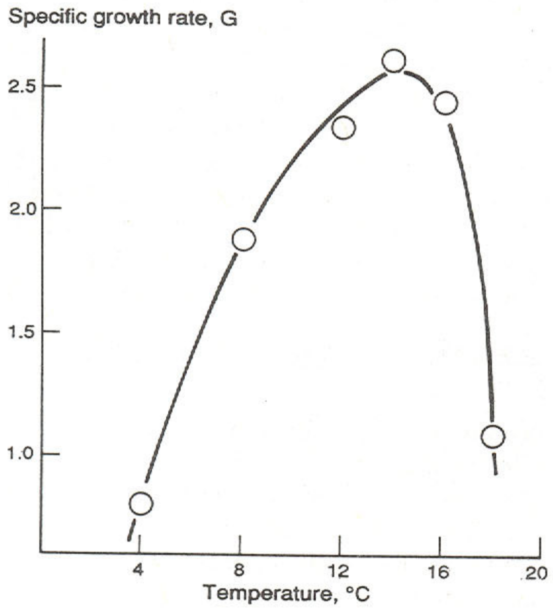


Figure 1. Effects of temperature on growth rates of Arctic charr fed in excess.

Figure 1: Effects of temperature on growth rates of Arctic charr fed in excess at juvenile stage (adapted from Jobling *et al.* 1992).

2.1.2 Digestibility and chemical composition in Arctic charr studies

Gudmundsson and Petursdottir (1998) carried out studies on the digestibility and growth of Arctic charr. The studies involved the use of celite on three age groups and three protein dietary compositions.

Their results indicated that protein digestibility was highly independent of the three diets given. The effect of temperature on protein digestibility was therefore limited and not significant (P>0.05). They also noted that the digestibility of fats was low and independent of water temperature at low protein or fat concentrations in feeds but increased as these nutrients increased in the diet making the fat digestibility significantly higher at 11°C than at 7°C. The effects on the digestibility of nitrogen free extract were small and there are no effects on the digestibility of ash (Gudmundsson and Petursdottir 1998).

In general they also found that as protein increases in the diet there is an increase in specific growth rate, but this is reduced as the fish grows. Similarly as the fat in the diet increases the specific growth rate increases in juvenile fish to the maximum fat concentration tested but this increase ceases around 15% dietary fat for older fish.

Gudmundsson and Petursdottir (1998) concluded, as is commonly believed, that digestibility of nutrients in Arctic charr is highest in the juvenile fish and decreases as the fish grow older. They also noted that dietary protein 50%, increased fat and carbohydrate digestibility. The optimum fat digestibility was between 15 and 20 % and that temperature had a minimal effect on digestibility in juvenile Arctic charr but significant effects started appearing as the fish grew. They also concluded that there is a need for standardising the apparent digestibility coefficient (ADC) determination in fish to give the best comparison between food ingredients and to minimise physiological and environmental factors was recognised.

Studies done in Norway on digestibility have shown that some fish will grow faster than others because they better digest and absorb amino acids. Torrissen and Shearer (1989) have done studies and found out that fish have trypsin isozyme (TRP-2(92) with special abilities to stimulate growth through better feed intake and digestibility. Jobling (1983) presented digestibility of various protein sources in Table 1 below.

Table 1: Digestibility of various protein source s (adapted from Willoughby 1999 after Jobling 1993).

Feed	Protein digestibility (%)
Fish meals	
Anchovy	85
Herring	87
Animal by products	
Blood meal	69
Meat meal	75
Poultry meal	70
Plant	
Wheat	68
Soybean	75
Alfalfa	61

2.1.3 Effects of protein replacement on the chemical composition of farmed Arctic charr

Tabachek (1986) evaluated the growth, body composition and feed utilisation for Arctic charr fed on a control diet and nine practical diets formulated to contain three protein levels (34, 44 and 54%) at each of three lipid levels (10, 15 and 20%). Fish were raised for 24 weeks at 12°C. He noted that increasing either dietary protein and/or lipid resulted in improved weight gain, feed efficiency and energy retained. Protein efficiency ratio and protein retained were directly related to dietary lipid and inversely related to dietary protein. A sparing effect of lipid on protein was evident where, at each protein level, Arctic charr utilised protein more efficiently with each increase in dietary lipid. Levels of 34% protein and/or 10% lipid were well below the requirements for Arctic charr (initial weight, 4.6 g). Within the range tested, the dietary combination of 54% protein with 20% lipid maximised weight gain and feed efficiency and resulted in fish with low liver and body lipid contents. This is important because the quality of the fish is improved in terms of shelf life of the farmed fish

Luo *et al.* (2006) studied the effect of solvent-extracted cottonseed meal (SCSM) as a partial or total replacement of fish meal in juvenile rainbow trout (*Oncorhynchus mykiss*). In this study, fish growth final body weight (FBW), weight gain rate (WGR) and specific growth rate (SGR) decreased in relation to the level of SCSM. However, a significant reduction of growth was only observed when fish meal was totally replaced. No significant difference was observed for feed intake (FI), except SCSMT that showed significantly lower value than other diets ($P < 0.05$). The data on the body composition of rainbow trout at the end of the growth trial indicated that a significant difference was found in the fish fed on the different experimental diets ($P > 0.05$), which means that SCSM had no significantly adverse effect on the body composition of rainbow trout (Luo *et al.* 2006)

2.2 Raw materials for fish feed formulation

2.2.1 Proteins

Protein is an important component in the diet because it supplies amino acids to organisms for growth. Protein can also be metabolised as an energy source. We are looking for the optimum energy for usage in (mmg) from alternative plant sources in the form of fats and proteins for optimal growth. Many studies have been carried out to determine protein requirements for fish, with estimated protein requirements ranging from 400 to 550 g kg for carnivorous fish (National Research Council 1983, Gao *et al.* 2005).

The difference in protein requirements is due to fish size. We are therefore looking for the optimum level for all size classes for Arctic charr by stepwise lowering of the protein ratio in our current research work. Studies on juvenile flounder on the effects of protein level on growth have indicated that specific growth rates in wet weight are affected by protein levels (Gao *et al.* 2005)

2.2.2 Fish meal/fish oil

The increasing demand for fish meal (FM) and fish oil (FO) combined with decreasing wild fish stocks indicate that an alternative product (or products) would be highly desirable. Fish meal production over the last decade has fluctuated between 6.3 and 7.4 million metric tonnes (MMT) per year, while fish oil production has ranged between 1.0 and 1.7 MMT. The poultry industry uses 24%, pigs 29%, farmed fish 35%, and ruminants 3% of the total global fish meal being consumed. With anticipation of a major increase in the production of both fish and chicken, global fish meal requirements are projected to double by 2010. Shortly thereafter, it is predicted that aquaculture alone would be able to consume all the available fish meal and fish oil production. Besides ecological and ethical opposition to the use of finite and valuable aquatic resources as feed ingredients for high value animal species, there is a growing economical concern about the uncertain availability and cost. An additional reason for concern is that fishery products may contain toxic compounds, as many fishing grounds have become increasingly contaminated with industrial pollution (e.g. mercury, PCBs, dio7çin, mycotoxins, pesticides etc.). Consequently, industries that use fish meal will be eventually forced to find alternatives, which are of high quality, nutritionally equivalent, and sustainable. In particular, European current agricultural practice is moving towards non-animal delivery forms of key nutrients, such as the n-3 fatty acids for terrestrial animal feeds (poultry, swine). Furthermore, for ruminant species, the use of animal meal and fish meal is now prohibited in several countries.

From the ethical point of view, reliance on animal products can also have a detrimental effect on public heath globally. For example, the use of fish meal and fish oil has devastated some fish fisheries that produce fish deemed undesirable for various reasons, but useful in the production of fish oil and fish meal. This fish oil and fish meal serves to feed other fish, and the oceans are being thrown out of balance by the widescale harvest of fish for use as the use as fish meal and fish oil.

The production of compound aqua feeds, particularly feeds for carnivorous finfish species and marine shrimp, has so far been dependent upon the use of fish meal and fish oil as cost-efficient sources of dietary protein and fat (Coutteau 2002). The world production of these two key ingredients is based on a yearly average catch of 30-36 MMT of low-valued fish species, which are processed into 6.6 MMT of fish meal and 1.25 MMT of fish oil (averages for 1990-2000). Although these average production data are good estimates of the FM/FO supply in most years, the aqua feed industry has to foresee the occurrence of “crisis years” such as 1998 when production fell 25-30% as a result of El Niño. Besides ecological and ethical opposition to the use of finite and valuable aquatic resources as a feed ingredient for high-value species, there is a growing economical concern about the uncertain market availability and cost of FM/FO. Severe increases of formulation cost of aqua feeds can only be avoided by a significant reduction of the inclusion levels of FM/FO.

The drastic replacement of FM/FO by standard sources of plant protein/fat in feeds for fish e.g. sea bream/sea bass reduces feed intake by this fish and in turn affects its growth and food conversion, affects liver function and fat deposition in the body, and may change the quality of the final product (Coutteau 2002). Nutritional imbalances and low palatability of low FM formulations can be compensated by the appropriate supplementation of nutrients and attractants. Specially designed finishing feeds may improve the final product quality of marine fish that have been grown on low FM/FO feeds. These “formulative solutions” could maintain the market pressure on marine resources within acceptable limits during the coming decade.

Figures 2 and 3 below show the prices of fish meal and oil in the recent past and thereby the need to reduce on the use of these products.

