Eldar Åsgard Bendiksen

# Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt

Dr. scient. avhandling 2003

Fakultet for naturvitenskap
og teknologi
Institutt for biologi

URN:NBN:no-6435

URN:NBN:no-6435

**ACKNOWLEDGEMENTS**

This thesis is based on work carried out from 1999 to 2003, when I attended a doctoral education programme at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway while being an associate member of the R&D department of BioMar AS in Trondheim. The work has been carried out with financial support from BioMar AS and the Norwegian Research Council.

This project would have failed without the support of dedicated researchers at several Norwegian research institutions. I wish to express my gratitude to my supervisors, Professor Ole Kr. Berg, at the Department of Biology, NTNU, and Professor Malcolm Jobling at Norwegian Fisheries College, University of Tromsø, for invaluable supervision and constructive criticism throughout the project period and for guiding me safely through the doctoral education programme. I have enjoyed much help from Arne M. Arnesen at Norwegian Institute of Fisheries and Aquaculture Research (Fiskeriforskning), who has arranged most of the practical work at Havbruksstasjonen AS, Tromsø, in an excellent way, especially the parts related to smolt physiology. Kjell A. Måsøval at BioMar AS has had a steady hand on the overall project management through these years, and has, in the position as R&D manager from 2001, ensured that I could devote most of my working hours to this project. Trygve Sigholt has been an inspiring discussion partner within the R&D department of BioMar AS and has helped me to convey the main results internally in the company. On the analytical side, Professor Per Carlsen, Department of Chemistry, NTNU, provided facilities for parts of the lipid analysis, which is greatly acknowledged. Einar S. Egeland and Frode Rougnø at Department of Chemistry, NTNU, and Håvard Hopen at Department of Biology, NTNU, are acknowledged for assistance during analysis.

I have also enjoyed knowledge and input from other members of BioMar R&D departments; Jørgen Holm (BioMar Denmark), Sigve Nordrum, Marie Hillestad, Ellinor Helland, Trine Galloway, Håvard Jørgensen, Pattrick Campbell, Richard Smullen and Dick Alderson. Finally, I wish to express my gratitude to the employees at the BioMar House in Trondheim and to other friends and family who have supported me throughout these years.

Trondheim, August 2003


URN:NBN:no-6435

URN:NBN:no-6435

CONTENTS

**Acknowledgements**

**List of papers**

**Abstract** 3

**Sammendrag [in Norwegian]** 5

**1 Introduction** 7

**2 Aims and questions addressed** 9

**3 Methodological considerations**

3.1 Environmental factors 11

3.2 Feed intake measurement 12

3.3 Test feeds

3.3.1 Proximate feed composition 14

3.3.2 Dietary oil sources 15

3.4 Experimental design and statistical methods 17

**4 Results and general discussion**

4.1 Feeding and growth

4.1.1 Thermal and dietary effects and their interactions 18

4.1.2 Lipostatic regulation of feeding and growth 22

4.1.3 Effects of dietary oil sources 24

4.2 Nutrient digestibility and retention efficiencies 25

4.3 n-3 and n-6 EFA retentions 28

4.4 Fatty acid deposition in polar and non-polar lipids 29

4.5 Dietary effects on seawater acclimation and growth 32

4.6 Conclusions 35

**5 Literature** 37

**Individual papers (Paper I-V)**

URN:NBN:no-6435

**LIST OF PAPERS**

This thesis is based on the following papers, which will be referred to by their Roman numerals:

**I.** Bendiksen, E.Å., Jobling, M. & Arnesen, A.M., 2002. Feed intake of Atlantic salmon parr *Salmo salar* L. in relation to temperature and feed composition. Aquaculture Research 33, 525-532.

**II.** Bendiksen, E.Å., Berg, O.K., Jobling, M., Arnesen, A.M. & Måsøval., K.A., 2003. Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. Aquaculture 224, 283-299.

**III.** Bendiksen, E.Å. & Jobling, M. Effects of rearing temperature and feed composition on deposition and retention of essential fatty acids (n-3 and n-6) of farmed Atlantic salmon (*Salmo salar* L.) parr. Submitted Fish Physiology and Biochemistry.

**IV.** Jobling, M. & Bendiksen, E.Å., in press. Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. Aquaculture Research.

**V.** Bendiksen, E.Å., Arnesen, A.M. & Jobling, M., 2003. Effects of dietary fatty acid profile and fat content on smolting and seawater performance in Atlantic salmon (*Salmo salar* L.). Aquaculture 225, 149-163.

URN:NBN:no-6435

**ABSTRACT**

The aim of this study was to investigate the effects of water temperature and salinity on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt. Salmon parr were held at low water temperature (2˚C) for six months while being fed feeds that differed in oil source (i.e. marine fish oil or vegetable oil blend) and concentration (low, 21% and high, 34%). The responses at low temperature were compared with those of fish held at 8˚C using full-factorial design.

Feeding and growth were maintained at 2˚C, although at lower rates than at 8˚C. Growth and feed utilisation improved over time, suggestive for a long-term acclimation response in fish held at low temperature. Overall feed efficiency was better at the lower temperature. A gradual decrease in growth rate and feed utilisation was seen at the higher temperature as the fish grew larger.

The fish compensated for reduced energy density by increasing feed intake. At the higher temperature, better growth was found for fish fed the low-fat feeds, and there was also a tendency for improved growth when vegetable oil was used. Thus, there were no signs that vegetable oils are inferior to marine fish oil in promoting growth of Atlantic salmon parr in fresh water.

Fish fed high fat feed were fatter than fish fed low fat feed, suggestive of lipostatic regulation of feed intake. Fat and protein digestibility were high at both 2˚C and 8˚C, although both fat and protein digestibility were lower at 2˚C. At the lower temperature, increased dietary fat level increased the fat digestibility, and improved protein digestibility were seen when vegetable oil was included in the feed. Protein retention was higher at the higher temperature irrespective of feed treatment, indicating that proteins were both readily digested and converted into new tissues.




URN:NBN:no-6435

The effects of feed treatment on low temperature acclimation responses were assessed from deposition of dietary fatty acids in fish tissues and from n-3 and n-6 essential fatty acid (EFA) budgets. Fatty acid composition of polar (membrane) and non-polar (storage) lipids in muscle, viscera and carcass were markedly influenced by the dietary oil, and non-polar lipids were more influenced than polar lipids. The retention n-6 EFAs was lower than for n-3 EFAs, and was independent of temperature. The retention of n-3 EFAs retention was higher at the 2˚C, especially amongst fish given the fish oil based diets. This may be a reflection of the importance of n-3 HUFAs during low temperature acclimation. However, the unsaturation (UFA:SFA ratio) of polar lipids was higher in fish fed the vegetable oils than for fish fed fish oil based feed. This may imply that vegetable oils produced fish that were better able to withstand exposure to low temperature, while having membrane lipids less susceptible to oxidative damage, due to the lower contents of n-3 HUFAs (mainly EPA and DHA).

The six months feeding period in freshwater was followed by parr-smolt transformation, and a subsequent 42-days on-growing in seawater. Feed history during freshwater rearing influenced on-growth of smolts. A positive effect of using a vegetable oil was indicated, but this effect was only seen when there was a shift to a high-lipid fish oil based feed at the time of transfer to seawater.

As such, it was evident that use of vegetable oils in freshwater feed did not interfere with low temperature acclimation or parr-smolt transformation of juvenile salmon, and subsequent on-growing in seawater was better when vegetable oil had been used in the feed. This indicates that fatty acid (lipid) requirement of Atlantic salmon are probably different in fresh water and seawater, and that these changes are linked to parr-smolt transformation. It could be speculated that that salinity may be more important than temperature as an environmental influence on the fatty acid requirements of Atlantic salmon.



URN:NBN:no-6435

**SAMMENDRAG**

Målet med dette studiet har vært å undersøke vanntemperaturens og saltholdighetens innvirkning på lipidernæringen hos parr og smolt av oppdrettet atlantisk laks (*Salmo salar* L.). Lakseparr ble holdt ved lav vanntemperatur (2ºC) i seks måneder mens de ble fôret med en av fire fôrtyper med ulike fettkilde (dvs. marin fiskeolje eller vegetabilsk olje) og ulik konsentrasjon (lav, 21% og høy, 34%). Responsene ved den lave temperaturen ble sammenlignet med responsene en fikk hos fisk holdt ved 8ºC i et full-faktorielt forsøksdesign.

Fôrinntak og vekst ble opprettholdt ved 2ºC, men var lavere enn ved 8ºC. Over tid ble vekst og fôrutnyttelse forbedret, noe som indikerer en langtids akklimeringsrespons hos fisken ved den lave temperaturen. Totalt sett var utnyttelsen av fôret bedre ved den laveste temperaturen. En kunne observere en gradvis reduksjon i veksthastighet og fôrutnyttelse ved den høyeste temperaturen ettersom fisken ble større.

Fisken kompenserte for lavere energitetthet i fôret ved å øke fôrinntaket. Ved den høyeste temperaturen var veksten bedre hos fisk fôret med lav-fett-fôrene. Det var også en tendens til forbedret tilvekst når vegetabilsk olje ble brukt. Det var ingen tegn til at vegetabilsk olje var dårligere enn marin fiskeolje til å fremme vekst hos lakseparr i ferskvann.

Fisken som ble fôret med høy-fett-fôr ble fetere enn den som fikk lav-fett-fôr. Det indikerer lipostatisk regulering av fôrinntak. Fett- og proteinfordøyeligheten var høy både ved 2ºC og 8ºC, selv om både fett- og proteinfordøyeligheten var lavest ved 2ºC. Ved den laveste temperaturen, ga økt fettinnhold en forbedret fettfordøyelighet, og bruk av vegetabilsk olje i fôret ga bedre proteinfordøyelighet. Proteinretensjonen var høyere ved den høyeste temperaturen uavhengig av fôrtype, noe som indikerer at proteinet ble både lett fordøyd og omdannet til nytt vev.



URN:NBN:no-6435

Effektene av fôrtype på akklimeringen til lav temperatur ble bestemt fra deponeringen av fettsyrer fra fôret i ulike vev og fra budsjetter for n-3 og n-6 essensielle fettsyrer (EFS). Fettsyresammensetningen i polare (membran) lipider og upolare (lagrings) lipider i muskel, innvoller og 'rest' ble tydelig påvirket av oljene i fôret, og de upolare lipidene ble mer påvirket enn de polare lipidene. Retensjonen av n-6 EFS var lavere enn for n-3 EFS, og var uavhengig av temperatur. Retensjonen av n-3 EFS var høyere ved 2℃, spesielt hos fisk som fikk et fiskeoljebasert fôr. Dette kan reflektere betydningen av n-3 HUFA fettsyrer i akklimeringen til lav temperatur. Imidlertid var de polare lipider hos fisk som ble gitt fôr med vegetabils olje, mer umettet (UFA:SFA forhold) enn hos fisk gitt fôr med marine fiskeoljer. Dette kan bety at vegetabilske oljer produserte fisk som var bedre i stand til å tåle eksponering til lav temperatur, samtidig som membranlipidene var mindre utsatt for oksidering som følge av et lavere innhold av n-3 HUFA fettsyrer (hovedsaklig EPA og DHA).

Etter seks måneder i ferskvann ble fisken smoltifisert, etterfulgt av en 42-dagers periode i sjøvann. Fôrhistorie i ferskvannsfasen påvirket påvekst hos smolt. En positiv effekt av vegetabilsk olje ble funnet, men denne effekten ble bare funnet i grupper som hadde et skifte til et høy-fett-fiskeoljefôr ved overføring til sjøvann.

Det var derfor tydelig at vegetabilsk olje ikke hadde negative konsekvenser for akklimering til lav temperatur eller for smoltifiseringen hos unglaks, og påfølgende tilvekst i sjøvann var bedre når vegetabilske oljer hadde blitt brukt. Dette indikerer at fettsyre (fett) behovet til atlantisk laks er forskjellig mellom ferskvann og sjøvann, og at forskjellene er knyttet til smoltifiseringen. Det kan derfor spekuleres i om saltholdigheten i miljøet er viktigere enn temperaturen i å bestemme fettsyrebehovet hos atlantisk laks.



URN:NBN:no-6435

# 1 INTRODUCTION

A large number of fish and shellfish species are currently cultured worldwide. In Norway, farmed Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) represent large export industries, with approximately 465.000 tonnes of Atlantic salmon and 83.000 tonnes of rainbow trout being produced in 2002 (Fiskeridirektoratet 2003). The increased production of farmed fish over the last three decades has necessitated a parallel increase in fish feed production, and a concomitant search for protein and oil sources to keep abreast of the increase in farmed fish production. Finding suitable protein and oil sources for feed production is considered a major challenge for the fish farming industry (Higgs & Dong 2000; Jobling et al. 2001; Opsahl-Ferstad et al. 2003).

Wild Atlantic salmon occur between 40 and 70ºN in the region bounded by North America, Scandinavia and the other countries of the western Europe (MacCrimmon & Gots 1979; Klemetsen et al. 2003). The species experiences marked seasonal variations in environmental conditions and food availability. The winter is regarded as a critical period (Cunjak & Power 1987; Berg & Bremset 1998), characterised by food scarcity, low growth, and lipolytic activity, when fish mobilise fat reserves deposited during the summer. Atlantic salmon are diadromous; they spawn in fresh water, and after a period of varying length undergo parr-smolt transformation and migrate to offshore marine habitats (reviewed by Boeuf 1993; Clarke 2000). Changes in photoperiod probably play a major role in initiation, timing and synchronisation of the physiological, morphological and behavioural changes associated with the parr-smolt transformation, and alterations in lipid metabolism are regarded as an integral part of the process (Sargent et al. 2002). The accretion of body tissue (growth) of salmon is flexible. In addition to fish genotype and environment (light, temperature and salinity) it is also influenced by the amount and nutritional quality of the feed.