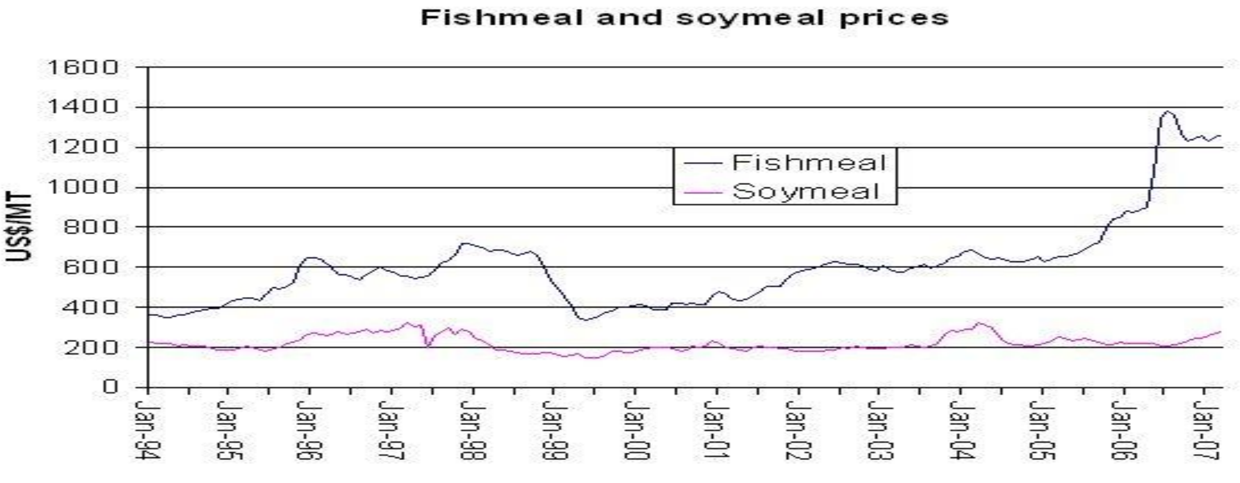


Figure 2: Trends in fish meal and soy meal prices in the recent past (Olafur 2007).

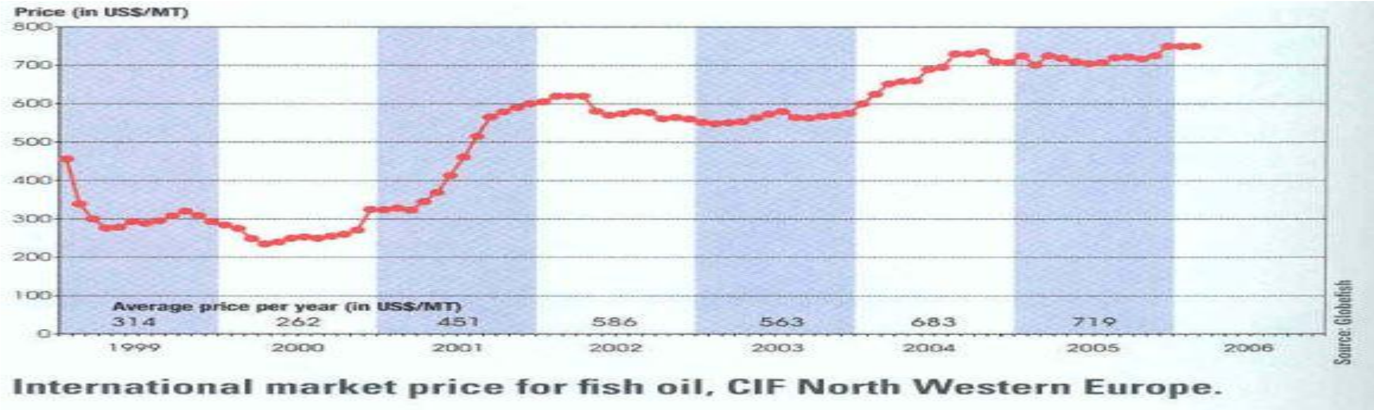


Figure 3: Trends in the price of fish oil in the recent past (Olafur 2007).

Figure 4 below shows the correlation between % protein feed and feed cost, meaning that if the current diet compostion of 42.5% were to be reduced to 35.5% the cost of feed would decrease from 100% to about 89%, thereby decreasing production costs and increasing profits, which was the underlying factor to carry out research in this field.

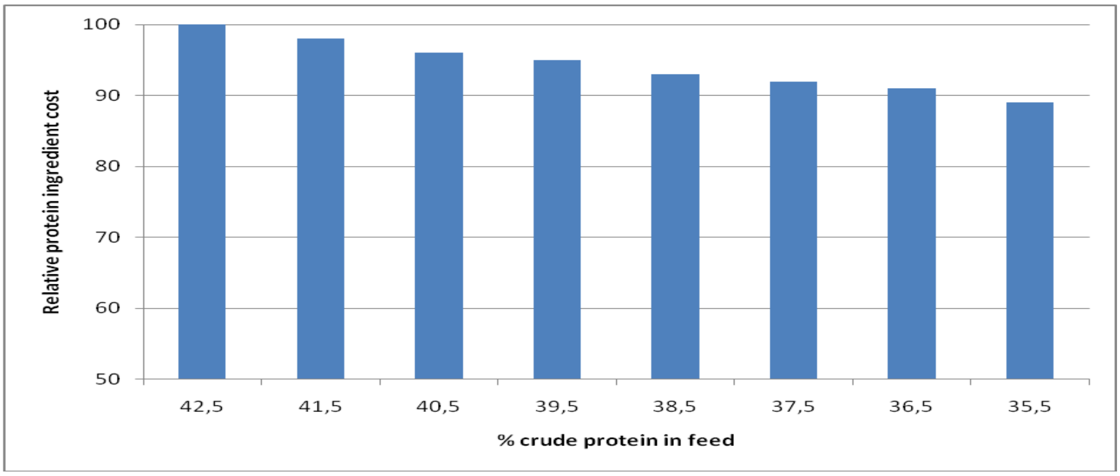


Figure 4: Correlation between % protein in feed and feed cost (Olafur 2007).

2.2.3 Farmed Arctic charr feed use

The primary concern associated with the use of fish meal and fish oil in aquaculture feed is the high input of wild fish compared to the amount of farmed fish produced. There are three components that need to be considered when determining the quantity of marine resources used to produce farmed fish: the amount of wild-caught fish used to create fish meal and fish oil; the inclusion rate, or the average percentage of fish meal and fish oil in Arctic charr feed; and the feed conversion ratio (FCR), or the average efficiency that Arctic charr convert feed to body weight.

Because the farming of Arctic charr is limited relative to other salmonids such as salmon and trout, there are few published estimates of inclusion rates for the feed used to raise Arctic charr.

They vary according to the age of the fish being raised as well as the feed manufacturer. Arctic charr are typically fed a modified trout/salmon feed, where fish meal is the primary source of protein and fish oil is used to provide a high lipid content and therefore higher energy levels for the fish (Hinshaw 1999).

Estimates of the percentage of fish meal and fish oil used in trout feed range from 30% - 35% fish meal and 15% - 20% fish oil (Naylor *et al.* 2000, Weber 2003). Therefore, the total inclusion rate for fish products in Arctic charr feed ranges from 45% - 55%. While the amount of fish meal in trout feed is expected to decrease to 25% by 2010, the input of other protein sources and the amount of fish oil is not expected to change (Weber 2003). The tendency to increase the fat % in feed is to increase energy and most of the proteins as feed should be used for growth.

<u>Feed conversion ratio (FCR)</u>
The general definition of FCR is the amount of dry feed required to produce one unit of wet fish (Weber 2003), and estimates of FCR vary with the aquaculture operation. Despite a low FCR of 1.2:1 for Atlantic salmon (Tacon and Forster 2000, Morris and Beatie 2003) two to five pounds of wild fish are used to produce one pound of farmed salmon because of high inclusion rates (SAMS 2002). Like other salmonids, Arctic charr can exhibit feed conversion ratios ranging from 1:1 (Summerfelt *et al.* 2004)

<u>Ratio of wild fish to farmed Arctic charr</u>
According to Naylor *et al.* (2000) the estimates of the ratio of wild fish to fed farmed fish are 3.16:1 for salmon and 2.46:1 for trout. The fish meal industry estimated in 2001 that the ratio of wild fish and fish parts used to produce fish meal and fish oil was 4.5:1 (FIN 2004). The following calculations were used to estimate the wild fish input to farmed fish output ratio.

<u>Conversion for fish meal</u>
(4.5 kg wild fish/1 kg fish meal) (0.325 kg fish meal/1 kg feed) (1.2 kg feed/1 kg Arctic charr) = 1.76 kg wild fish/1 kg Arctic charr

<u>Conversion for fish oil</u>
(8.3 kg wild fish/1 kg fish oil) (0.175 kg fish oil/1 kg feed) (1.2 kg feed/1 kg Arctic charr) = 1.74 kg wild fish/1 kg Arctic charr

These calculations were not added together, as that would result in double-counting the wild fish inputs required to grow the farmed fish. Instead, the larger of the two values, 1.76, represents the ratio of wild fish input to farmed Arctic charr output.

2.2.4 Arctic charr feeding behaviour

The optimum feed particle size is 1.6-1.7% of fork length for 73-110 mm Arctic charr, and 2.0-2.4% of fork length for 121-400 mm Arctic charr (Tabachek 1993). The greatest weight gain and feed efficiency was obtained with diets containing 54% protein and 20% lipid; diets containing 44% protein and 20% lipid resulted in a minor reduction in weight gain but at a lower cost per kilogram of weight gain (Tabachek 1993). The current study will be testing for this.