One reason for the commercial success of Norwegian salmon farming relates to the thermal requirements of the species. Salmonids are cold-water tolerant with growth optima at 12-



URN:NBN:no-6435

17ºC (Kestemont & Baras 2001). This enables farming of these species in coastal areas of temperate and polar regions where temperatures below 4ºC are regularly encountered during winter months. Temperature influences metabolic rate by its influence on molecular activation of the components of the metabolic chain. Feeding and growth increase with increasing temperature up to a certain point, and then fall as the upper thermal limits are approached (Brett 1979; Jobling 1994; Kestemont & Baras 2001).

Commercial feeds for carnivorous fish traditionally contain large amounts of meals and oils produced from pelagic marine fish. Marine fish oils of commercial importance are obtained from 'oily fish', e.g. herring (*Clupea harengus*), pilchard (*Sardinia ocellata*), Atlantic menhaden (*Brevoortia tyrannus*) and anchovy (*Engraulis encrasicolus*). Polyunsaturated fatty acids (PUFAs) of the n-3 series are characteristic of marine fish oils and the major PUFAs are usually 20:5 (eicosapentaenoic acid; EPA) and 22:6 (docosahexaenoic acid; DHA)(Gunstone et al. 1994; Steffens 1997; Arts et al. 2001; Higgs & Dong 2000).

Most pelagic fisheries are finite, are fully exploited and they may also show fluctuations over years. One example is the collapse of the anchoveta fisheries off the coast of South America that may occur at 7-12 year intervals during El Niño events. Supplies of fish oils for aquaculture production are expected to become limiting by year 2005 to 2010 (Bell & Sargent 2003). To increase sustainability of cultured fish products, protein and lipid sources of vegetable origin have attracted interest for commercial aquafeeds. In the case of dietary oils, soybean (*Glycine max*), palm (*Elaeis* sp.) and rapeseed/canola (*Brassica* sp.) oil are the most widely available (Higgs & Dong 2000; Sargent et al. 2002). Vegetable oils are generally dominated by one or a few C16 and C18 fatty acids, usually palmitic (C16:0), oleic (C18:1n-9), linoleic (C18:2n-6) and/or linolenic (C18:3n-3) acid, and they have insignificant contents of EPA and DHA (NRC 1993; Gunstone et al. 1994). The latter are often designated as HUFAs (highly unsaturated fatty acids).



URN:NBN:no-6435

There is little information about bioenergetics of salmonids held at temperatures below 4ºC, or about the importance of feed composition at such temperatures. There are, however, indications that quantitative and qualitative aspects of lipid nutrition may be of importance when rearing fish at low temperatures. Lipid metabolism increases during low temperature acclimation, and cell membrane fatty acid compositions change when ectotherms are exposed to low temperature (Hochachka & Somero 2002). Therefore, adequate dietary supplies of lipids and their fatty acids are potentially an important determinant of the ability of animals to adapt to environmental changes. Whether oils of vegetable origin would be able to support adaptation of Atlantic salmon to changes in temperature and salinity are largely unknown.

This thesis describes studies on environmental influences on qualitative and quantitative aspects of lipid nutrition of farmed Atlantic salmon parr and smolt. In **Paper I**, the design and testing of a feed monitoring system is described. The feed monitoring system was used in studies of feed intake, growth and nutrient utilisation of salmon parr (**Paper I** & **Paper II**), and the deposition and retention efficiencies of n-3 and n-6 series fatty acids (**Paper III**) in relation to temperature and feed composition. In **Paper IV**, the interacting effects of temperature and feed composition on the deposition of fatty acids in polar and non-polar lipids of three tissue compartments are discussed. **Paper V** examines the effects of feed history during freshwater rearing on parr-smolt transformation, and on subsequent growth and seawater acclimation of Atlantic salmon smolts.

## 2 AIMS AND QUESTIONS ADDRESSED

The aims and main questions addressed were:

1. To investigate the effects of temperature on feed intake, growth and nutrient utilisation of Atlantic salmon parr, and examine the importance of feed composition (fat content and oil source) at low temperatures:
   - How does temperature (2ºC and 8ºC) influence feed intake and growth of Atlantic



URN:NBN:no-6435

salmon parr, and does feed composition influence the responses?

- How does low temperature influence nutrient utilisation?
- Do dietary effects on feed intake, growth and nutrient utilisation differ with temperature?

These issues are treated in **Paper I** and **Paper II**.

2. To investigate the effects of rearing temperature and feed composition (fat content and oil source) on fatty acid deposition and retention efficiencies of n-3 and n-6 essential fatty acids (EFAs) in Atlantic salmon parr:
   - Do retentions of n-3 and n-6 EFAs differ?
   - Is n-3 EFA retention efficiency increased at 2°C compared to at 8°C?
   - Is n-3 EFA retention higher when fish are given feed low in n-3 HUFAs, i.e. when inclusion of vegetable oils in feeds reduces n-3 HUFA concentration relative to when marine fish oils are used as lipid source?
   - How is deposition of fatty acids affected by temperature and feed treatment, and is the deposition of fatty acids in polar and non-polar lipids of muscle, viscera and 'carcass' different at 2°C and 8°C?

These issues are treated in **Paper III** and **Paper IV**.

3. To investigate the importance of lipid content and composition during freshwater rearing on parr-smolt transformation and subsequent on-growing in seawater:
   - Do dietary-induced effects on body composition during freshwater rearing affect parr-smolt transformation?
   - Do feed history during freshwater rearing, and feed composition during the seawater on-growing, influence the performance of smolts?

These issues are treated in **Paper V**.



URN:NBN:no-6435

# 3 METHODOLOGICAL CONSIDERATIONS

## *3.1 Environmental factors*

In the wild, fish are exposed to a complex array of interacting biotic and abiotic factors that are difficult to reproduce in the laboratory. Temperature may be classified as a lethal, controlling or directive factor for fish (Wootton 1998). In addition, living organisms possess endogenous rhythms many of which may be synchronised to the prevailing environment by zeitgebers (Bünning 1973). Light, or photoperiod, is one such important zeitgeber. In fish, the pineal gland is a 'photoneuroendocrine transducer' that converts information about light period (day-length) to nervous and endocrine signals (Falcón & Collin 1989). This synchronises physiological processes via the light-pituitary axis (Koumourdjian et al. 1976; Zachmann et al. 1992). Light and temperature cycles reinforce each other, although temperature is commonly regarded as being secondary to light in importance as a zeitgeber (Max & Menaker 1992; Liu et al. 1998). Few studies have investigated the interacting effects of light and temperature cycles. However, among temperature, photoperiod and salinity, temperature had the greatest influence on the growth of sockeye (*Oncorhynchus nerka*), coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tschawytscha*) salmon fry. For the coho salmon, effects of temperature and photoperiod were significant and there was also an interaction between the effects of temperature and photoperiod (Clarke et al. 1981). This indicates that the effect of temperature differs depending on the prevailing light regime.

Measures were taken to account for the possibility of confounding effects of interacting photoperiodic and thermal cues. Light conditions (day-length) were gradually decreased from continuous light (LD24:0) to cycles of 12 hours light:12 hours dark (LD12:12) in the months prior to the experiments, and an accompanying decrease in water temperatures provided the fish with 'winter' stimuli. The 'short day' light regime (LD12:12) was maintained during the feeding trial. The short day light regime also enabled induction of parr-smolt transformation subsequent to the termination of the feeding trial. This enabled studies of the importance of feeding history on parr-smolt transformation and early on-



URN:NBN:no-6435

growing in seawater to be undertaken. Parr-smolt transformation was induced by increasing the photoperiod (LD12:12→LD24:0) and water temperature from 2ºC to 8ºC. Thereafter the smolts were transferred directly into seawater at 33‰ salinity.

One aspect of nutritional studies that has received little attention is the duration of the experiment (Shearer 2000a). The time (duration) aspect may be of particular importance in the present context, because adaptation to low temperature may take several weeks (Jobling 1994), so a relatively long feeding period (six months) was used in an attempt to ensure thermal adaptation and adequate accumulation of body constituents, including lipids. This procedure enabled comparison of 'size-matched' fish (Rasmussen & Ostenfeld 2000) reared at the higher and lower temperature; by comparing fish grown to same size at two different temperatures (2 and 8ºC), any confounding effects of body mass on the investigated response parameters could be minimised.

### *3.2 Feed intake measurement*

In order to gain information about feed consumption, a feed intake monitoring system was developed (Figure 1). The system is based on collection of uneaten pellets filtered from the tank outlet water (Helland et al. 1996). This system enabled assessments of feed intake to be made without disturbing the fish, and the assessment of feed intake enabled estimates of retention efficiencies of feed and specific nutrients (protein, energy and essential fatty acids (EFAs)) to be made on a tank basis.

The efficiency of the system was tested prior to the feeding experiment to determine dry matter losses from each test feed. The tests revealed similar dry matter recoveries for all feeds (74-78%; calculation is given in **Paper I**), and these data were used as a correction factor when estimates of feed and nutrient intake were made. A detailed description of design, installation and testing of the system is given in **Paper I**.



URN:NBN:no-6435

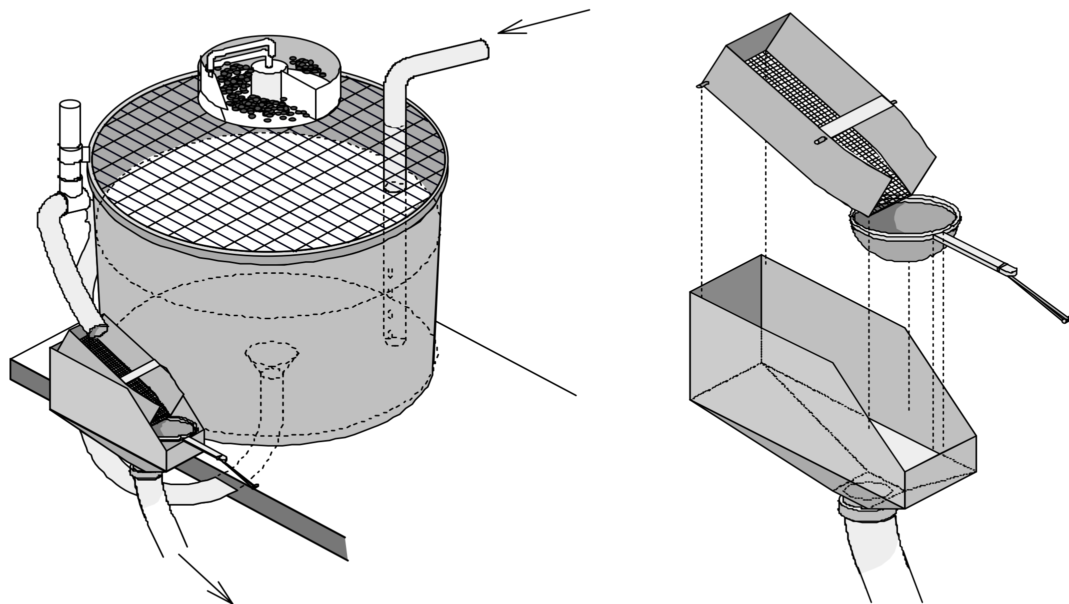

Figure 1. Schematic diagram of the feed intake monitoring system. General overview (left) and feed waste collector (right) (Figure 1 from **Paper I**).

### *3.3 Test feeds*

The development of 'optimal' winter feeds for Atlantic salmon was not the aim of the work. As such, the work should be considered to encompass 'nutritional challenge studies', emphasising qualitative and quantitative aspects of lipid nutrition, rather than strict requirement studies. The feeds were produced with high-quality fish meal (Ultra Flash, Fiskernes Fiskeforbund A.M.B.A., Skagen, Denmark) and ground wheat as the major ingredients, and vitamin and mineral premixes (F. Hoffman-La Roche Ltd., Basel, Switzerland) were added according to the commercial standards of BioMar AS. Feed pellets (2.5 mm) were produced by extrusion technology and either marine fish oil or a vegetable oil blend was added at low or high levels. The feeds were designated LFFO, LFVO, HFFO and HFVO according to fat level (LF-low fat; HF-high fat) and oil source (FO-fish oil; VO-vegetable oil). The gross compositions of the test feeds are shown in Table 1.



URN:NBN:no-6435

### 3.3.1 Proximate feed composition

The replacement of protein by fat, thereby altering dietary protein, gross energy and protein-to-energy ratio, is common practice in nutritional research (e.g. Lee & Putnam 1973; Kaushik & Oliva-Teles 1985; Dias et al. 1998; Hillestad et al. 1998). Increased dietary fat content to achieve efficient high-energy diets has also been a common trend in commercial fish feeds (Sargent et al. 2002).

Table 1. Analysed proximate composition and gross energy content of the test feeds

| | **LFFO** | **LFVO** | **HFFO** | **HFVO** |
|---|---|---|---|---|
| *Proximate composition, g 100 g DM$^{-1}$* | | | | |
| Dry matter | 94.5 | 94.1 | 96.4 | 96.3 |
| Crude protein | 50.2 | 50.4 | 40.3 | 40.2 |
| Crude fat | 20.7 | 21.4 | 33.5 | 33.9 |
| Ash | 9.1 | 9.3 | 10.3 | 10.4 |
| Gross energy, MJ kg$^{-1}$ | 22.5 | 22.5 | 24.8 | 24.5 |

Codes are as follows; LF, low fat (21%); HF, high fat (34%); FO, marine fish oil (100% of added oil); and VO, vegetable oil (rapeseed:linseed oil at 7:3 by weight, 100% of added oil). Carbohydrate contents of the feeds were not determined.