It has also been noted that Arctic charr take feed from the bottom and in the water column (Jørgensen and Jobling 1989, 1990; This benthic feeding behaviour is better suited for tank culture rather than net pens, where a large proportion of the feed would be lost (Heasman and Black 1998). At one farm in Iceland, Arctic charr are raised in tanks with salmon, where the charr mainly consumed the food that has settled at the bottom of the tank, thereby minimising the waste of fish feed (Georgsson and Fridleifsson 1996).

3 MATERIALS AND METHODS

3.1 Study area

The present study was carried out in Holar which is beautifully located in the valley Hjaltadalur in the Skagafjordur district of the mid-northern part of Iceland on land based facilities at both Holalax farm (with fresh water Arctic charr facilities) and At Verid Hus at Holar University College aquaculture facility Saudarkrokur 32 km from Holar (salt water facilities) between November 2007 and February 2008.

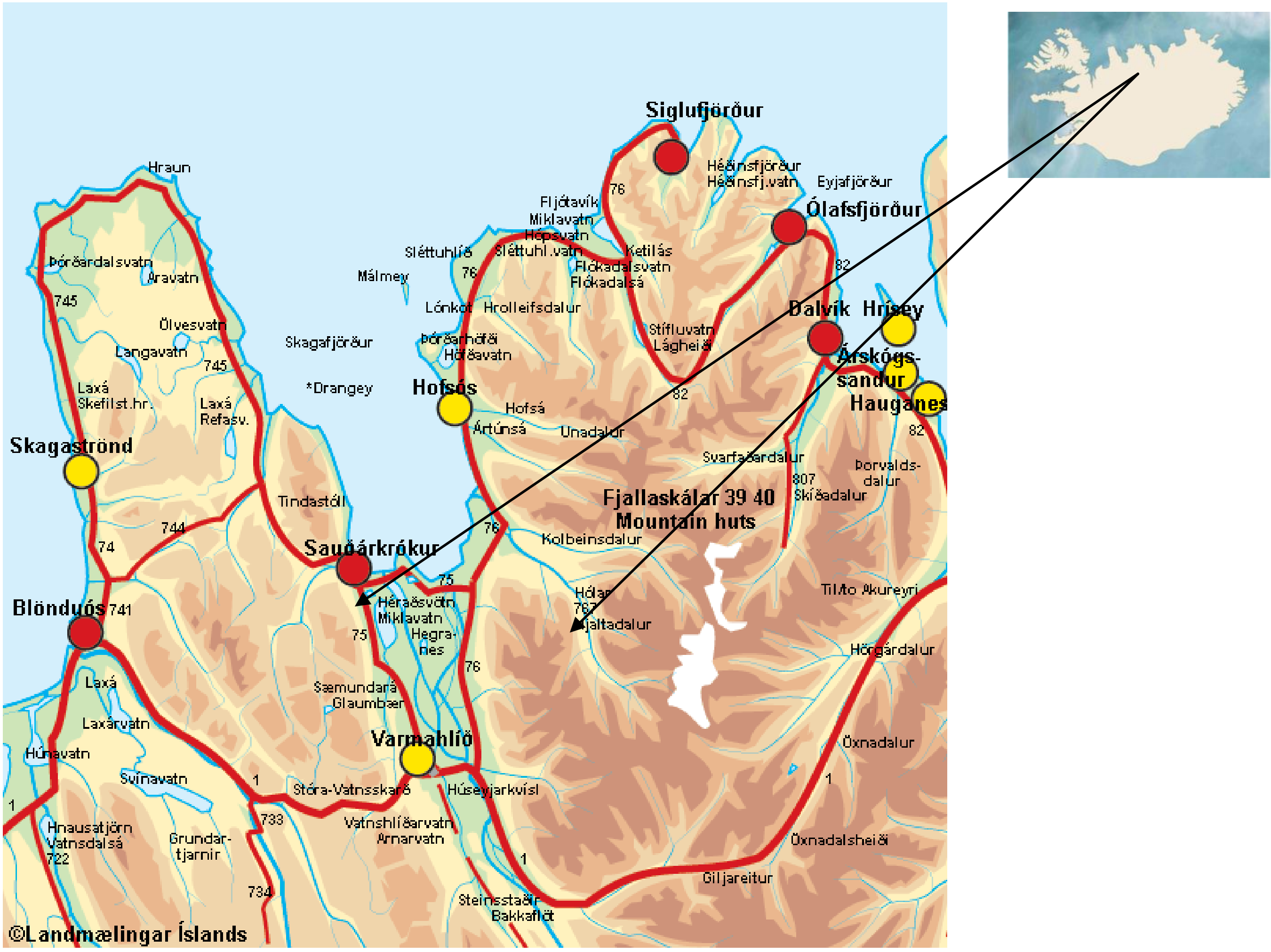


Figure 5: Map showing the location of study facilities (northwest Iceland)(www. Skagafjordur.is).

3.2 Materials

The following Equipments were required: Hanna pocket thermometer, YSI oxygen meter Model 57, Soxtec Avanti automatic system 2050, Metller sensitive balance (4DP).

3.3 Experimental setups

The experiments involved two setups, one was done for the salt water environment and the other was for the fresh water setup. The water quality measurements including oxygen temperature and salinity were measured. These were monitored on a daily basis and measurements were taken. Measurements of oxygen were taken using a YSI oxymeter model 57 and the temperature measurements were taken using the pocket Hanna thermometer and salinity was measured using YSI probes as well.

3.3.1 Verid Hus in Saudarkrokur (saline environment) setup description

This setup comprised of 12 tanks of average volume 680 l (dimensions L*W*H of 1 m by 1 m by 0.68 m respectively) and were operated on a recirculation system each containing 45 fish of starting weight 250-/+40 g which were tagged with pit tags and placed in each of the twelve tanks at a temperature of 8°C, gradually increasing salinity to attain a maximum of 17 0/00 and monitoring for all water quality parameters was done. Each two tanks acted as replicas of each other. Figure 6 below shows the setup.

Figure 6: The experimental set up in the salt water environment at Verid Hus, Saudarkrokur.

3.3.2 Holalax farm for fresh water environment

In this experimental setup 18 tanks were used. The fish measuring an average weight 230-/+ 10 g, n=120 were placed in tanks of average volume 1400 l (dimensions L*W*H of 2 m by 2 m by 0.34 m respectively). Each three tanks acted as triplicate samples for each of the given six protein dietary compositions. A flow through system was setup in the fresh water model. The set up was as in Figure 7 below.

Figure 7: **The experimental setup in Holalax farm for fresh water.**

3.4 Growth, food, feeding, digestibility, and chemical composition determination

The fish were fed to satiety to the six different dietary compositions daily in terms of proteins and oils protein to fat compositions of 29%-28.5%, 33%-26.5, 37%-25.3%, 41%-24%, 45%-22.8% and 49%-21.6% respectively as in Tables 2 and 3 below. The feed was placed on an automatic feeder, which released food over a 24 hour period and the pelleted feed remaining was collected the next day by hand counting method. The feeding regime depended more on how many pellets were left the previous day and food was decreased or increased in accordingly. Then after 4- 6 weeks the weight and length measurements were taken to determine the growth and condition factor rate respectively in terms of mm and grams respectively.

Table 2: The mixture of ingredients fish protein meal and fish oil meal composition fed to the fish in salt water in Verid and fresh water at Holalax fish farm in Holar.

	Ingredients							
feed no	Fish meal	Wheat	fish-oil	celite (marker)	Premix	Ash	Colour	MonoCal
712	34.95	36.35	24.94	1	1	0.032	0.027	1.71
713	40.89	34.62	22.43	1	1	0.032	0.027	0
714	47.33	29.94	20.67	1	1	0.032	0.027	0
715	53.77	25.26	18.92	1	1	0.032	0.027	0
716	60.21	20.57	17.16	1	1	0.032	0.027	0
717	66.65	15.89	15.4	1	1	0.032	0.027	0

Table 3: The calculated composition of ingredients fed to the fish in salt water in Verid and fresh water at Holalax fish farm in Holar.

	Calculated content					brutto energy
feed no	dry%	Protein	Fat	Starch	Ash	GE
712	92.4	29	28.5	21	8.2	21.7
713	92.3	33	26.5	20	7.2	21.7
714	92.5	37	25.3	17.3	7.8	21.7
715	92.7	41	24	14.7	8.6	21.7
716	92.9	45	22.8	12.1	9.3	21.7
717	93.1	49	21.6	9.3	9.9	21.7

3.4.1 Determination of FCR, growth rate and condition factor

<u>FCR was given by:</u>
FCR = (feed consumed)/ (weight gained)

<u>SGR was given by:</u>
SGR, % day = (log e W_2-Log e W_1)/t_2-t_1)*100- where $W_1$and W_2 are mean live weight of the fish and t $_1$and t_2 are time of weighting.

<u>The condition factor was determined from the equation given by:</u>
$K = W/L^3$ where W is weight expressed in g and L is length expressed in cm.

3.4.2 Determination of digestibility

Digestibility was established by the marker digestibility method using celite. The celite was incorporated into the diet at a rate of 1% into feed. Gastric evacuation (stripping method) was done to collect samples of the faeces by pressing the belly and causing it to escape from the gut. About 60 g per sample were placed in the vial tube. 30 samples were drawn for both the fresh water and seawater environments. 12 samples were drawn from the saline water and 18 samples being drawn from the fresh water environment.

The total dry matter digestibility was obtained and the individual protein and fat content was established in addition to ash content.

Digestability coefficient for dry matter (%DC) is calculated as:

100 x (1- (concentration (%) marker in feed / concentration (%) marker in faeces)).