Table 2. Analysed amino acid composition of the test feeds

| | **LFFO** | **LFVO** | **HFFO** | **HFVO** |
|---|---|---|---|---|
| *Essential amino acids, g 100 g DM$^{-1}$* | | | | |
| Methionine | 1.56 | 1.56 | 1.22 | 1.20 |
| Threonine | 2.27 | 2.26 | 1.72 | 1.67 |
| Valine | 2.92 | 2.87 | 2.28 | 2.29 |
| Isoleucine | 2.40 | 2.50 | 1.99 | 1.91 |
| Leucine | 4.00 | 4.00 | 3.10 | 3.10 |
| Phenylalanine | 2.18 | 2.16 | 1.70 | 1.67 |
| Histidine | 1.32 | 1.36 | 1.07 | 1.06 |
| Lysine | 4.00 | 4.00 | 3.09 | 3.12 |
| Arginine | 2.93 | 2.91 | 2.28 | 2.28 |

Codes are as follows; LF, low fat (21%); HF, high fat (34%); FO, marine fish oil (100% of added oil); and VO, vegetable oil (rapeseed:linseed oil at 7:3 by weight, 100% of added oil). Tryptophan contents of the feeds were not determined.

Carnivorous fish, such as salmonids, require 40-55% protein (NRC 1993), and the requirement is higher in small than in large fish (NRC 1993, Einen & Roem 1997; Wilson 2002). The EAA contents are given in Table 2. As expected, the high fat feeds contained lower levels of EAAs. Thus, essential amino acids (EAAs) and protein of the high fat feeds



URN:NBN:no-6435

(Table 1) may have influenced growth depending on the availability of the EAAs and the obtained feed conversion ratios (FCRs, g feed eaten g gained$^{-1}$).

### *3.3.2 Dietary oil sources*

Two oil sources were used in the experimental feeds; sandeel (*Ammodytes* spp.) oil and a blend of rapeseed (*Brassica* sp.) and linseed (*Linum* sp.) oil (7:3 ratio on a weight basis). The vegetable oils were refined, i.e. neutralised, bleached and de-odorised. The rapeseed oil was double-low quality i.e. low contents of glucosinolates (<30$\mu$mol g$^{-1}$) and erucic acid (C22:1n-9; <3% of total fatty acids). The fatty acid compositions of the test feeds are given in Table 3.

Table 3. Feed oil sources in recipe and the relative fatty acid composition of test feeds

| | **LFFO** | **LFVO** | **HFFO** | **HFVO** |
|---|---|---|---|---|
| *Feed oil composition, % of recipe* | | | | |
| Sandeel oil | 14.0 | | 27.0 | |
| Rapeseed oil | | 10.4 | | 20.0 |
| Linseed oil | | 3.9 | | 7.0 |
| *Fatty acid composition, %* | | | | |
| 14:0 | 5.7 | 1.5 | 6.0 | 0.8 |
| 16:0 | 13.4 | 7.0 | 13.6 | 5.7 |
| 18:0 | 2.0 | 2.3 | 1.9 | 2.3 |
| Σ SAFA[1] | 21.4 | 11.6 | 21.9 | 9.9 |
| 16:1 | 5.1 | 1.2 | 5.4 | 0.7 |
| 18:1n-9 | 8.2 | 38.4 | 7.3 | 43.3 |
| 20:1 | 10.8 | 3.1 | 11.4 | 2.2 |
| 22:1 | 15.9 | 3.8 | 17.1 | 2.0 |
| Σ MUFA[2] | 43.1 | 49.3 | 44.3 | 50.6 |
| 18:2n-6 | 3.1 | 15.1 | 2.4 | 16.8 |
| 18:3n-3 | 2.0 | 15.7 | 1.6 | 18.3 |
| 18:4 | 4.2 | 0.9 | 4.4 | 0.5 |
| 20:4n-6 | 0.6 | 0.2 | 0.5 | 0.1 |
| 20:5n-3 | 10.1 | 2.1 | 10.8 | 1.1 |
| 22:6n-3 | 12.6 | 4.3 | 11.8 | 2.2 |
| Σ PUFA[3] | 35.5 | 39.1 | 33.8 | 39.5 |
| n-3:n-6 ratio | 6.7 | 1.4 | 8.2 | 1.3 |

Codes are as follows; LF, low fat (21%); HF, high fat (34%); FO, marine fish oil (100% of added oil); and VO, vegetable oil (rapeseed:linseed oil at 7:3 by weight, 100% of added oil).
[1]Saturated fatty acids, i.e. fatty acids without double bonds in the carbon chain, [2] monounsaturated fatty acids, i.e. fatty acids with a single double bond in the carbon chain, [3] polyunsaturated fatty acids, i.e. fatty acids with several double bonds in the carbon chain.



URN:NBN:no-6435

The main differences were in PUFA compositions (Table 3). Northern hemisphere fish oils are characterised by high contents of EPA and DHA, which are the main n-3 HUFAs (fatty acids with >4 double bonds in the carbon chain), and long-chain MUFAs, mainly C20:1 and C22:1 isomers (Gunstone et al. 1994; Arts et al. 2001). Low-erucic rapeseed oil is particularly rich in C18:1n-9 and has also high relative contents of C18:2n-6 and less C18:3n-3, while linseed oil is characterised by a high concentration of the latter (NRC 1993; Gunstone et al. 1994). The vegetable oil blend gave feeds with balanced contents of n-3 and n-6 fatty acids dominated by C18 PUFAs, and with high contents of C18 MUFAs (Table 3). The fish meal in the feeds provided sufficient n-3 HUFAs to meet the EFA requirements of Atlantic salmon parr as indicated by Ruyter et al. (1998). Juvenile salmon are exposed to a 'freshwater food web' in the wild and there are resemblances between the fatty acids in freshwater and terrestrial food webs (Hanson et al. 1985; Bell et al. 1994; Higgs et al. 1995; Goedkoop et al. 2000; Bendiksen et al. 2003; see Figure 2).

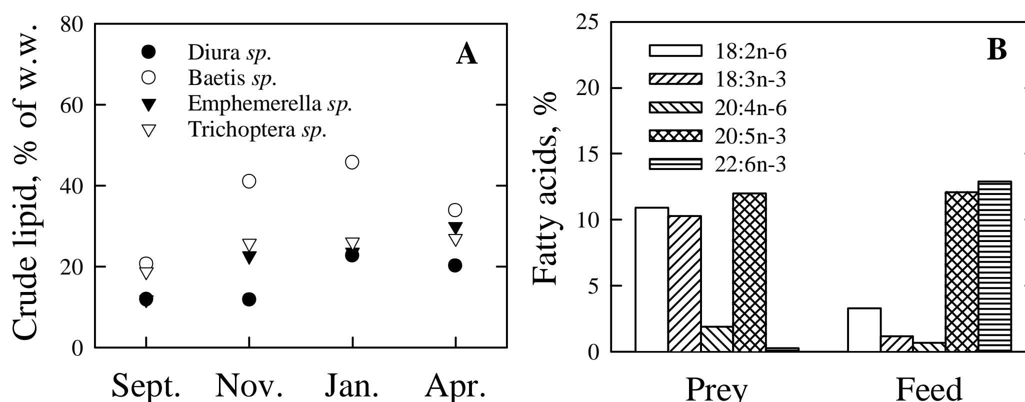


Figure 2. Changes in fat content of natural prey dominating stomach content of small salmon in the River Stjørdalselva (63°25'N) collected during winter (A), and mean PUFA composition of natural prey and a marine fish oil based commercial feed (B) (from Bendiksen et al. 2003).

There is evidence to suggest that Atlantic salmon parr readily elongate and desaturate C18 precursors of n-3 and n-6 series fatty acids to the biologically active forms of essential fatty acids (n-3 and n-6 HUFAs)(Sargent et al. 1995; 1999; 2002). Information about lipid



URN:NBN:no-6435

nutrition of wild juvenile Atlantic salmon during winter is sparse, but Bendiksen et al. (2003) found that prey of salmonids inhabiting a high-latitude river had a low content of the n-3 HUFA DHA (Figure 2). The minor contribution of DHA to the fatty acid content of the prey implies that the requirement of the salmon parr for this fatty acid is largely met by elongation and desaturation of C18/C20 n-3 precursors (Bendiksen et al. 2003). As such, salmon parr are expected to a have high tolerance for vegetable oils.

### *3.4 Experimental design and statistical methods*

The statistical methods used in aquaculture studies have been the subject of recent scrutiny (e.g. Searchy-Bernal 1994; Smart et al. 1998; Shearer 2000a; Ruohonen et al. 2001; Ling & Cotter 2003). Analysis of variance (ANOVA) is the most common technique used to analyse experimental data in biology because it is readily adaptable to complex multifactor designs (Ling & Cotter 2003). A full-factorial $2^3$ completely randomised factorial design with triplicate replications (fish tanks) for each treatment was adopted for analysis of data collected in experiments reported in the thesis. Data were mainly analysed using fixed factor models within the GLM procedure of SPSS for Windows (version 10.0). In such models independent variables are selected arbitrarily and systematically, thereby limiting generalizations to the treatment effects observed with the treatment conditions selected (Zolman 1993). A repeated measure ANOVA was used when series of individual observations were available, and in these cases replicate tank was hierarchically nested within dietary treatments (Ling & Cotter 2003). A sub-population of about 60 fish in each tank was tagged (FTF-69, Floy Tag and Manufacturing, Seattle, WA) to give information about growth of individuals.

In factor experiments, both simple main effects and interactions between treatment factors are possible. Interaction effects were of interest as these could reveal whether the responses to feed treatment differed between temperatures. ANOVA tests were backed-up by (unplanned) *post-hoc* multi-comparisons using Tukey's HSD test, or alternatively, an



URN:NBN:no-6435

equivalent non-parametric test. In addition, simple correlations (Pearson's r) were used for assessing strength of associations between test variables.

The National Animal Research Authority of Norway approved the experiments.

# 4 RESULTS AND GENERAL DISCUSSION

## *4.1 Feeding and growth*

### 4.1.1 Thermal and dietary effects and their interactions

Feeding and growth were maintained at 2ºC, but at lower rates than at 8ºC. The suppressive effect of low temperature was progressively reduced, suggestive of a long-term thermal acclimation response (**Paper I** & **Paper II**).

Temperature influences rates of feeding and growth directly by affecting metabolic rate (Brett 1979; Elliott 1982; Jobling 1994). Brett (1979) suggested that growth at low temperatures is limited by the reduction in available energy caused by low feeding rates, while other studies have shown that the growth reduction may be caused by impaired protein digestion (Hardewig & van Dijk 2003) or by inhibition of protein synthesis (West & Driedzic 1999). Reduced rates of protein synthesis would lead to a decrease in energy demand and thus reduced appetite (West & Driedzic 1999).

Feeding rates were markedly affected by a reduction in temperature from 8ºC to 2ºC (**Paper I** & **Paper II**), with feed intake (g $fish^{-1}$) over the same two months period being about five times higher for fish held at 8ºC than at 2ºC. There was a two-fold increase in weight after six months of feeding at the lower temperature, while a five-fold weight increase was seen at the higher temperature. An additional four months of feeding was required for a doubling of weight of 19g salmon parr at 2ºC compared to at 8ºC (Figure 3; **Paper I** and **Paper II**).



URN:NBN:no-6435

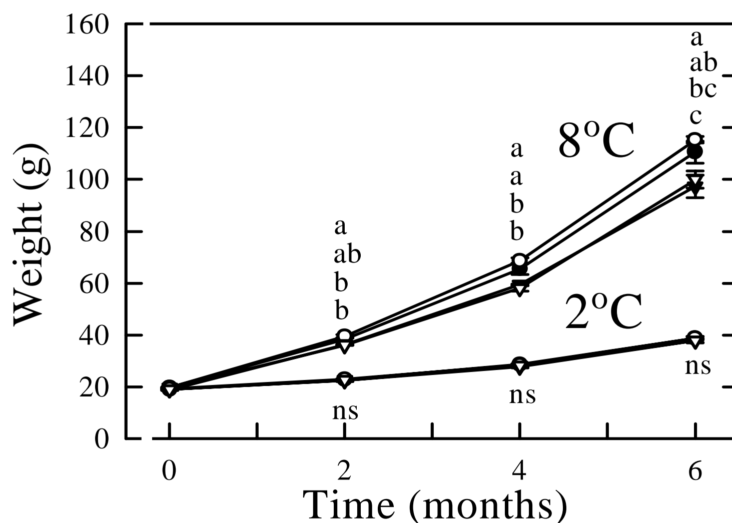


Figure 3. Growth of salmon parr held at 2˚C and 8˚C while being fed one of four feeds. Feed treatments are as follows; LFFO, low fat – fish oil (filled circle); LFVO, low fat – vegetable oil (open circles); HFFO, high fat – fish oil (filled triangles); and HFVO, high fat – vegetable oil (open triangles). Data are presented as mean ± S.E. (n=3 per treatment). Different letters indicate significant differences between dietary treatments within sampling times. Lines and symbols may be hidden (Figure 1 from **Paper II**).

Feeding and growth were maintained at low temperature, and the lower limit for feeding was below 2ºC (**Paper I** & **Paper II**). In line with this, feed intake and growth occur at low temperature in several salmonids both in the wild and in captivity (Brännäs & Wicklund 1992; Fraser et al. 1993; Heggenes et al. 1993; Koskela et al. 1997a,b). For example, Koskela et al. (1997a,b) found that both Atlantic salmon and brown trout (*Salmo trutta*) continued to feed at 2ºC, and the lower thermal limit for feed intake was estimated to be just above 0ºC (Koskela et al. 1997a).

Although the suppressive effect of reduced temperature on feed intake and growth was pronounced, the differences in rates of feed intake and growth between fish at the higher and lower temperatures were not constant over time.



URN:NBN:no-6435

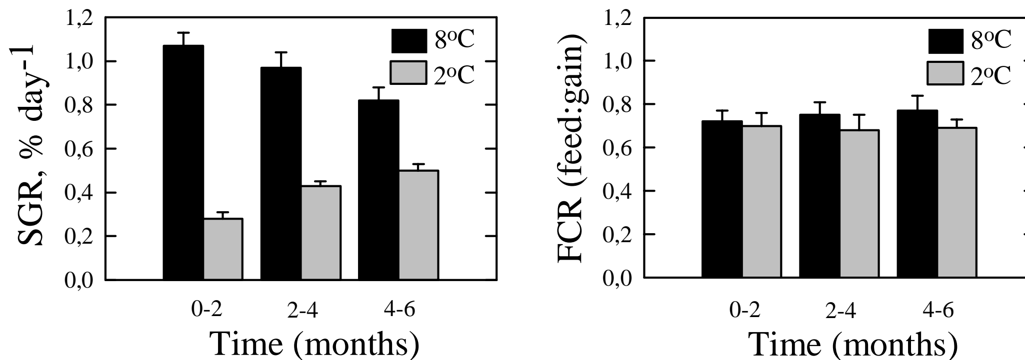


Figure 4. Temporal changes in growth (SGR, % day$^{-1}$) and FCR (feed:gain) of salmon parr reared at 2˚C and 8˚C for six months while being fed four different feeds. Data are presented as tank mean ± S.D. (n=12 per treatment).

A temporal increase in feed intake and growth was seen in fish held at the lower temperature (Figure 4; **Paper I** and **Paper II**). This is in accord with Koskela et al. (1997c) and Jobling et al. (1998) who found that rates of feed intake and growth tended to increase with time in Baltic salmon (*Salmo salar* L.) and brown trout held at constant low temperature under continuous light. For fish held at the lower temperature, feed conversion rate (FCR, feed:gain, calculation is given in **Paper I**) was better than in those at the higher temperature both when examined for size-matched groups of fish (**Paper I**) and for the whole six months growth period (**Paper II**). Specific growth rate (SGR, % day$^{-1}$, calculation is given in **Paper II**) and FCRs decreased over time at the higher temperature (Figure 4). Previous reports on the effect of temperature on feed utilisation are equivocal, but Alanärä (1992) reported a linear decrease in feed efficiency in rainbow trout as temperature increased. This agrees with the present results (**Paper I** and **Paper II**). Both rates of feed intake and growth are size-dependent, so it is possible that reduced growth rate and FCRs over time at the higher temperature (Figure 4) was largely the result of the fish becoming larger.

A temporal increase in 'temperature-corrected growth' (TGC; calculation is given in **Paper I**) was seen at the lower temperature, while constant TGCs were found at the higher



URN:NBN:no-6435

temperature (**Paper I**). TGC is not constant over the entire temperature range at which growth is possible and growth predictions are problematic (Jobling 2003). Despite such problems, a stable difference in TGC between fish held at the two temperatures would be predicted, but this was not the case (see Figure 5A and 5B in **Paper I**). It is suggested that the temporal increase in TGC for fish held at the lower temperature reflected a long-term acclimation response (**Paper I**) and that the fish at the lower temperature were able to respond more effectively to low temperature as time progressed.

Differences in performance were seen between dietary groups at the higher temperature, but not at the lower temperature. At the higher temperature the fish grew better when fed the low fat feeds, and there was also a tendency for improved growth when vegetable oils were used (Figure 3; **Paper II**). It is evident that the effect of low temperature masked any potential effects of feed treatment; diet-related growth differences observed at the higher temperature were diminished at the lower temperature. This constitutes a challenge when information about nutritional requirements for fish held at very low temperature is sought, as weight gain is a frequently used response parameter in such studies.

The high fat feeds induced higher whole body fat contents than the low-fat feeds (**Paper II**), and according to the lipostatic theory (Kennedy 1953; see section 4.1.2) increased body fat would exert a negative feedback on the hypothalamic regions involved in appetite regulation, resulting in reduced feed intake. However, growth of fish is also dependent upon dietary protein and salmonids require 40-55% of dietary protein (NRC 1993; Wilson 2002). Essential amino acid (EAA) contents of the test diets were high, due to the inclusion of high quality fish meal, although more EAAs were available in the low fat feeds (Table 2).

There is little evidence that protein requirements differ between fish held at different water temperatures (NRC 1993; Wilson 2002), although higher protein requirement at high temperatures has been reported for chinook salmon fingerlings (DeLong et al. 1958) and



URN:NBN:no-6435

striped bass (*Morone saxatilis*; Millikin 1982). No differences in protein requirement are reported for rainbow trout at temperatures ranging from 9 to 18°C (NRC, 1993).

### 4.1.2 Lipostatic regulation of feeding and growth

Differences in feed energy density invoked compensation in feed intake to maintain energy and nutrient intake, indicating regulation of feed intake and growth (Figure 5 right panel; **Paper I** and **Paper II**). The increase in dietary fat from 21 to 34% increased the energy density from about 23 to 25 kJ $g^{-1}$. Consumption of low-fat feeds was higher than that of high-fat feeds, and this was seen at both temperatures (**Paper I** and **Paper II**), and across oil sources (**Paper II**). These results are consistent with the idea that the fish compensate for differences in feed energy density to maintain energy and nutrient intake (e.g. Lee & Putnam 1973; Shearer et al. 1997; Yamamoto et al. 2000; Sæther & Jobling 2001).

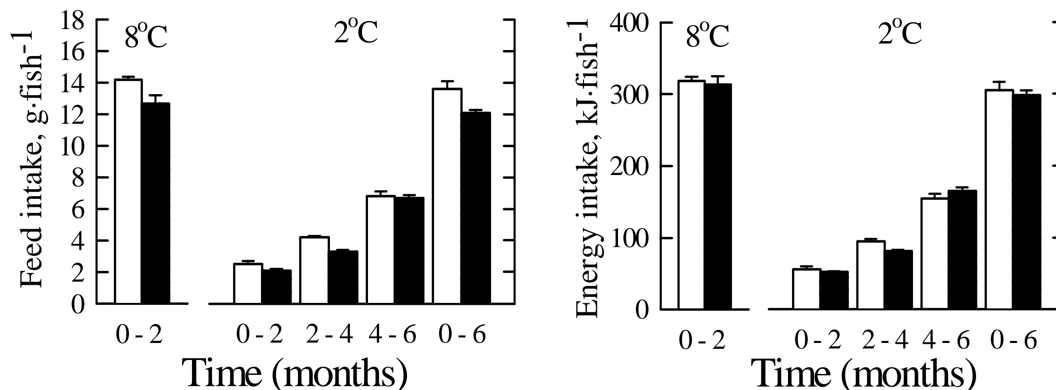


Figure 5. Feed intake in mass (left) and energy (right) of salmon parr growing from c. 19g to c. 38g at 2°C and 8°C. Data for fish fed LFFO (open bars) and HFFO (shaded bars) feeds are presented. Data are given as mean ± S.E. (n=3 per treatment)(from **Paper I**).

Current understanding of long-term energy homeostasis involves regulatory systems involving sensors, feedback loops, and compensatory mechanisms (Weigle 1994; Woods & Seeley 2000). Deviations of body energy in a positive or negative direction invoke hypophagic or hyperphagic responses to restore body energy reserves. Fat is the major form of stored chemical energy in living organisms, and body fat mass is involved in long-term



URN:NBN:no-6435

regulation of energy balance. A possible link between circulating factors and the regulation of appetite was suggested 50 years ago (lipostatic theory; Kennedy 1953), but it was not until 1994 that Zhang and co-workers identified a circulating feedback signal (leptin)(Zhang et al. 1994). Leptin, and other adiposity signals, provide an index of body fat, establishing a link between fat stores (adipose tissues) and the central hypothalamic regions involved in the regulation of feeding and energy expenditure (Weigle 1994; Woods & Seeley 2000). A 'leptin-like factor' seems to be present in fish (Johnson et al. 2000) and is recently reported in Atlantic salmon (Vegusdal et al. 2003).

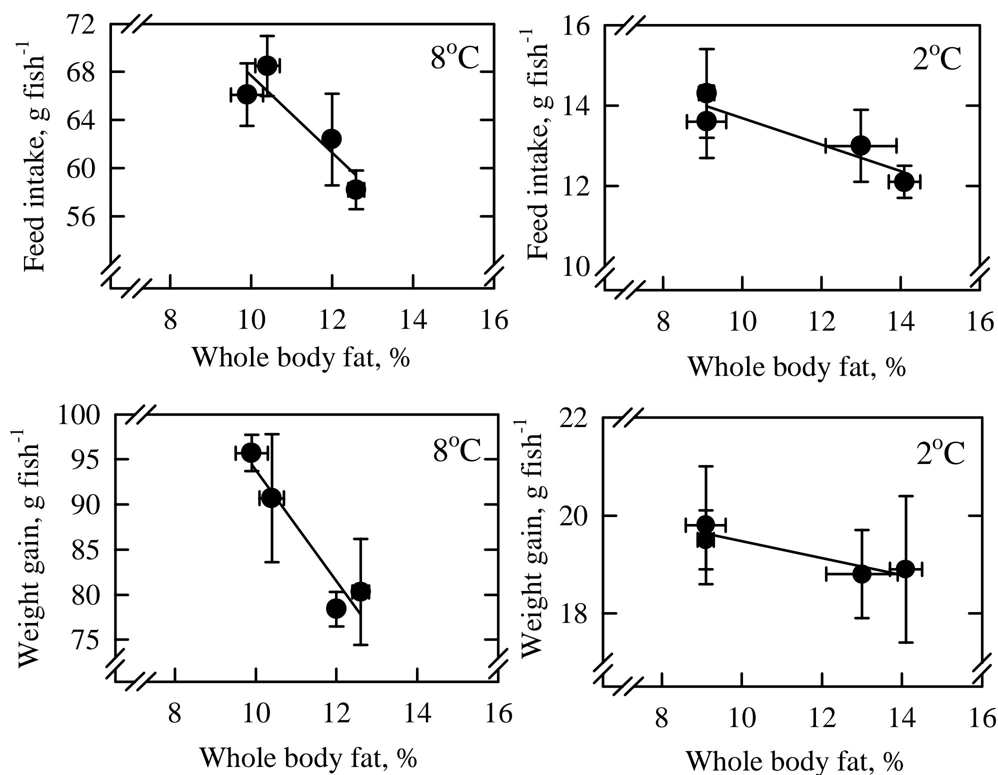


Figure 6. Association between feed intake and whole body fat (upper panels), and between weight gain and whole body fat (lower panels) of fish held for six months at water temperatures of 8°C (left panels) and 2°C (right panels) while being fed four different feeds (see Table 1). Data are presented as mean ± S.D. (n=3 per treatment)(based on data from Table 2 in **Paper II**). All regressions are significant at the $P$<0.05 levels.



URN:NBN:no-6435

It is possible that the differences in feed intake between the salmon parr fed the low and high fat feeds were related to differences in body fat content (Figure 6; **Paper I** and **Paper II**), as the size of fat depots seems to be involved in feed intake regulation in salmonids (Jobling & Miglavs 1993; Metcalfe & Thorpe 1992). For example, the appetite of over-wintering juvenile salmon increased when fat reserves fell, but declined once the reserves had been replenished (Metcalfe & Thorpe 1992). This may indicate that both size of fat stores and the rates of their depletion and replenishment are important factors in regulation of appetite in fish. At the higher temperature, the low fat feeds resulted in higher weight gain than the high fat feeds (Figure 3; **Paper I** and **Paper II**). Although weight differences that were introduced between dietary groups at 8°C could explain some of the difference in feed intake, there were also differences in feed intake between fish fed low and high fat feeds at the lower temperature. Therefore, it is concluded that the reduction in feed intake for fish fed the high fat feed was not merely a size-effect, but was rather a consequence of regulatory mechanisms, possibly related to increased accumulation of body fat.

#### 4.1.3 Effects of dietary oil sources

There were no indications that vegetable oils were inferior to marine fish oils in supporting growth of the salmon parr (**Paper II**). Feeds with vegetable oils had lower contents of the n-3 HUFAs EPA and DHA (Table 3). Dietary lipid may affect whole-animal physiology, and n-3 HUFAs, especially DHA, are considered important when fish adapt to low temperature (Hazel 1979; 1984). Cold tolerance of juvenile red drum (*Sciaenops ocellatus*), expressed as lower median lethal temperature, was affected both by the levels and kinds of dietary lipids. Fish fed diets rich in n-3 HUFA were able to survive temperatures 3.5 to 4.5°C lower than fish fed diets low in these fatty acids (Craig et al. 1995). Thus, it might be hypothesised that winter performance of fish could suffer when high levels of vegetable oils are included in feeds, due primarily to the low n-3 HUFA content.

This assumption was not supported by our studies. The HUFA-depleted vegetable oil was not found to be inferior to marine fish oil as a lipid source even when high inclusions were



URN:NBN:no-6435

tested at low temperature for prolonged periods (**Paper II**). At the higher temperature there was a tendency for improved performance when vegetable oils were used (**Paper II**).

Dosanjh et al. (1998) found no effect on growth of replacing 47% of fish oil with canola oil in feed for post-smolt Atlantic salmon. Bell et al. (2001) tested rapeseed oil inclusion at 0, 10, 25, 50 and 100% of dietary oils in feeds for Atlantic salmon post-smolts. No differences in growth and feed conversion were found, although fish fed 100% rapeseed oil had the lowest final weights and SGRs, indicating an upper limit for the inclusion of rapeseed oil. The suitability of crude palm oil for Atlantic salmon post-smolts was tested in feeds with 0, 25, 50 and 100% crude palm oil as total added oil and there was found no effects of diets on SGR or FCR (Bell et al. 2002). On the other hand, growth-promoting effects have been reported when n-3 and n-6 EFAs are provided as n-3 and n-6 HUFAs rather than as C18 n-3 and n-6 fatty acids (Takeuchi & Watanabe 1979; Ruyter et al. 2000).