3.4.3 Chemical composition

In addition, the chemical composition and qualitative properties of the faeces/feeds for fish of sized 250 -/+40 g (at start feed) after one to one and half months of feeding were established by proximate analysis by the Soxtec and Keldjal method in accordance the ACOAC 1990 standard methods in reference to the AOCS official methods Ba -3-38 with modifications and ISO 5987-1997 for fat and proteins respectively. This was done for the salt water environment only.

3.5 Statistical analysis

Data was coded and tabulated into excel sheets and was subjected to the Statistica release 8 STATSOFT to find out if there were significant differences between treatments/fish groups related to the different dietary compositions. Graphical analysis was done with the same program. In addition the data was subjected to SPSS GLM analysis for nested treatments.

4 RESULTS

4.1 Water quality monitored parameters

4.1.1 Fresh water setup at Holalax farm

As a monitoring instrument the researcher took readings for the different water quality parameters in the two environments. The results in the fresh water environment, over the first five week sampling period showed that the temperature varied between 3°C degrees and 6°C with the highest recorded being about 9.2°C registered during week 4 and the temperatures were on average 6°C as seen in Figure 8 below. In addition, values were taken once for every tank to observe if conditions varied within tanks (Refer to appendices). These showed no variation within the tanks.

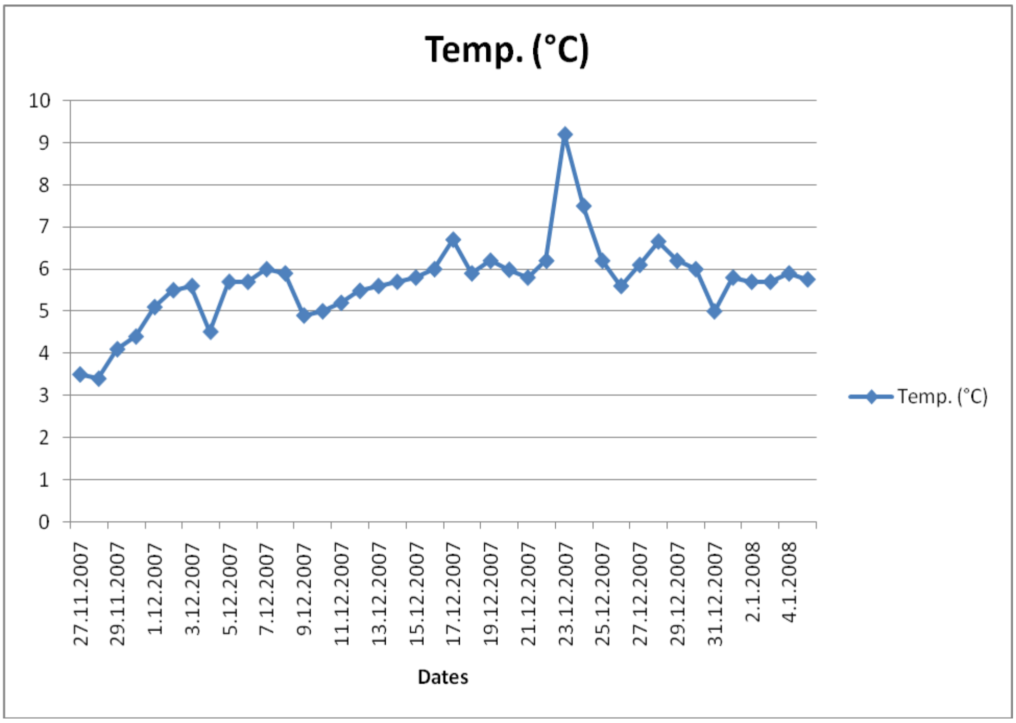


Figure 8: Daily temperature variations in the fresh water environment.

4.1.2 Salt water setup at Verid - Hus, Saudarkrokur

In the salt water environment, over the first sampling period the temperature varied between 6°C and 10°C with the average being recorded as 6°C as shown in Figure 9 and the salt concentration was about 8 ppm on average but varied between 8 and 10 ppm. In addition all measurements were taken for the entire system but results presented here show one of the three systems making up the Recirculation Aquaculture System (RAS) (for the other figs refer to appendix).

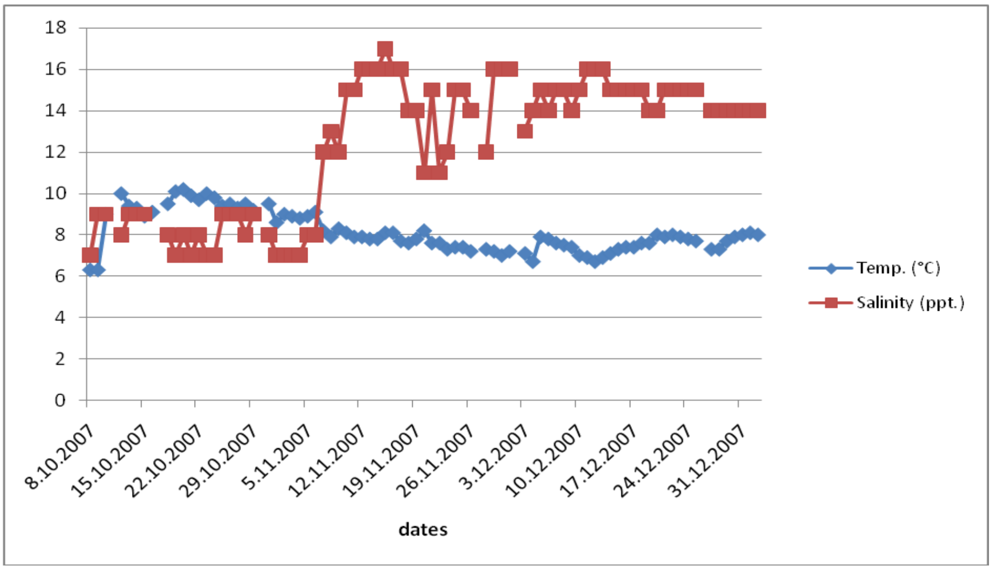


Figure 9: Variation of temperature and salinity in the seawater environment.

4.2 Chemical composition

4.2.1 Chemical composition in feed diets

The chemical composition of the feed was analysed to find out if it complied with the desired composition. Results in Table 4 below indicated a slight variation in diet composition in 712 and 713 from the stated 28.5% fat and 26.5% composition respectively.

Table 4: Ingredients and chemical composition of the feed as per Matis Chemical Analytical Laboratories, Iceland.

Feed number	% water	%Ash	%Fat composition
712	6.4 ±0.4	7.7 ±0.5	25.6 ±0.4
713	7.0 ±0.4	7.5 ±0.5	25.6 ±0.4
714	6.7 ±0.4	7.8 ±0.5	23.8 ±0.4
715	6.3 ±0.4	8.4 ±0.5	23.5±0.4
716	5.9 ±0.4	8.9 ±0.5	23.0 ±0.4
717	5.9 ±0.4	9.8 ±0.5	21.8 ±0.4

4.2.2 Chemical composition in faecal matter

Results for faecal matter chemical composition show a decrease from over 20% fat composition to less than 1% indicating that Arctic charr in seawater were taking up the fats into their body. This is as seen in Table 5 below.

Table 5: Ingredients and chemical composition of the faeces per tank replicates per feed for Arctic charr in seawater as per Matis Chemical Analytical Laboratories Iceland.

Feed number	% water	%Ash	%Fat composition
712A	86.3 ±0.4		0.8 ±0.4
712B	85.0 ±0.4		0.3 ±0.4
713A	86.1 ±0.4		0.4 ±0.4
713B	85.3 ±0.4		0.4 ±0.4
714A	86.3 ±0.4		0.4 ±0.4
714B	86.3 ±0.4		0.3 ±0.4
715A	85.7 ±0.4		0.2 ±0.4
715B	85.8±0.4		0.3±0.4
716A	84.3 ±0.4		0.3 ±0.4
716B	86.6 ±0.4		0.2±0.4
717A	87.3 ±0.4		0.2 ±0.4
717B	86.3 ±0.4		0.3 ±0.4

4.3 Feed consumption and weight gain

4.3.1 Feed consumption and weight gain in saline water

Results for feed consumption and weight gain in fresh water indicated a variation in feed intake and weight gain shown in Fig 10 below, however there was no marked significant difference in the consumption and weight gain in the charr raised in saline water toward the different diet compositions. ANOVA, df =5, $p > 0.05$

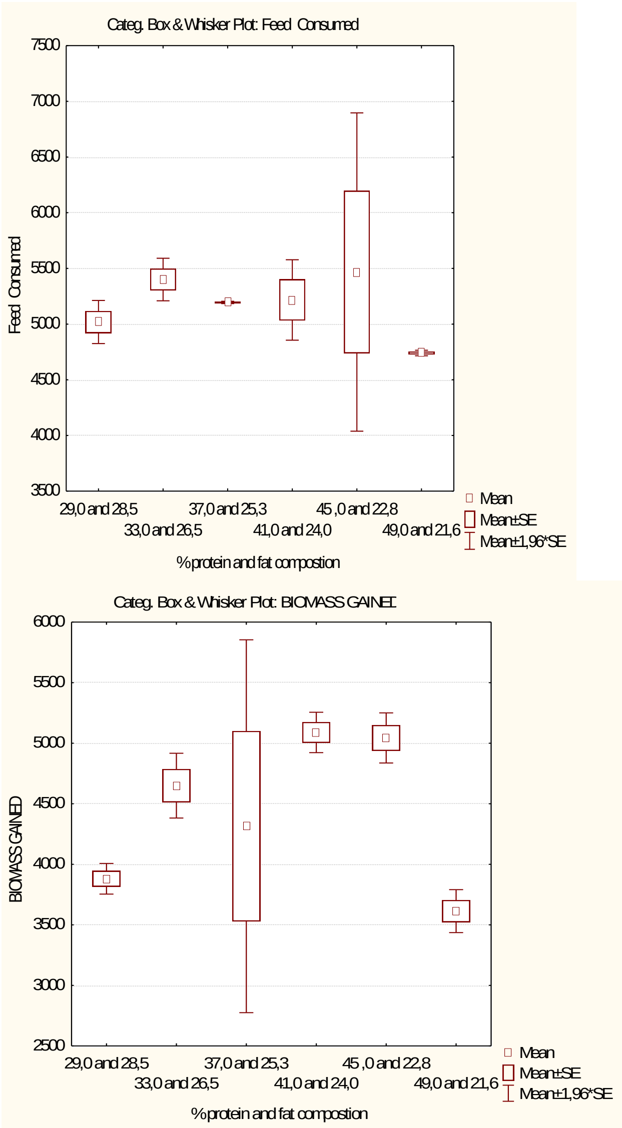
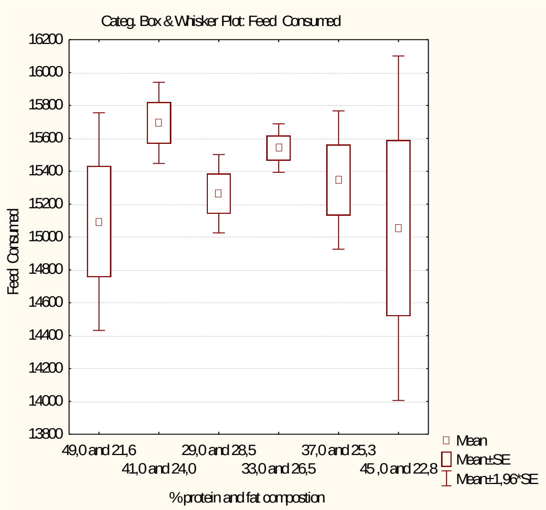


Figure 10: Trends for food consumption and weight gain for charr reared in saline water fed on the different diet compostions.

4.3.2 Feed consumption and weight gain in fresh water

Results for feed consumption and weight gain in fresh water indicated a variation in feed intake and weight gain as shown in Figures 11 and 12. However, there was no marked significant difference in the consumption and weight gain in the charr raised in fresh water towards the different diet compositions. However, in the results of weight gain with feed consumption as a covariate a marked significance was observed for fresh water df =5, $p < 0.00001$.

Based on estimated marginal means:
* The mean difference is significant at the 05 level.
a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).



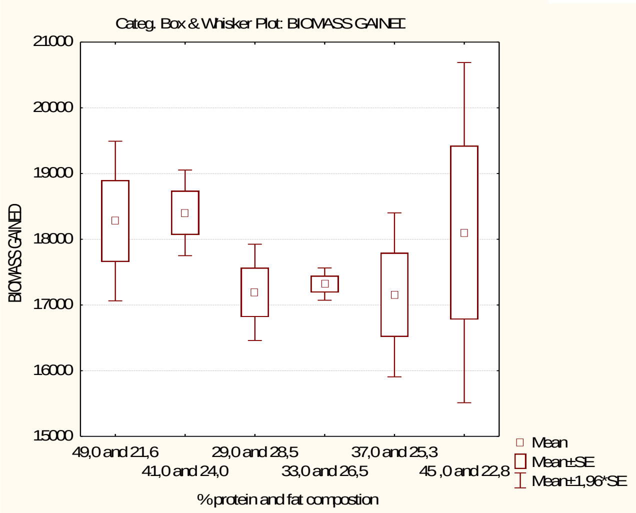


Figure 11: Trends for food consumption and weight gain for charr reared in fresh water fed on the differeent diet compostions.

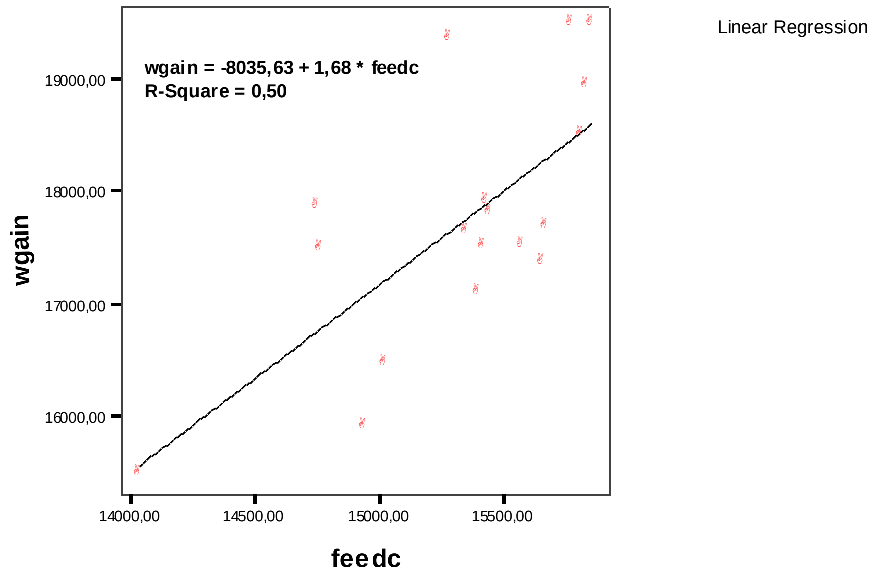


Figure 12: Correlation between weight gain and feed consumption for charr raised in fresh water.

4.4 Effects of varying protein and oil ratios on the growth of Arctic charr

4.4.1 Feed conversion ratios (FCR)

4.4.1.1 FCR for Arctic charr reared in seawater environment fed on six different dietary compositions

The results in Figure 13 show that the feed conversion ratio for Arctic charr reared in seawater indicates that variations occur in the rate at which they could utilise the food. No marked significant differences were observed in FCR as the P- value for ANOVA was df=5, $p < 0.05$.

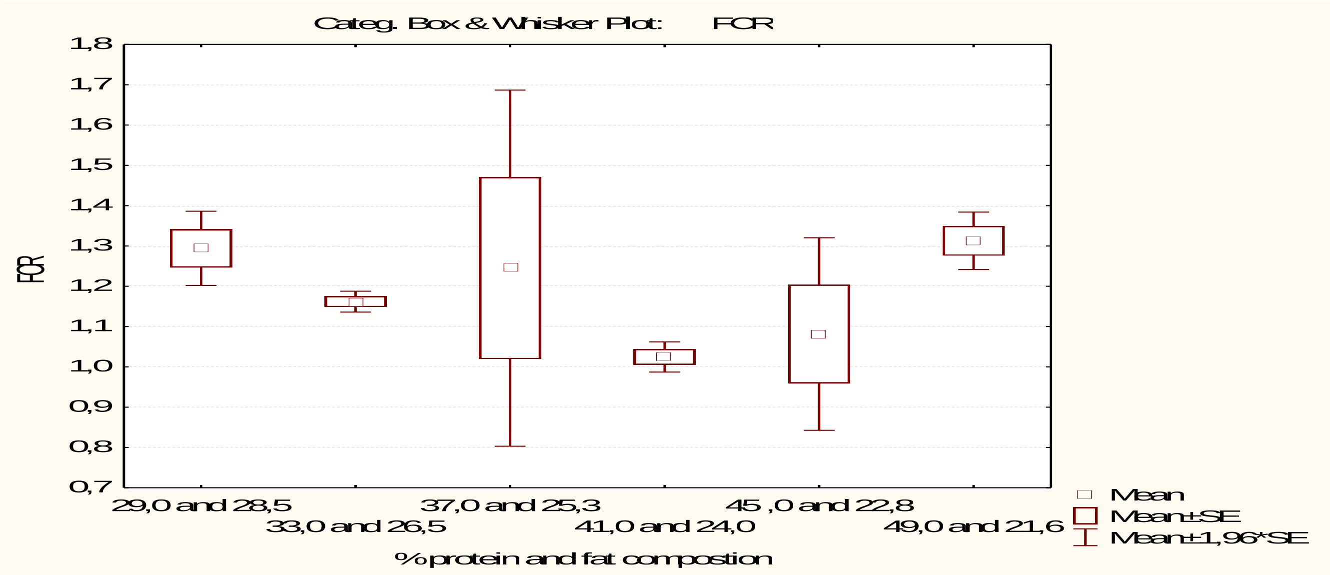


Figure 13: Variation of FCR with different dietary compositions in seawater.