There are few studies of long-term effects of vegetable oil at low temperatures (**Paper II**). Grisdale-Helland et al. (2002) investigated the influence of high contents of dietary soybean oil on post-smolt salmon reared at 5 and 12°C. The fish grew well at both temperatures on high-energy, fish meal-based diets containing up to 100% supplementary soybean oil (Grisdale-Helland et al. 2002). Together, the results indicate a high tolerance for vegetable oils in juvenile Atlantic salmon, and low temperature does not seem to be a major impediment for extensive use of vegetable oils during freshwater growth (**Paper II**).

### *4.2 Nutrient digestibility and retention efficiencies*

Protein and fat digestibilities were reduced at the lower temperature, but effects of feed treatment on fat and protein digestibilities were more pronounced at the low temperature: At the lower temperature, increased dietary fat level resulted in higher fat digestibility, and improved protein digestibility was seen when vegetable oil was included in the feed (Figure 7; **Paper II**).



URN:NBN:no-6435

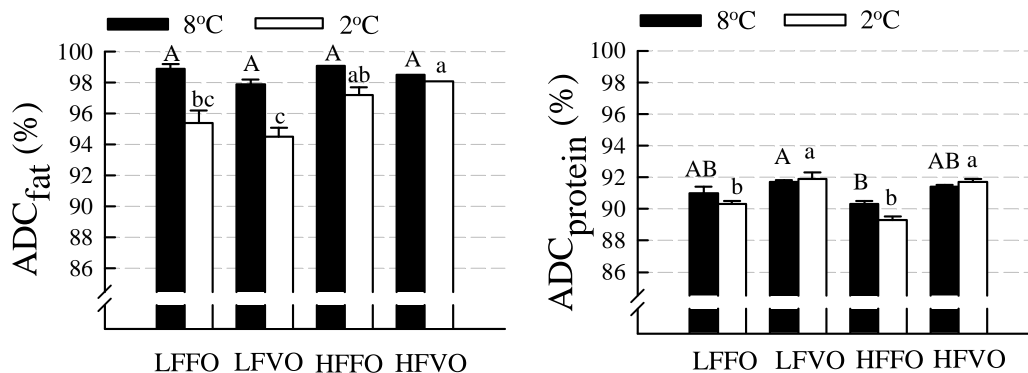


Figure 7. Apparent digestibility coefficients for fat (left) and protein (right) of salmon parr held at 2 and 8 °C while being fed one of four feeds. Feed codes are as follows; LF, low fat; HF, high fat; FO, fish oil; VO, vegetable oil. Data are presented as mean ±S.E. (n=3 per treatment). Different upper and lower case letter indicate significant differences between dietary treatments at 8 and 2°C, respectively (Figure 2 from **Paper II**).

Fat digestibility depends upon the degree of hydrogenation of the oil (e.g. Austreng et al. 1979; Torstensen et al. 2000). Vegetable oil was not more digestible than the fish oil although contents of saturated fatty acids (SAFAs) were lower (Table 3),implying that feeds with about 22% SAFAs are readily digested and utilised by Atlantic salmon parr (**Paper II**). Rates of gastric evacuation in fish are slowed both by reductions in temperature (Fauconneau 1983; Jobling 1994), and by increased feed fat and energy content (Jobling 1980; 1994), presumably giving more time for digestive lipases to act and increase fat digestion. Although there was a co-operative effect of low temperature and feed fat level on fat digestibility in salmon parr (Figure 7; **Paper II**), the overall finding of increased nutrient digestibility at higher temperature is in general accord with previous reports from salmonid species (Watanabe et al. 1996a,b; Azevedo et al. 1998; Olsen & Ringø 1998).

Protein retention efficiency (PRE: [g protein increase g protein ingested$^{-1}$] × 100) was better at the higher temperature. In line with the results of the digestibility trial, protein retention efficiency was generally high, but was significantly higher at 8°C than at 2°C (see Table 2 and Table 3 in **Paper II**). This indicates that the feed proteins were both readily digested and deposited as fish tissue (**Paper II**). Protein retention efficiencies may have



URN:NBN:no-6435

been slightly overestimated due to the indirect method used to assess fish proteins, but contents of carbohydrates are very low in fish (0.1-0.5% of body wet weight, Jonsson & Jonsson 1997). Even with some error in estimation, the differences recorded between dietary groups and temperatures would still be valid.

The difference in protein retention efficiency between fish held at different temperatures reflected increased digestibility at the higher temperature, suggestive of a close relationship between these two parameters. Azevedo et al. (1998) reported a significant effect of temperature on protein digestibility in rainbow trout, but no differences in protein retention efficiencies were found between temperatures. Their experiment was conducted within the thermal range 6-15ºC under a constant 12 h light: 12 h dark regime (Azevedo et al. 1998).

High feed fat content improved protein retention, indicating 'protein sparing' as previously seen in other studies (Shearer 2000b). However, one consequence of adding fat to a diet may be that additional fat is deposited in the fish (Shearer 2000b); 'protein sparing' was accompanied by an increase in body fat content (**Paper II**). At the higher temperature, vegetable oils gave a 'protein sparing' effect that was accompanied by a tendency for improved SGR and FCR pointing to a general positive effect of using vegetable oils. As such, vegetable oils seemed to be promising candidates to provide a protein sparing without giving excessive fat accumulation.

In contrast to PRE, there was a tendency for higher energy retention (ERE: [kJ gain kJ ingested$^{-1}$] $\times$ 100) in fish held at the lower temperature, and energy retention was also significantly higher for fish fed the high fat feeds. The results may indicate an effect of temperature on energy partitioning, with a larger proportion of the dietary energy being directed towards fat storage at the lower temperature, and increased protein deposition at the higher temperature.



URN:NBN:no-6435

### *4.3 n-3 and n-6 EFA retentions*

Retention of n-3 EFAs was higher than n-6 EFAs, and low temperature induced higher n-3 EFA retention (**Paper III**). Fish, in common with other vertebrates, cannot synthesise polyunsaturated fatty acids of the n-6 and n-3 series *de novo*. This is due to their deficiency of Δ12 and Δ15 desaturases, which insert double bonds at the n-6 and n-3 positions in the fatty acid carbon chain. Consequently, n-3 and n-6 fatty acids are considered essential fatty acids (EFAs), and adequate amounts must be delivered in the food for normal growth and development. Both C18 and C20/C22 members of the n-3 and n-6 fatty acid series have the potential to meet EFA requirements of salmonids (Sargent et al. 1995; 1999; 2002). C20/C22 EFAs dominated in the fish oil based feeds, while EFAs of vegetable oil based feeds comprised mainly C18 fatty acids (Table 3).

Retentions of both n-3 and n-6 EFAs were high (**Paper III)**, indicating that EFAs of both series were protected against excessive metabolic degradation, a suggestion in keeping with previous reports on the selectivity of mitochondrial and peroxisomal oxidation of fatty acids in fish (Henderson & Sargent 1985; Kiessling & Kiessling 1993). In addition, selective mechanisms that favour PUFA digestion, absorption and deposition appear to exist in fish (Olsen & Ringø 1998; Johnsen et al. 2000). For example, PUFAs are more efficiently absorbed from the digesta than monounsaturated and saturated fatty acids by turbot (*Scophthalmus maximus*)(Koven et al. 1994), and n-3 HUFAs were efficiently absorbed by Atlantic salmon post-smolts (Johnsen et al. 2000).

The consistently higher retention efficiencies of n-3 EFAs compared to n-6 EFAs may be a reflection of a higher requirement for n-3 EFAs than for n-6 EFAs, with a larger proportion of n-6 fatty acids being metabolised. The n-3 EFA requirement is well defined in a variety of salmonid species, while the requirement for n-6 EFAs is less certain (Sargent et al. 2002). Increased requirements for arachidonic acid (AA, 20:4n-6) are probably related mainly to periods of environmental stress (Sargent et al. 2002; Bell & Sargent 2003). Temperature influenced n-3 EFA retention, with higher retention being found at 2°C. The



URN:NBN:no-6435

retention of n-6 EFAs was unaffected by temperature, but increased dietary fat level gave higher n-6 EFA retention. This may indicate that deposition of n-6 fatty acids is increased when they are freely available from the diet (**Paper III**).

At the lower temperature, n-3 EFA retention was higher in fish fed diets with a high n-3 HUFA content, i.e. the fish oil diets. Such high retention may reflect an adaptation to low temperature (**Paper III**). Lipid structures of a given membrane impact properties that may be responsible for a variety of cell functions including enzyme regulation (Williams 1998). Ectotherms deposit long-chain PUFAs in membrane lipids during cold acclimation and the importance of DHA fatty acid in such processes is often highlighted (e.g. Hazel & Williams 1990; Fodor et al. 1995; Logue et al. 2000). Thus, efficient retention of n-3 HUFAs due to selective absorption and reduced oxidative degradation may be mechanisms that operate to ensure maintenance of membrane function in cold environments. Whether this indicates a higher n-3 HUFA requirement in Atlantic salmon in a cold environment remains to be elucidated. Links between dietary fat composition and thermal biology are indicated both for ectotherms (Craig et al. 1995; Simandle et al. 2001) and endotherms (Florant et al. 1993).

### *4.4 Fatty acid deposition in polar and non-polar lipids*

Fatty acid compositions of polar (membrane) and non-polar (storage) lipids in fish tissues were influenced by dietary fatty acids, and exposure to low temperature gave lipids with greater unsaturation (UFA:SFA ratio). From this it is evident that dietary lipids and temperature interacted to influence tissue fatty acid composition (**Paper IV**).

Fatty acids of polar (phospholipids, PLs) and non-polar (neutral lipids, NLs) lipids were determined in three body compartments (muscle, viscera and ‘carcass’) of fish given the four test feeds (Table 2 and Table 3). Comparisons of tissue fatty acid composition were made when the fish had doubled weight from 19g to 38g i.e. after two and 6 months of feeding at 8ºC and 2ºC, respectively. Fatty acid compositions of both the polar and the non-



URN:NBN:no-6435

polar lipids were strongly influenced by dietary fatty acids (**Paper IV**), as seen previously (e.g. Thomassen & Røsjø 1989; Green & Selivonchick 1990; Polvi & Ackman 1992; Arzel et al. 1994; Guillou et al. 1995; Higgs & Dong 2000). High concentrations of n-3 HUFAs were found in PLs from fish given fish oil, while PLs from the fish fed vegetable oil had higher concentrations of MUFAs. PLs were less influenced by dietary fatty acids than NLs, indicating a greater regulation of the fatty acid composition of membrane lipids (PLs) than of storage lipids (NLs). This is not unexpected, since fatty acids found at the sn-3 position in NL storage TAGs are incorporated directly from the dietary fatty acids (Arts et al. 2001). In contrast, a limited number of fatty acids tend to dominate in the PLs, with either a SAFA, such as C16:0, or a MUFA, e.g. C18:1n-9, being found in the sn-1 position of the glycerol backbone, and a MUFA or a polyene, such as EPA and DHA, being found in position sn-2 (Henderson & Tocher 1987; Arts et al. 2001; Higgs & Dong 2000; Sargent et al. 2002).

Temperature had a greater influence on the fatty acid composition of PLs than of NLs (**Paper IV**). At the lower temperature the differential deposition of fatty acids in PLs resulted in a reduction in unsaturated to saturated fatty acids ratio (UFA:SFA ratio), implying that compensatory mechanisms were operating. This is interpreted as a thermal acclimation response that would contribute to the maintenance of membrane fluidity at reduced temperature (**Paper IV**). Reduced temperature invokes compensatory changes in membrane phospholipids, a phenomenon denoted 'homeoviscous adaptation'. This was first described in bacteria (Sinensky, 1974), and later in other ectotherms including fish (Hazel 1979; 1984).

These changes relate to three components; acyl chain composition, fatty acid distribution within the phospholipids, and relative PL composition (Hazel 1984; Hochachka & Somero, 2002). Changes in acyl chain composition are usually associated with a relative reduction in proportions of saturated fatty acids (SAFA) and a corresponding increase in unsaturated fatty acids (UFA)(e.g Hazel 1984; Tiku et al. 1996; Logue et al. 2000; Truman et al. 2000;



URN:NBN:no-6435

Farkas et al. 2001; Hsieh et al. 2003). These changes result in greater unsaturation of the phospholipids i.e. the UFA:SAFA ratio is increased. Several of these studies highlight the importance of n-3 HUFAs for maintenance of membrane fluidity on exposure to low ambient temperature (e.g. Hazel &Williams 1990; Fodor et al. 1995; Logue et al. 2000).

In contrast to higher UFA:SAFA ratio, indicating greater unsaturation, the UIs (i.e. the number of unsaturated double bonds per 100 fatty acids molecules) of the salmon parr phospholipids seemed to be independent of temperature. The numerical value of the UI is strongly influenced by n-3 HUFAs, mainly EPA and DHA, so the finding of UIs being independent of temperature was unexpected given the putative role of n-3 HUFAs in cold acclimation. The finding also seems paradoxical given the higher n-3 EFA retentions in the salmon held at the lower temperature (**Paper III**). Taken together, the results indicate that the UFA:SAFA ratio and UIs provide different sorts of information regarding membrane properties (**Paper IV**).

There are several studies in which minor changes in EPA and DHA of polar lipids have been found during low temperature acclimation (Ingemansson et al. 1993; Labbe et al. 1995; Cordier et al. 2002; Grisdale-Helland et al. 2002). For example, the change in fatty acid composition of rainbow trout muscle lipids seemed to be greater between 19ºC and 12ºC than between 12ºC and 5ºC (Ingemansson et al. 1993). It could be speculated that selective n-3 HUFA incorporation is more important during acclimation to lower temperature within the moderate to high range, than at low ambient temperature (**Paper IV**). In keeping with this suggestion, Skuladottir et al. (1990) found that temperature had only minor effects on fatty acid compositions of muscle, heart and liver PLs of Atlantic salmon exposed to two low temperatures (-1.4 vs. 6.5ºC).

In general, the UFA:SFAs were higher in fish fed feeds containing vegetable oils, perhaps indicating greater membrane fluidity in these fish. By contrast, PLs of fish fed on fish oil



URN:NBN:no-6435

had higher concentrations of n-3 HUFAs, which may have made them more prone to peroxidative damage (**Paper IV**).

### *4.5 Dietary effects on seawater acclimation and growth*

The importance of the dietary fat content (LF vs. HF) and fatty acid profile (FO vs. VO) for seawater acclimation and growth were tested in Atlantic salmon parr that had been held at 2ºC in fresh water. The parr-smolt transformation was induced by light and temperature manipulation. Eight different feed combinations during freshwater and seawater rearing were obtained by providing the fish with four feeds during freshwater rearing and either LFFO or HFFO feed from seawater entry onwards (i.e. LFFO→LFFO; LFFO→HFFO; LFVO→LFFO; LFVO→HFFO; HFFO→LFFO; HFFO→HFFO; HFVO→LFFO and HFVO→HFFO).

Freshwater feed did not affect parr-smolt transformation, but feed history had an effect on early on-growing of smolts in seawater: Improved seawater growth was found for fish fed the LFVO (i.e. low fat – vegetable oil) feed during freshwater rearing. Parr- smolt transformation has previously been reported to be relatively unaffected by dietary manipulations during the freshwater period (e.g. Higgs et al. 1992; Helland & Grisdale-Helland et al. 1998; Nordgarden et al. 2002).

Changes in lipid metabolism may be an integral part of the parr-smolt transformation, and dietary fatty acids may be of importance for seawater acclimation in Atlantic salmon (Bell et al. 1997). Thus, it is argued that adjusting the dietary fatty acid profile to a 'terrestrial-like food web' type by adding vegetable oil to the feed would benefit the fish (Bell et al. 1994; Ghioni et al. 1997; Sargent et al. 1999; Sargent et al. 2002). Despite the pronounced differences in body composition that arose from the feed treatments provided during freshwater rearing (see Table 4 and Table 5 in **Paper V**), all groups of fish accomplished parr-smolt transformation as adjudged by assessments of gill $Na^{+}K^{+}$-ATPase, muscle water and plasma chloride following a seawater challenge test (see Figure 1 in **Paper V**).



URN:NBN:no-6435

Fatty acids may be of importance for seawater acclimation in Atlantic salmon and a direct influence on osmoregulation in hypersaline environments has been indicated (Bell et al. 1997). Eicosanoids are hormone-like compounds produced from C20 fatty acids, mainly EPA and AA. They are known to be involved in regulation of water and ion fluxes in gills and kidney (Mustafa & Srivastava 1989), and eicosanoid synthesis may be altered by dietary manipulation (Bell et al. 1997; Tocher et al. 2000). The conversion of C18 precursors to AA is antagonised by EPA and its eicosanoid derivatives (Bell et al. 1989). This interaction may be crucial since AA requirements are associated with stressful periods (Bell & Sargent 2003) and the requirement could be expected to increase during seawater acclimation. The increase in fatty acid elongation and desaturation activity during parr-smolt transformation increases the production of C20 and C22 HUFAs from C18 precursors (Bell et al. 1997; Tocher et al. 2000), but the increase is significantly reduced upon feeding oils rich in n-3 HUFAs (Bell et al. 1997; Tocher et al. 2001).

In our study, there was no clear evidence that the growth promoting effect of feeding vegetable oil in fresh water was the result of osmoregulatory improvements. No differences in gill $Na^+$,$K^+$-ATPase activity, muscle water and plasma chloride were found between feed treatment groups following 24 h seawater tests conducted during the smolt induction period (see Figure 1 in **Paper V**), or at the end of the 42 days seawater period.

Growth was low during the first period in seawater, but smolts previously fed the LFVO feed (i.e. low fat – vegetable oil) during freshwater rearing gained weight during the total 42 days seawater period. This indicated a positive effect of adding vegetable oils to the parr feed (Figure 8; **Paper V**), in line with previous suggestions (e.g. Bell et al. 1994; 1997). Improved growth of smolts may be related to energy metabolism. Rapeseed oil is abundant in oleic acid, which is a good substrate for ß oxidation (e.g. Henderson & Tocher 1987; Kiessling & Kiessling 1993). The fat stores of the fish fed feeds with vegetable oil may



URN:NBN:no-6435

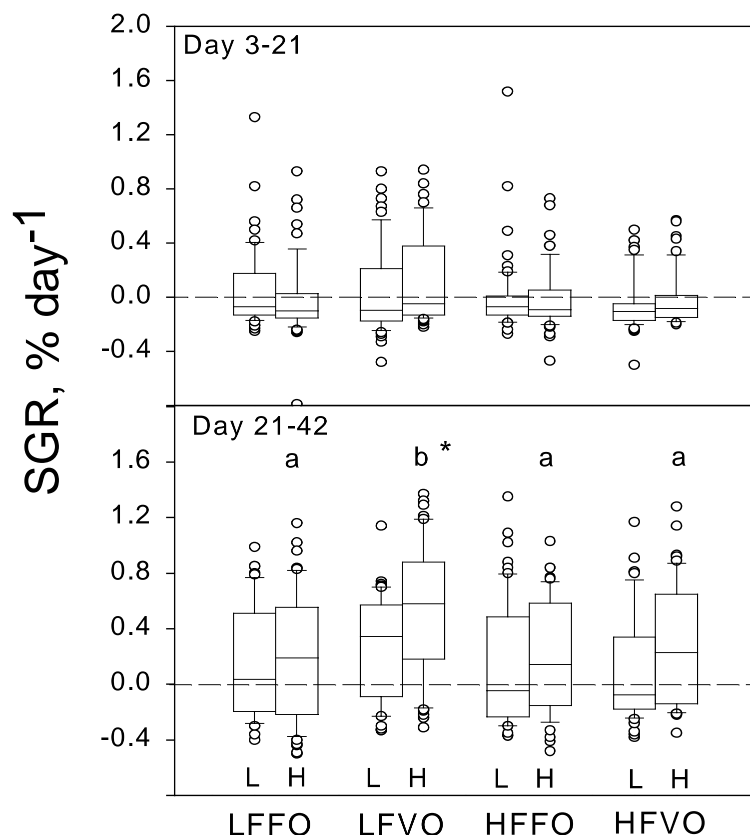


Figure 8. Box-plots (n=50-60 in each plot) showing growth rates in seawater of fish fed four different feeds during rearing in fresh water (LFFO, LFVO, HFFO and HFVO), and subjected to new feeds at seawater entry (L=LFFO, H=HFFO). The box contains 50% of the data (90% of data when whiskers are included), while circles indicate extreme values. The horizontal line within each box indicates the median. An asterisk (*) indicates significant differences between L and H treatments, whereas different lower case letters indicate significant differences between freshwater feed groups within L or H treatments (data from **Paper V**).

have furnished these fish with readily available energy substrate during the early period in seawater when the fish were feeding poorly. Significantly better growth in smolts was, however, only seen in the group in which a shift in both lipid source and feed fat content had been applied (Figure 8; **Paper V**). This indicates the importance, not only of freshwater feed, but also of composition of the feed provided during the seawater period. The finding



URN:NBN:no-6435

may reflect a higher energy requirement in seawater due to higher maintenance costs in the marine environment (Boeuf & Payan 2001). It is also possible that this effect reflects differences in n-3 HUFA requirement in freshwater-adapted salmon and salmon smolts, and that the smolt benefit from increased supply of n-3 HUFAs upon seawater transfer. In natural ecosystems, water salinity seems to be an important determinant for EPA and DHA deposition in animals, with higher contents of n-3 HUFAs being found in marine animals than in those from freshwater environments (Steffens 1997; Art et al. 2001; Makhutova et al. 2003). Lipid composition of food to a high degree determines food web interactions, individual and population growth (Brett & Müller-Navarra 1997). The nutritional regulation of desaturase genes has recently been indicated (Seiliez et al. 2002), and this may have consequences for EFA requirements and how dietary oils in feeds for farmed fish are designed, as indicated in the present study (**Paper V**). As such, the results may indicate that both dietary fat content and fatty acid composition may be of importance for early seawater on-growth of salmon, pointing to a positive effect of using vegetable oil during freshwater periods. Whether the recorded effect of the HFFO feed was the result of increased supply of n-3 HUFAs or dietary energy, or a combination of both factors, could, however, not be determined (**Paper V**), and requires further investigation.

## 4.6 CONCLUSIONS

Based on the aims and questions addressed the following main conclusions can be drawn:

**Aim 1:** Atlantic salmon (*Salmo salar* L.) parr maintain feeding and growth at 2ºC, but at lower rates than at 8ºC. There seems to be a link between the body fat and the control of appetite, with reduced appetite and growth being related to an increase in body fat. Acclimation to low temperature seems to occur relatively slowly, but exposure to low temperature does not seem to induce poorer feed utilisation. Both proteins and fats are less readily digested when ambient temperatures are low. Protein seems to be less efficiently utilised at low temperature, but low temperature induced higher energy retention efficiency. The inclusion of vegetable oils in the feed induces better protein digestion and utilisation,



URN:NBN:no-6435

and this effect is more pronounced at low temperature. An increase in dietary fat level improve fat digestibility when the ambient temperature is low.

**Aim 2:** The fish retain n-3 and n-6 EFAs efficiently, but retention is higher for n-3 EFAs than for n-6 EFAs. The n-3 EFA retention seems to increase at low temperature, while n-6 EFA retention is unaffected by temperature. Retention of n-3 HUFAs, mainly EPA and DHA is high, which may indicate that selective mechanisms favour n-3 HUFAs at reduced ambient temperature. Deposition of fatty acids in muscle, viscera and 'carcass' were markedly influenced by dietary treatment, but non-polar (storage) lipids were more influenced by the diet than the polar lipids. This indicates stronger regulation of the composition polar lipids. Vegetable oils induce higher unsaturation (UFA:SFA ratio) of polar lipids than fish oils. This may imply that vegetable oils produce fish that are better able to withstand exposure to low temperature, while having membrane lipids less susceptible to oxidative damage, due to the lower contents of n-3 HUFAs.

**Aim 3:** Parr-smolt transformation in Atlantic salmon is resistant to manipulation of dietary lipid composition. Seawater acclimation and on-growing of smolts are improved when the fish are fed a diet containing vegetable oil during freshwater rearing, but an increase in dietary n-3 HUFA and/or energy content upon seawater entry seems to benefit seawater growth. This may indicate that changes in lipid metabolism are an integral part of parr-smolt transformation, and that changes in water salinity are an important determinant for lipid requirements in Atlantic salmon.

In summary, vegetable oils can replace marine fish oils entirely in fish meal based feeds for juvenile Atlantic salmon during the freshwater rearing phase without detrimental effects on fish performance, parr-smolt transformation and subsequent on-growing in seawater. This indicates a high tolerance for vegetable oil in juvenile Atlantic salmon even when they are held for prolonged periods at low temperature. Based on the data presented in this thesis, it appears that changes in water salinity or ontogenetic life-history stage is important in



URN:NBN:no-6435

determining dietary lipid requirements in Atlantic salmon, and is of more importance than changes in water temperature.

## 5 LITERATURE


Alanärä, A., 1992. Demand feeding as a self-regulating feeding system for rainbow trout (*Oncorhynchus mykiss*) in net-pens. Aquaculture 108, 347-356.

Arts, M.T., Ackman, R.G., Holub, B.J., 2001. 'Essential fatty acids' in aquatic ecosystems: A crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58, 122-137.

Arzel, J., Cardinal, M., Cornet, J., Metailler, R., Guillaume, J.C., 1994. Effect of dietary lipid on growth performance and body composition of brown trout (*Salmo trutta*) reared in seawater. Aquaculture 123, 361-375.

Austreng, E., Skrede, A., Eldegard, Å., 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agric. Scan. 29, 119-126.

Azevedo, P.A., Cho, C.Y., Leeson, S., Bureau, D.P., 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquat. Living Resour. 11, 227-238.

Bell, J.G., Ghioni, C., Sargent, J.R., 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): A comparison with commercial diets. Aquaculture 128, 301-313.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Smullen, R.P., Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J. Nutr. 132, 222-230.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid composition and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535-1543.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: Current status and future opportunities. Aquaculture 218, 491-499.

Bell, J.G., Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W., Sargent, J.R., 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation. Lipids 32, 515-525.

Bell, J.G., Youngson, A., Mitchell, A.I., Cowey, C.B., 1989. The effects of enhanced intake of linoleic acid on the fatty acid composition of tissue polar lipids of post-smolt Atlantic salmon (*Salmo salar*). Lipids 24, 240-242.




URN:NBN:no-6435

Bendiksen, E.Å., Bergan, M., Nystad, B., Berg, O.K., Arnekleiv, J.V., 2003. Natural winter prey and commercial feeds for juvenile Atlantic salmon differ in fatty acid composition. EAS Special Publication no. 33, p. 123-124.

Berg, O.K., Bremset, G., 1998. Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. J Fish Biol. 52, 1272-1288.

Boeuf, G., 1993. Salmonid smolting: A pre-adaptation to oceanic environment. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 105-133.

Boeuf, G., Payan, P., 2001. How should salinity influence fish growth? Comp. Biochem. Physiol. 130C, 411-423.

Brännäs, E., Wicklund, B.-S., 1992. Low temperature growth potential of Arctic charr and rainbow trout. Nord. J. Freshwater Res. 67, 77-81.

Brett, J.R., 1979. Environmental factors and growth. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.) Fish Physiology, Volume XIII. Bioenergetics and Growth. Academic Press, New York, pp. 599-675.