4.4.1.2 FCR for Arctic charr reared in fresh water environment fed on six different dietary compositions

There was a variation in the utilisation for the different diet composition in fresh water as shown in Figure 14. However, there were no marked significant differences in feed conversion in fresh water. Anova, df=5, p> 0.05

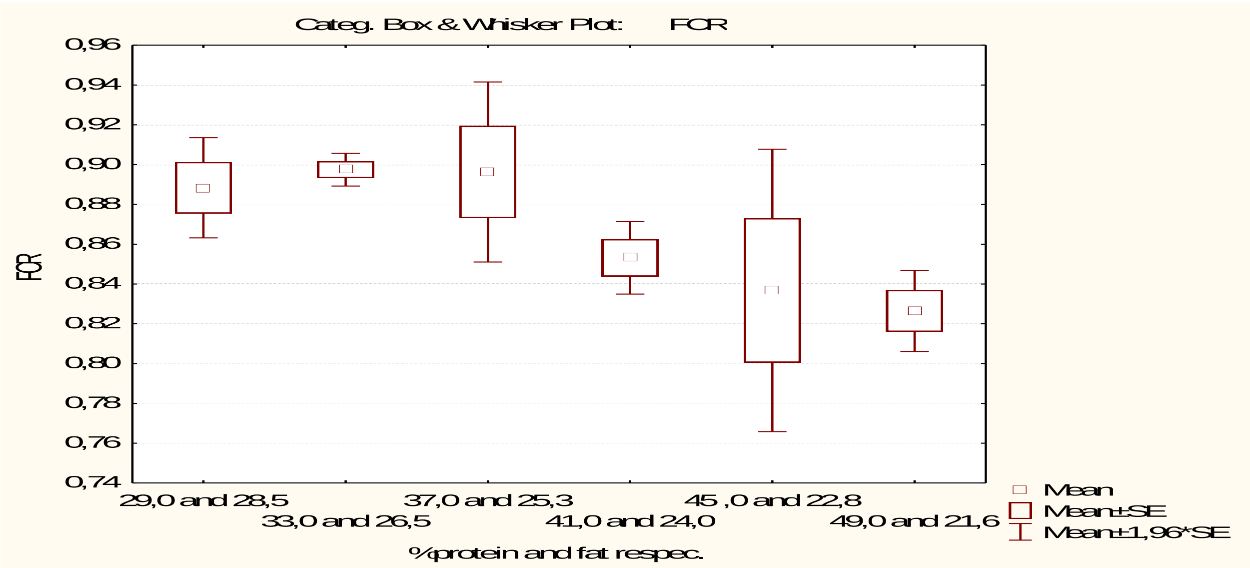


Figure 14: Variation of FCR with different dietary compositions in Arctic charr reared in fresh water.

4.4.2 Specific growth rates (SGR) for charr raised in saline water

Trends in specific growth rates observed for Arctic charr reared in saline water.
The results showed variation in growth as shown in Figure 15. However, no marked differences were observed SGR amongst the different diet compositions. Anova, df=5, p>0.05

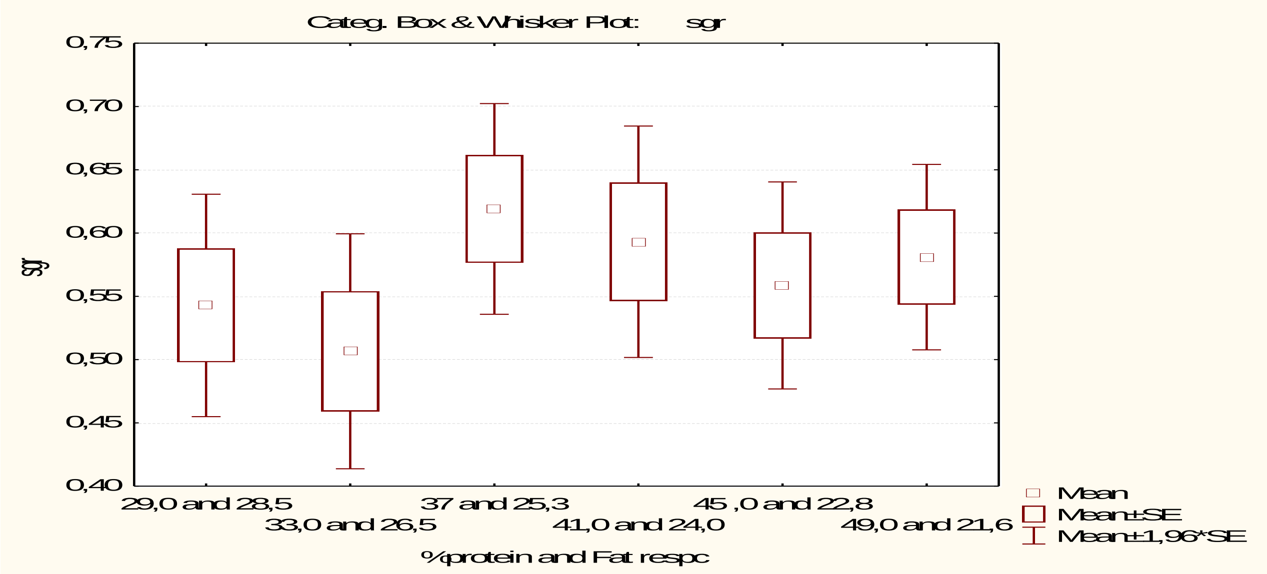


Figure 15: Trends for specific growth rate for charr rasied in saline water after 112 days.

4.4.3 Weight of Arctic charr raised in saline water

4.4.3.1 Results for the weight of Artic charr raised in saline water

Results showed no variations in the weight over the entire sampling period as shown in Figure 16. There is no marked significant difference in weights in all the Artcic charrs fed the different diet compostions. Anova, df=5, p>0.05

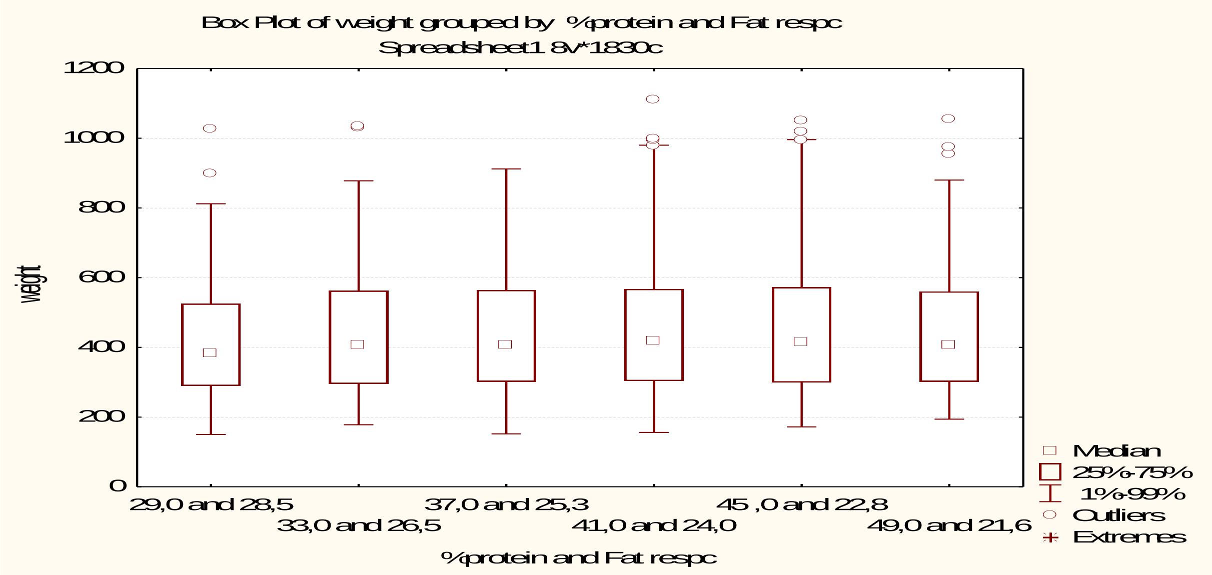


Figure 16: Trends for weight for Arctic charr raised in saline water.

4.4.3.2 Results for weights of Arctic charr raised in fresh water
Results for weight in fresh water showed no variations in the weight over the entire sampling period as seen in Figure 17. There is no marked significant difference in weights in all the Artcic charrs fed the different diet compostions. Anova, df=5, p>0.05

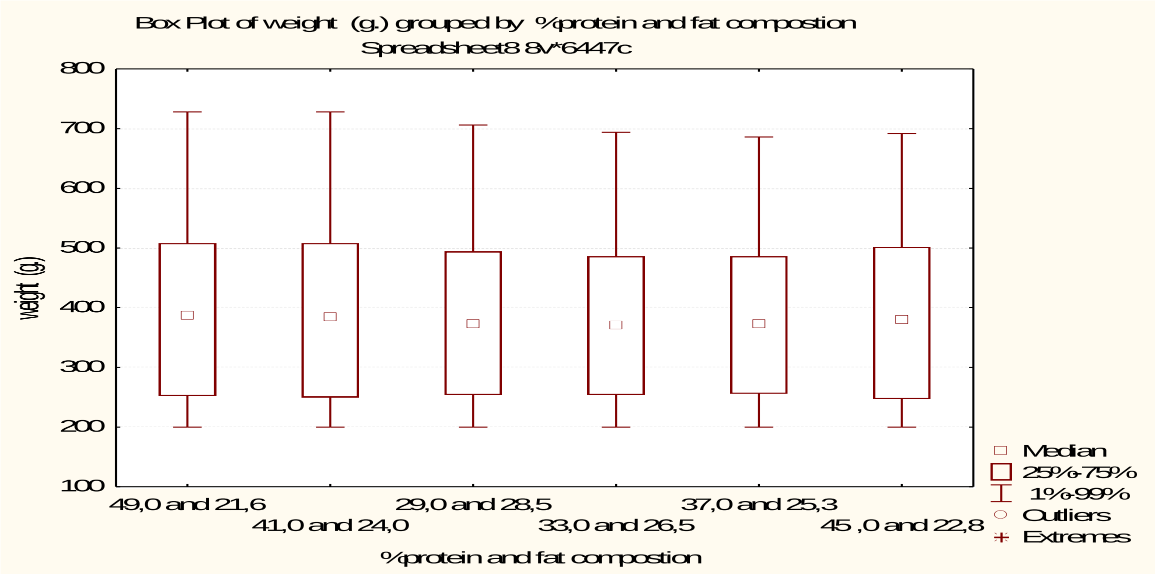


Figure 17: Variation in weight for Arctic charr raised in fresh water.