Bünning, E., 1973. The Physiological Clock. Springer-Verlag, New York. 258 pp.

Clarke, W.C., 2000. Smolting. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. John Wiley & Sons, New York, pp. 879-884.

Clarke, W.C., Shelbourne, J.E., Brett, J.R., 1981. Effect of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho (*Oncorhynchus kisutch*), Chinook (*O. tshawytscha*) and sockeye (*O. nerka*) salmon. Aquaculture 22, 105-116.

Cordiner, M., Brichon, G., Weber, J.-M., Zwingelstein, G., 2002. Changes in the fatty acid composition of phospholipids in tissues of farmed sea bass (*Dicentrarcus labrax*) during an annual cycle. Role of environmental temperature and salinity. Comp. Biochem. Physiol. 133B, 281-288.

Craig, S.R., Neill, W.H., Gatlin, D.M. III., 1995. Effects of dietary lipid and environmental salinity on growth, body composition, and cold tolerance of juvenile red drum (*Sciaenops ocellatus*). Fish Physiol. Biochem. 14, 49–61.

Cunjak, R.A., Power, G., 1987. Winter habitat utilization by stream resident brook trout (*S. fontinalis*) and brown trout (*S. trutta*). Can. J. Fish. Aquat. Sci. 43, 1970-1981.

DeLong, D.C., Halver, J.E., Mertz, E.T., 1958. Nutrition of salmonid fishes. VI. Protein requirements of chinook salmon at two water temperatures. J. Nutr. 65, 589-599.

Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Corraze, G., Bautista, J.M., Kaushik, S.J., 1998. Regulation of hepatic lipogenesis by dietary protein/energy ratio in juvenile European seabass (*Dicentrarcus labrax*). Aquaculture 161, 169-186.



URN:NBN:no-6435

Dosanjh, B.S., Higgs, D.A., McKenzie, D.J., Randall, D.J., Eales, J.G., Roeshandeli, N., 1998. Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal status of Atlantic salmon (*Salmo salar*) in sea water. Fish Physiol. Biochem. 19, 123-134.

Einen, O., Roem, A.J., 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: Growth, feed utilization and slaughter quality. Aquacult. Nutr. 3, 115-126.

Elliott, J.M., 1982. The effects of temperature and ration size on the growth and energetics of the salmonids in captivity. Comp. Biochem. Physiol. 73B, 81-91.

Falcón, J., Collin, J.-P., 1989. Photoreceptors in the pineal gland. Functional aspects. Experientia (Basel) 45, 909-913.

Farkas, T., Fodor, E., Kitajka, K., Halver, J.E., 2001. Response of fish membranes to environmental temperature. Aquacult. Res. 32, 645-655.

Fauconneau, B., Choubert, G., Blanc, D., Breque, J., Luquet, P., 1983. Influence of environmental temperature on flow rate of food stuffs through the gastrointestinal tract of rainbow trout. Aquaculture 34, 27-39.

Fiskeridirektoratet, 2003. Statistikk oppdrett 2002. Fiskeridirektoratet, Bergen, Norway, 30 pp.

Florant, G.L., Hester, L., Ameenuddin, S., Rintoul, D.A., 1993. The effect of a low essential fatty acid diet on hibernation in marmots. Am. J. Physiol. 264, R747-R753.

Fodor, E., Jones, R.H., Buda, C., Kitajka, K., Dey, I. , Farkas, T., 1995. Molecular architecture and biophysical properties of phospholipids during thermal adaptation in fish: An experimental and model study. Lipids 30, 1119-1126.

Fraser, N.H.C., Metcalfe, N.B., Thorpe, J.E., 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. London, Ser B 252, 135-139.

Ghioni, C., Bell, J.G., Sargent, J.R., 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. Comp. Biochem. Physiol. 114B, 161-170.

Goedkoop, W., Sonesten, L., Ahlgren, G., Boberg, M., 2000. Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: Seasonal variation and throphic indications. Can. J. Fish. Aquat. Sci. 57, 2267-2279.

Greene, D.H.S. & Selivonchick, D.P., 1990. Effects of dietary vegetable, animal and marine lipids on muscle lipid and hematology of rainbow trout (*Oncorhynchus mykiss*) Aquaculture 89, 165-182.



URN:NBN:no-6435

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.G., Standal, H., Røsjø, C., 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquaculture 207, 311-329.

Guillou, A., Soucy, P., Khahil, M., Adambounou, L., 1995. Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook trout (*Salvelinus fontinalis*). Aquaculture 136, 351-362.

Gunstone, F.D., Harwood, J.L., Padley, F.B., 1994. The Lipid Handbook (second edition), Chapman & Hall, London, England. 1273 pp.

Hanson, B.J., Cummins, K.W., Cargill, A.S., Lowry, R.R., 1985. Lipid content, fatty acid composition and the effect of diet on fats of aquatic insects. Comp. Biochem. Physiol. 80B, 257-276.

Hardewig, I., van Dijk, P.L.M., 2003. Is digestive capacity limiting growth at low temperatures in roach? J. Fish Biol. 62, 358-374.

Hazel, J.R., 1979. The influence of thermal acclimation on membrane lipid composition of rainbow trout liver. Am. J. Physiol. 236, R91-R101.

Hazel, J.R., 1984. Effects of temperature on the structure and metabolism of cell membranes in fish. Am. J. Physiol. 246, R460-R470.

Hazel, J.R., Williams, E.E., 1990. The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog. Lip. Res. 29, 167-227.

Heggenes, J., Krog, O.M.W., Lindås, O.R., Dokk, J.G., Bremnes, T., 1993. Homeostatic behavioural responses in a changing environment: Brown trout (*Salmo trutta*) become nocturnal during winter. J. Animal Ecol. 62, 295-308.

Helland, S.J., Grisdale-Helland, B., 1998. The influence of replacing fish meal in the diet with fish oil on growth, feed utilization, and body composition of Atlantic salmon (*Salmo salar*) during the smoltification period. Aquaculture 162, 1-10.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture 139, 157-163.

Henderson, R.J., Sargent, J.R., 1985. Chain length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in livers of rainbow trout. Comp. Biochem. Physiol. 82B, 79–85.

Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lip. Res. 26, 281-347.



URN:NBN:no-6435

Higgs, D.A., Dong, F.M., 2000. Lipids and fatty acids. In: Stickney, R.R. (Ed.) Encyclopedia of Aquaculture. John Wiley and Sons, New York, USA, pp. 476–496.

Higgs, D.A., Dosanjh, B.S., Plotnikoff, J.R., Markert, D., Lawseth, J.R., McBride, J.R., Buckley, J.T., 1992. Influence of dietary protein to lipid ratio and lipid composition on the performance and marine survival of hatchery reared Chinook salmon (*Oncorhynchus tshawytscha*). Bull. Aquacul. Assoc. Canada 92, 46-48.

Higgs, D.A., MacDonald, J.S., Levings, C.D., Dosanjh, B.S., 1995. Nutrition and feeding habits in relation to life history stage. In: Groot, C., Margolis, L., Clarke, W.C. (Eds.), Physiological Ecology of Pacific Salmon. UBC Press, Vancouver, Canada, pp. 161-315.

Hillestad, M., Johnsen, F., Austreng, E., Åsgård, T., 1998. Long-term effects of dietary fat level and feeding rate on growth, feed utilization and carcass quality of Atlantic salmon. Aquacult. Nutr. 4, 89-97.

Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation. Oxford University Press, Oxford, England. 466 pp.

Hsieh, S.L., Chen, Y.N., Kuo, C.M., 2003. Physiological responses, desaturase activity, and fatty acid composition in milkfish (*Chanos chanos*) under cold acclimation. Aquaculture 220, 903-918.

Ingemansson, T., Olsson, N.U., Kaufmann, P., 1993. Lipid composition of light and dark muscle of rainbow trout (*Oncorhynchus mykiss*) after thermal acclimation: A multivariate approach. Aquaculture 113, 153-165.

Jobling, M., 1980. Gastric evacuation in plaice, *Pleuronectes Platessa* L.: Effects of dietary energy level and food consumption. J. Fish Biol. 19, 187-196.

Jobling, M., 1994. Fish Bioenergetics. Chapman & Hall, London, England. 309 pp.

Jobling, M., 2003. The thermal growth coefficient (TGC) model of fish growth: A cautionary note. Aquac. Res. 34, 581-584.

Jobling, M., Gomes, E., Dias, J., 2001. Feed types, manufacture and ingredients. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.) Food Intake in Fish. Blackwell Science, Oxford, England, pp. 25-48.

Jobling, M., Koskela, J., Pirhonen, J., 1998. Feeding time, feed intake and growth of Baltic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, reared in monoculture and duoculture at constant low temperature. Aquaculture 163, 73-84.

Jobling, M., Miglavs, I. 1993. The size of lipid depots – a factor contributing to the control of food intake in Arctic charr, *Salvelinus alpinus*? J. Fish Biol. 43, 487-489.



URN:NBN:no-6435

Johnsen, R.I., Grahl-Nielsen, O., Roem, A., 2000. Relative absoprtion of fatty acids by Atlantic salmon from different diets, as evaulated by multivariate statistics. Aquacult. Nutr. 6, 255-261.

Johnson, R.M., Johnson, T.M., Londraville, R.L., 2000. Evidence for leptin expression in fishes. J. Exp. Biology 286, 718-724.

Jonsson, N., Jonsson, B., 1997. Energy allocation in polymorphic brown trout. Func. Ecol. 11, 310-317.

Kaushik, S.J., Oliva-Teles, A., 1985. Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture 50, 89-101.

Kennedy, G.C., 1953. The role of depot fat in hypothalamic control of food intake in the rat. Proc. R. Soc. London, Ser B 140, 578-592.

Kestemont, P., Baras, E., 2001. Environmental factors and feed intake: Mechanisms and interactions. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.) Food Intake in Fish. Blackwell Science, Oxford, England, pp. 131-156.

Kiessling, K.-H., Kiessling, A., 1993. Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. Can. J. Zool. 71, 248-251.

Klemetsen, A., Amundsen, P.-A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E., 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): A review of aspects of their life histories. Ecol. Freshwater Fish 12, 1-59.

Koskela, J., Pirhonen, J., Jobling, M., 1997a. Growth and feeding responses of a hatchery population of brown trout (*Salmo trutta* L.) at low temperatures. Ecol. Freshwater Fish 6, 116-121.

Koskela, J., Pirhonen, J., Jobling, M., 1997b. Effect of low temperature on feed intake, growth rate and body composition of juvenile Baltic salmon. Aquacult. Int. 5, 479-488.

Koskela, J., Pirhonen, J., Jobling, M., 1997c. Variations in feed intake and growth of Baltic salmon and brown trout exposed to continuous light at constant low temperature. J. Fish. Biology 50, 837-845.

Koumourdjian, M.P., Fenwick, J.C., Saunders, R.L., 1976. Evidence for the role of growth hormone as a part of the 'light-pituitary' axis' in growth and smoltification of Atlantic salmon (*Salmo salar*). Can J. Zool. 54, 544-551.

Koven, W.M., Henderson, R.J., Sargent, J.R., 1994. Lipid digestion in turbot (*Scophthalmus maximus*). I: Lipid class and fatty acid composition of digesta from different segments of the digestive tract. Fish. Physiol. Biochem. 13, 69-79.



URN:NBN:no-6435

Lee, D.J., Putman, G.B., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr. 103, 34-39.

Ling, N., Cotter, D., 2003. Statistical power in comparative aquaculture studies. Aquaculture 224, 159-168.

Liu, Y., Merrow, M., Loros, J.L., Dunlap, J.C., 1998. How temperature changes reset a circadian oscillator. Science 281, 825-829.

Logue, J.A., DeVries, A.L., Fodor, E., Cossins, A.R., 2000. Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure. J. Exp. Biol. 203, 2105-2115.

MacCrimmon, H.R., Gots, B.L., 1979. World distribution of Atlantic salmon, *Salmo salar*. J. Fish. Res. Board Can. 36, 422-457.

Makutova, O.N., Kalachova, G.S., Gladyshev, M.I., 2003. A comparison of the fatty acid composition of *Gammarus lacustris* and its food sources from a freshwater reservoir, Bugach, and the saline Lake Shira in Siberia, Russia. Aquatic Ecology 37, 159-167.

Max, M., Menaker, M., 1992. Regulation of melatonin production by light, darkness, and temperature in the trout pineal. J. Comp. Physiol. 170A, 479-489.

Metcalfe, N.B., Thorpe, J.E., 1992. Anorexia and defended energy levels in over-wintering juvenile salmon. J. Animal Ecology 61, 175-181.

Millikin, M.R., 1982. Effects of dietary protein concentration on growth, feed efficiency, and body composition of age-0 striped bass. Trans. Am. Fish. Soc. 111, 373-378.

Mustafa, T., Srivastava, K.C., 1989. Prostaglandines (eicosanoids) and their role in ectothermic organisms. Adv. Comp. Env. Physiol. 5, 157-207.

National Research Council (NRC), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, USA. 114 pp.

Nordgarden, U., Hemre, G.-I., Hansen, T., 2002. Growth and body composition of Atlantic salmon (*Salmo salar* L.) parr and smolt fed diets varying in protein and lipid contents. Aquaculture 207, 65-78.

Olsen, R.E., Ringø, E., 1998. The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. Aquacult. Res. 29, 695-701.

Opsahl-Ferstad, H.-G., Rudi, H., Ruyter, B., Refstie, S., 2003. Biotechnological approaches to modify rapeseed oil composition for applications in aquaculture. Plant Science 165, 349-357.

Polvi, S.M., Ackman, R.G., 1992. Atlantic salmon (*Salmo salar*) muscle lipids and their response to alternative dietary fatty acid sources. J. Agric. Food Chem. 40, 1001-1007.