4.4.4 Condition factor (K)

4.4.4.1 Variation of the condition factor for Arctic charr reared in saline water

The results of the K in seawater for the Arctic charr are shown in Figure 18. There was no variation in this factor for charr raised in saline water except some extreme values were observed and some outliers as well. Results were also not significantly different between the different treatment groups. Anova , df=5, p>0.05

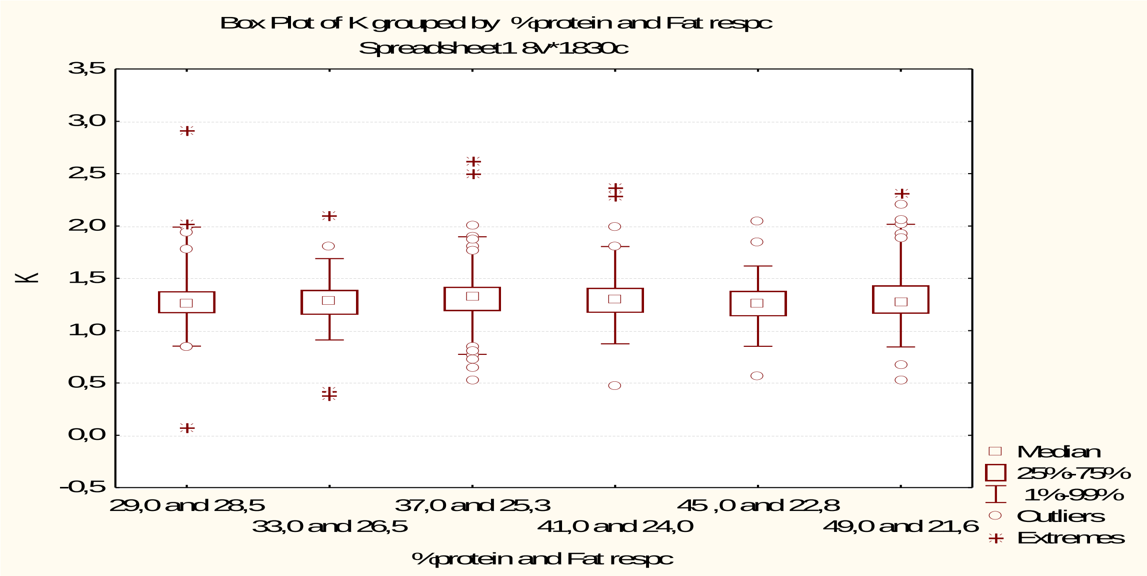


Figure 18: Variation of the condition factor for Arctic charr reared in saline water.

4.4.4.2 Variation of the condition factor for Arctic charr reared in freshwater

The results for K in seawater for Arctic charr are shown in Figure 19. There was no variation in this factor for charr raised in fresh water except some extreme values were observed and some outliers as well. Results were also not significantly different between the different treatment groups. Anova, df=5, p>0.05

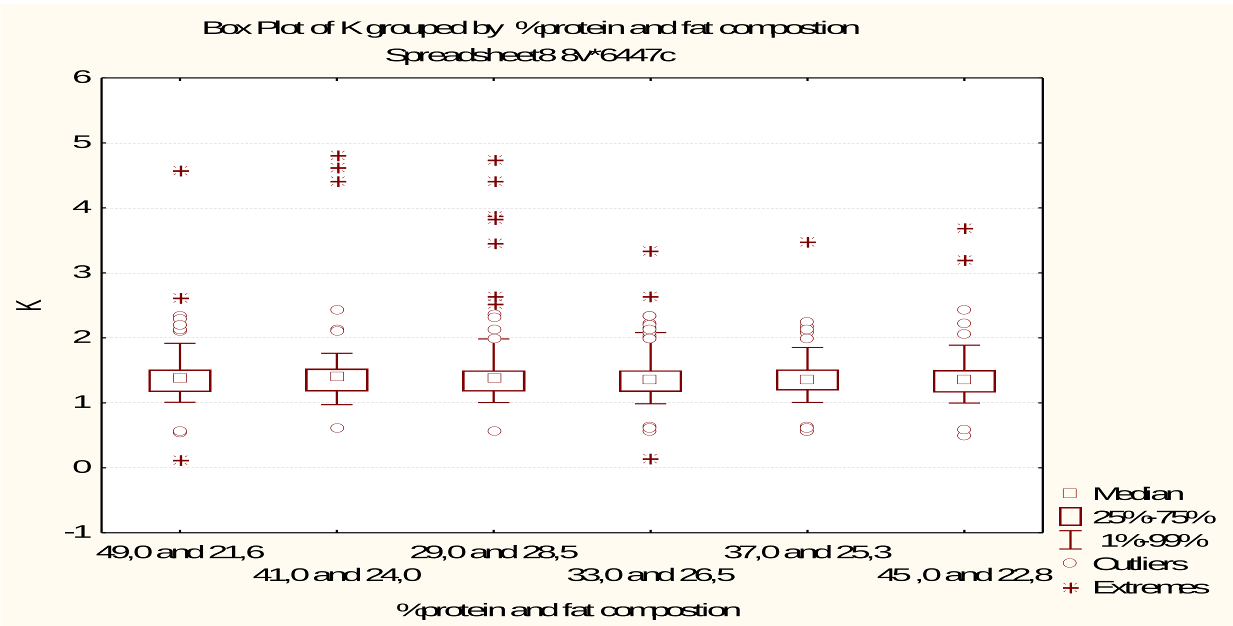


Figure 19: Variation of the condition factor for Arctic charr reared in freshwater.

4.4.5 Digestibility results

4.4.5.1 Variation in digestibility for Arctic charr reared in saline water

Comparison between different diet compositions shows that the fish were able to digest the different compositions but with the ability decreasing at very high protein and low fat (45% protein, 22.8% fat levels). It is also observed that the digestibility was lower at low protein and high fat composition (29% protein, 28.5% fat composition) as in Figure 20 below. The results also indicated marked significant differences in digestibility for the different diet compositions. Anova, df=5, $p < 0.05$

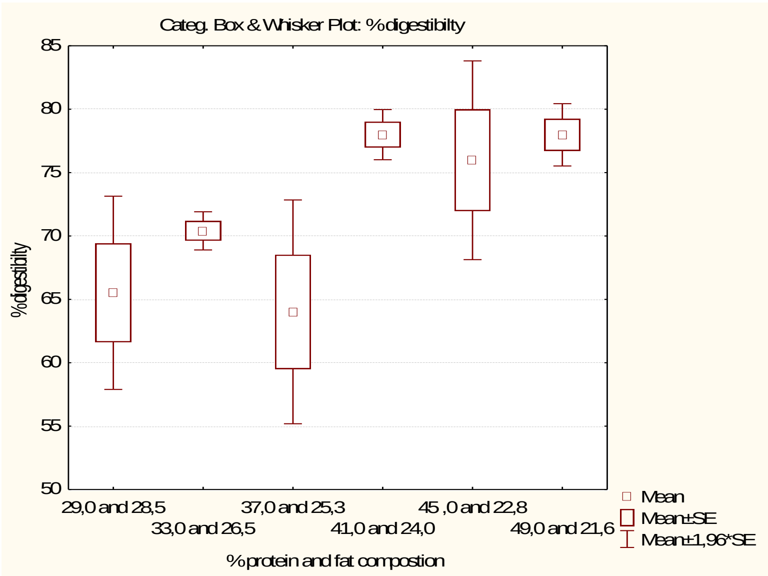


Figure 20: Trends for the digestibility of the six dietary compositions in saline water.

4.4.5.2 Variation in digestibility for Arctic charr reared in fresh water

Comparison between different diet compositions shows that the fish were able to digest the different compositions. There were no marked differences in the digestibility of the diets in fresh water.

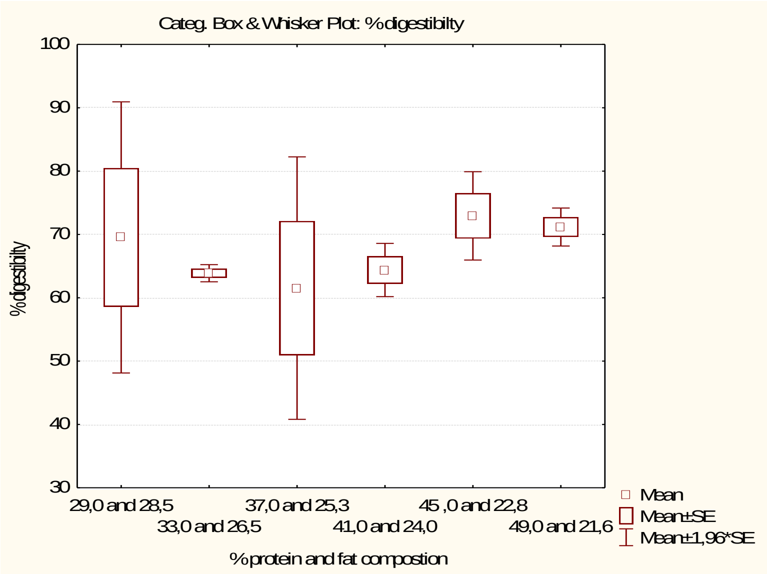


Figure 21: Trends for the digestibility of the six dietary compositions in fresh water.

5 DISCUSSION

The results obtained indicate that in terms of feed conversion ratio and digestibility, the fish were able to uptake and utilise either for maintenance or growth all the different dietary compositions in both saline and fresh water. The purpose of this study was to pave the way for reducing the use of high protein and oil ratios to reduce the costs of feeds and increase the farmer's profits. This may also have ethical implications in that the use of trash fish to produce high valued species will be reduced.

The exploitation of the world's fishing stocks is ever increasing with the population increase and the growing need for food. Already many of the fish stocks are being depleted or even in danger of extinction. At the time that the world's total fish catch has stagnated or only slightly increased, the demand for farmed of fish and other marine species has been steadily growing. Already the farming of fish is an important trade the world over, with the production of freshwater species dominating and farming of marine species increasing in recent years. It can be expected that this development will continue and aquaculture will be of growing importance in the procurement and production of seafood.

Aquaculture holds the key to supplying the deficit created by fisheries levelling off with the anticipated likely increase of the human population by 2050. However, aquaculture as an enterprise will require reducing on the amount of fish meal and oil that is needed to produce aquaculture feeds vis-à-vis the increased costs. Arctic charr are carnivorous and therefore have high protein requirements. In light of this, an optimal protein ratio needs to be identified for Arctic charr which is carnivorous in feeding habits and whose costs for production in terms of feed costs exceed the profit that can be attained at the moment.

5.1 Water quality parameters

The water quality parameters in the experimental set up tended to vary on a daily basis in accordance with the environmental conditions. However, the average required temperature of 6°C was maintained by supplying warm water at both facilities. Therefore I believe that the effects of the water quality were minimal although on some days when it snowed and the streams which acted as sources for the fresh water were clogged and sediments ended up in the tanks, this affected the feeding patterns of the fish but this may not have been significant in the growth of the fish since Arctic charr are known to tolerate conditions of environmental fluctuations to a great extent.

5.2 Effects of varying protein and oil ratios on the growth of Arctic charr

.
In seawater they were able to utilise as low as 29% protein and 28.5% fat at almost the same rate as 49% protein and 21% fat while in fresh water they efficiently utilised 33% protein and 26.5 % fat. The present study has identified these ratios as the ones for the fish at start feed 250 g and seems like these ratios could be adopted for the formulation of feed for the sea and fresh water environment at 6 °C.

In terms of growth Arctic charr reared in seawater showed growth at 37% protein, 25.3% fat that was comparable to that at attained at 45% protein, 22.8% fat. It looks likely that charr in seawater will give good growth results at the ratio of 37%, 25.3 % protein and fat respectively. This is suitable for the age range which was examined.

The findings of the present study concur with those of Tabachek (1986) and Luo *et al.* (2006) that levels of protein less than 34% and 10% fat were well below the limit. However, it is worth noting that the protein requirements of the charr depend on the size range to which the feed is administered.

In terms of chemical composition of fat in the faecal matter for charr reared in seawater, it seems that charr utilise the fats taken in efficiently as the levels obtained in all cases showed that the fat lost in form of waste is well below 1%.

This gives an indication that it utilised either for maintenance or growth which fits in well with the digestibility results obtained for seawater indicating most of it is channelled into maintenance at the expense of growth.

Also the fact that they prefer a diet with a low percentage of proteins and a high percentage of fat (Hardy 2000) states that in salmonids lipids are used as the energy source and may in part explain why this age range may prefer this ratio.

On the other hand, Arctic charr reared in fresh water were also able to uptake and utilise the different dietary compositions although at very varied rates. This concurs with the findings of Gudmundsson and Petursdottir (1998) that in general they also found that as protein increases in the diet there is an increase in specific growth rate, but this is reduced as the fish grows and similarly as the fat in the diet increases the specific growth rate increases in juvenile fish to the maximum fat concentration tested but this increase ceases around 15% dietary fat for older fish. In fresh water we see that the Arctic charr have a high SGR at fast sampling but it decreases, at second sampling although it is higher when compared to the seawater raised charr. Also in term of optimal protein ratios for growth, charr reared in fresh water seem to attain a good weight with the use of 45% protein and 22.85% fat although we could reduce as far as 41% protein, 24% fat to reduce the costs of production. This is line with Tabachek (1993) who found that in optimum feed the particle size is 1.6-1.7% of fork length for 73-110 mm Arctic charr, and 2.0-2.4% of fork length for 121-400 mm Arctic charr (Tabachek 1993). The greatest weight gain and feed efficiency was obtained with diets containing 54% protein and 20% lipid; diets containing 44% protein and 20% lipid resulted in a minor reduction in weight gain but at a lower cost per kilogram of weight gain (Tabachek 1993). The current study is trying to address this as well.

In terms of well being all the fish in the two environments were healthy as on average they attained 1 and above meaning they were healthy.

6 CONCLUSIONS

The present study results indicated that terms of identification of the optimal protein ratio during the study period there is no significant difference in growth of Arctic charr fed on different dietary composition. However, these preliminary studies indicate that Arctic charr reared in seawater had a higher digestibility than those in fresh water.

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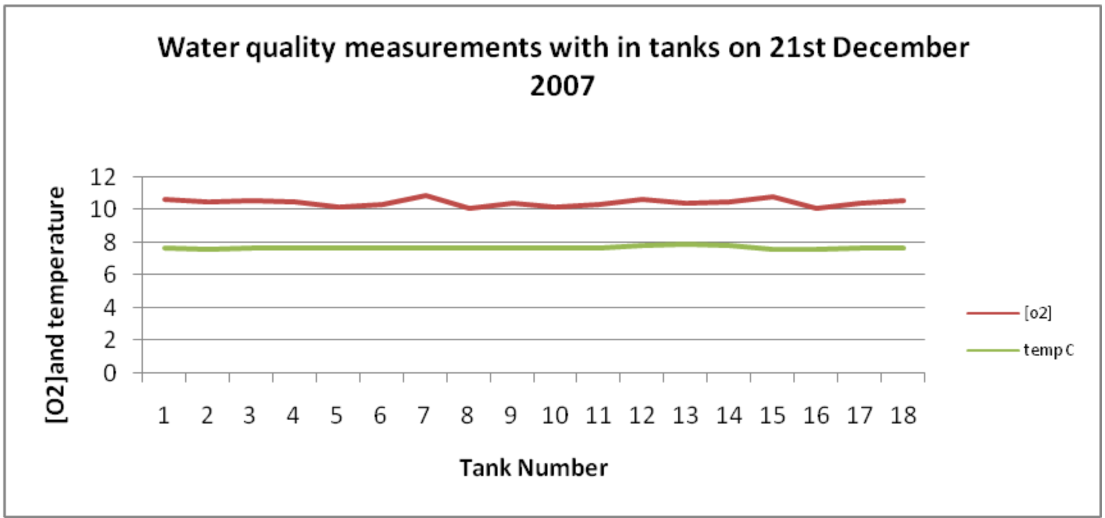
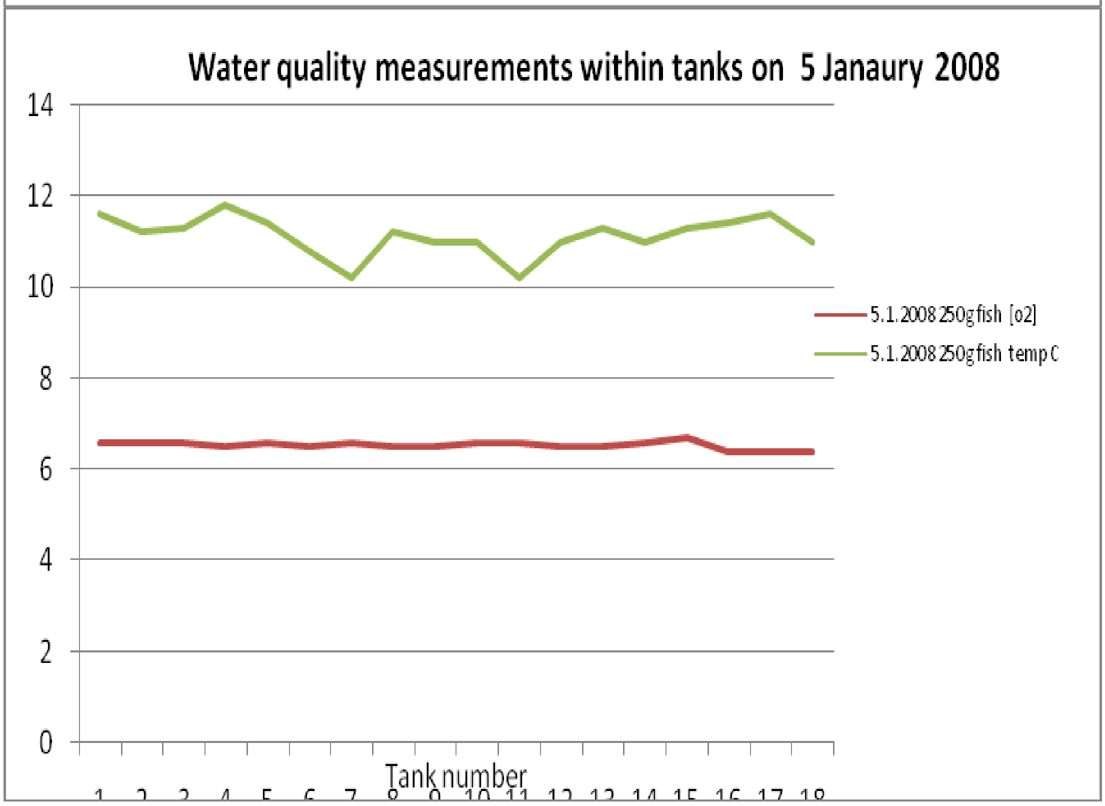
APPENDICES

1.0 Water quality measurements at Holalax farm within all the tanks





2.0 Sea -water water quality monitored parameters for RAS setup 2 and 3