URN:NBN:no-6435

Rasmussen, R.S., Ostenfeld, T.H., 2000. Effect of growth rate on quality traits and feed utilisation of rainbow trout (*Oncorhynchus myksiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture 184, 327-337.

Regost, C., Arzel, J., Cardinal, M., Laroche, M., Kaushik, S.J., 2001. Fat deposition and flesh quality in seawater reared, triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. Aquaculture 193, 325-345.

Ruohonen, K., Kettunen, J., King, J., 2001. Experimental design in feeding experiments. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.) Food Intake in Fish. Blackwell Science, Oxford, England, pp. 88-107.

Ruyter, B., 1998. Fatty acid metabolism in Atlantic salmon. A focus on essential fatty acids. Doctorial thesis at University of Oslo, Norway. 154 pp.

Ruyter, B., Røsjø, C., Einen, O., Thomassen, M.S., 2000. Essential fatty acids in Atlantic salmon: Effects of increasing dietary doses of n-6 and n-3 fatty acids on growth, survival and fatty acid composition of liver, blood and carcass. Aquacult. Nutr. 6, 119-127.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191-199.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J., Tocher, D.R., 1995. Requirement criteria for essential fatty acids. J. Appl. Ichthyol. 11, 183-198.

Sargent, J.R., Tocher, D.R., Bell, J.G. 2002. In: Halver, J.E., Hardy, R.W., Fish Nutrition (Third edition). Academic Press, San Diego, USA, pp. 181-257.

Searchy-Bernal, R., 1994. Statistical power and aquaculture research. Aquaculture 127, 371-388.

Seiliez, I., Panserat, S., Corraze, G., Kaushik, S., Bergot, P., 2002. Cloning and nutritional regulation of a partial Δ6-desaturase-like in gilthead sea beam (*Sparus aurata*). Abstract book 10$^{th}$ International Symposium on Nutrition & Feeding in Fish, Rhodes, Greece, p.67.

Shearer, K.D., 2000a. Experimental design, statistical analysis and modeling of dietary requirement studies for fish: A critical review. Aquacult. Nutr. 6, 91-102.

Shearer, K.D., 2000b. The effect of diet composition and feeding regime on the proximate composition of farmed fish. In: Kestin, S.C., Warriss, P.D. (Eds.) Farmed Fish Quality, (Cap. 4). Fishing News Books, Blackwell Science, Oxford, England, p. 31- 41.

Shearer, K.D., Silverstein, J.T., Dickhoff, W.W., 1997. Control of growth and adiposity of juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 157, 311-323.

Sidell, B.D., Crockett, E.L., Driedzic, W.R., 1995. Antarctic fish tissues preferentially catabolize monoenoic fatty acids. J. Exp. Biology 271, 73-81.



URN:NBN:no-6435

Simandle, E.T., Espinoza, R.E., Nussear, K.E., Tracy, C.R., 2001. Lizards, lipids, and dietary links to animal function. Physiol. Biochem. Zool. 74, 625-640.

Sinensky, M., 1974. Homeoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 71, 522-525.

Skuladottir, G.V., Schiöth, H.B., Gudmundsdottir, E., Richards, B., Gardarsson, F., Jonsson, L., 1990. Fatty acid composition of muscle, heart and liver lipids in Atlantic salmon, *Salmo salar*, at extremely low environmental temperature. Aquaculture 84, 71-80.

Smart, T.S., Riley, J., Edwards, P., 1998. Statistical aspects of aquaculture research: Sample sizes for pond experiments. Aquac. Res. 29, 373-379.

Steffens, W., 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture 151, 97-119.

Sæther, B.-S, Jobling, M., 2001. Fat content in turbot feed: Influence on feed intake, growth and body composition. Aquac. Res. 32, 451-458.

Takeuchi, T., Watanabe, T., 1979. Effects of excess amounts of essential fatty acids on growth of rainbow trout. Bull. Jap. Soc. Scient. Fish. 45, 1745-1752.

Thomassen, M.S., Røsjø, C., 1989. Different fats in feed for salmon: Influence on sensory parameters, growth rate and fatty acids in muscle and heart. Aquaculture 79, 129-135.

Tiku, P.E., Gracey, A.Y., Macartney, A.I., Beynon, R.J., Cossins, A.R., 1996. Cold-induced expression of $\Delta^9$-desaturase in carp by transcriptional and posttranslational mechanisms. Science 271, 815-818.

Tocher, D.R., Bell, J.R., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D., Morris, P.C., 2000. Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr-smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59-73.

Tocher, D.R., Bell, J.G., MacGlaughlin, P., McGhee, F., Dick, J.R., 2001. Hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition of the liver in salmonids: Effects of dietary vegetable oil. Comp. Biochem. Physiol. 130B, 257-270.

Torstensen, B.E., Lie, Ø., Frøyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.) – effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35, 653-664.

Trueman, R.J., Tiku, P.E., Caddick, M.X., Cossins, A.R., 2000. Thermal thresholds of lipid restructuring and $\Delta^9$-desaturase expression in the liver of carp (*Cyprinus carpio* L.). J. Exp. Biol. 203, 641-650.

Vegusdal, A., Sundvold, H., Gjøen, T., Ruyter, B., 2003. An in vitro method for studying the proliferation and differentiation of Atlantic salmon preadipocytes. Lipids 38, 289-296.



URN:NBN:no-6435

Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996a. Digestible energy: methodological influences and the mode of calculation. Fisheries Sci. 62, 288-292.

Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996b. Digestible crude protein contents of various feedstuffs determined with four freshwater fish species. Fisheries Sci. 62, 278-282.

Weigle, D.S., 1994. Appetite and the regulation of body composition. FASEB J. 8, 302-310.

West, J.L., Driedzic, W.R., 1999. Mitochondrial protein synthesis in rainbow trout (*Oncorhynchus mykiss*) heart is enhanced in sexually mature males but impaired by low temperature. J. Exp. Biol. 202, 2359-2369.

Williams, E.E., 1998. Membrane lipids: What membrane physical properties conserved during physiochemically-induced membrane restructuring? Amer. Zool. 38, 280-290.

Wilson, R.P., 2002. Amino acids and protein. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition (Third edition). Academic Press, San Diego, USA, pp. 143-179.

Woods, S.C., Seeley, R.J., 2000. Adiposity signals and the control of energy homeostasis. Nutrition 16, 894-902.

Wootton, R.J., 1998. Ecology of Teleost Fishes (Second edition). Kluwer Academic Publishers Fish and Fisheries Series 24, Dordrecht, The Netherlands. 386 pp.

Yamamoto, T., Shima, T., Unuma, T., Shiraishi, M., Akiyama, T., Tabata, M., 2000. Voluntary intake of diets with varying digestible energy contents and energy sources, by juvenile rainbow trout *Oncorhynchus mykiss,* using self-feeders. Fish. Sci. 66, 528-534.

Zachmann, A., Ali, M.A., Falcón, J., 1992. Melatonin and its effects in fishes: An overview. In: Ali, M.A. (Ed.) Rhythms in Fishes. NATO-ASI series A vol. 236, Plenum Press, New York, USA, pp. 149-165.

Zhang, J., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J., 1994. Positional cloning of the mouse obese gene and its human homolog. Nature 372, 425-432.

Zolman, J.F., 1993. Analysis of variance (ANOVA). In: Zolman, J.F. (Ed.) Biostatistics. Experimental design and statistical interference (Cap. 6), Oxford University Press, Oxford, p. 101-130.



URN:NBN:no-6435

# Paper I

URN:NBN:no-6435

URN:NBN:no-6435

Bendiksen, E. Å, Jobling, M. & Arnesen, A. M., 2002. Feed intake of Atlantic salmon parr *Salmo salar L.* in relation to temperature and feed composition. Aquaculture Research 33: 525-532.

Paper not included due to copyright restrictions.

# Paper II

URN:NBN:no-6435

URN:NBN:no-6435

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Aquaculture 224 (2003) 283–299

Aquaculture

www.elsevier.com/locate/aqua-online

# Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source

E.Å. Bendiksen[a,b,*], O.K. Berg[a], M. Jobling[c,d], A.M. Arnesen[c], K. Måsøval[b]

[a] *Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim N-7491, Norway*
[b] *BioMar AS, Kjøpmannsgata 50, Trondheim N-7484, Norway*
[c] *Norwegian Institute of Fisheries and Aquaculture Research, Tromsø N-9291, Norway*
[d] *NFH, University of Tromsø, Tromsø N-9037, Norway*

Received 3 December 2002; received in revised form 10 March 2003; accepted 10 March 2003

**Abstract**

An experiment was conducted to investigate the effects of temperature, feed fat content and dietary oil source on growth and nutrient utilisation of Atlantic salmon parr ( ~ 19 g). The fish were reared in freshwater at 2 or 8 °C for 6 months at light/dark cycles of 12 h:12 h. Each of four feeds was provided to triplicate groups of fish at each temperature. The feeds were formulated with marine fish oil or a blend of rapeseed and linseed oil at low or high inclusion levels to give feeds with 340 g $kg^{-1}$ fat and 400 g $kg^{-1}$ protein or 210 g $kg^{-1}$ fat and 500 g $kg^{-1}$ protein. Fish weights doubled over the 6 months at the lower temperature, whereas a fivefold increase was seen over the same period at the higher temperature. At the lower temperature, growth was similar for fish in all four dietary groups (SGR; $0.40 \pm 0.01\%$ $day^{-1}$), whereas significantly better growth was found for fish fed the low fat feeds at the higher temperature (SGR; $0.99 \pm 0.01\%$ vs. $0.93 \pm 0.01\%$ $day^{-1}$). Feed efficiencies were higher for fish at the lower temperature. Apparent fat and protein digestibilities were high at both temperatures, but fat digestibility was significantly lower at the lower temperature ($ADC_{fat}$; $96.3 \pm 0.5\%$ vs. $98.2 \pm 0.4\%$). Fat digestibility was higher for the high fat feeds, but significant differences between the groups were found only at the lower temperature. Protein digestibility was also lower at the lower temperature ($ADC_{protein}$; $90.8 \pm 0.4\%$ vs. $91.2 \pm 0.4\%$), and was significantly improved when vegetable oils were used in the feed. Protein retention efficiency (PRE: [g protein gain g protein ingested$^{-1}$] $\times$ 100) was significantly higher at 8 °C than at 2 °C (PRE; $52 \pm 1$ vs. $49 \pm 2$), and high feed fat content improved protein retention. Energy retention

* Corresponding author. BioMar AS, Kjøpmannsgata 50, Trondheim N-7484, Norway. Tel.: +47-7387-1116; fax: +47-7387-1119.
*E-mail address:* eldar.bendiksen@biomar.no (E.Å. Bendiksen).

0044-8486/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0044-8486(03)00218-7

URN:NBN:no-6435

(ERE: [kJ gain kJ ingested$^{-1}$] × 100) tended to be higher for fish at the lower temperature (ERE; 55 ± 2 vs. 50 ± 1). Energy retention was also significantly higher for fish fed the high fat feeds. There was no evidence that vegetable oils were inferior to marine fish oils at either temperature, and at low temperature vegetable oil enhanced protein digestibility.
© 2003 Elsevier Science B.V. All rights reserved.

*Keywords:* Low temperature; Salmonids; Digestibility; Nutrient retention; Vegetable oil; Winter performance

## 1. Introduction

Salmonid species of interest for commercial culture are coldwater stenotherms. They have growth optima at 12–17 °C (Brett, 1971; Elliott, 1976; Farmer et al., 1983; Koskela et al., 1997a; Kestemont and Baras, 2001), and maintain feeding and growth at temperatures approaching 0 °C (Brännäs and Wicklund, 1992; Fraser et al., 1993; Heggenes et al., 1993; Koskela et al., 1997b). Although salmonid farming is mostly conducted at high latitudes, where light and temperature change markedly throughout the year, information about nutrition–environment interactions is scarce. There are some indications that lipid oxidation capacity is enhanced during cold acclimatization in rainbow trout (Cordiner and Egginton, 1997; Thibault et al., 1997), and increased feed fat may give a protein-sparing effect (Lee and Putman, 1973; Medland and Beamish, 1985; Cho and Kaushik, 1990; Einen and Roem, 1997; Hillestad et al., 1998). Several authors have examined how fats of marine and terrestrial origin influence the performance of salmonids (e.g. Hardy et al., 1987; Skonberg et al., 1993; Torstensen et al., 2000; Bell et al., 2001; Grisdale-Helland et al., 2002; Jobling et al., 2002), but most studies have been carried out at moderate-to-high water temperatures. Cell membrane fatty acid compositions change when ectotherms are exposed to low temperature (Hazel, 1984; Wallaert and Babin, 1994; Fodor et al., 1995; Farkas et al., 2001). As such, winter performance might be affected when high levels of vegetable oils are included in feeds for salmonids that are farmed at high latitudes, due primarily to the low $n-3$ highly unsaturated fatty acid (HUFA) concentrations in these oils. Consequently, both qualitative and quantitative aspects of lipid nutrition may be of importance when rearing of Atlantic salmon under winter conditions.

The influence of feed fat content and fatty acid composition on growth of Atlantic salmon parr was examined in a feeding trial that incorporated investigations of feed intake, nutrient digestibility and nutrient partitioning in fish held at 2 and 8 °C. A full-factorial design was used to investigate whether the responses to feed treatments differed between temperatures.

## 2. Materials and methods

Four dry extruded feeds (2.5 mm diameter) were produced at BioMar Technology Center, Brande, Denmark. Sand eel (*Ammodytes* spp.) oil or a blend of rapeseed (*Brassica* sp.) oil and linseed (*Linum* sp.) oil (ratio 7:3 by weight) were used as fat sources. The vegetable oils were neutralised, bleached and de-odorised oils. Fish meal and wheat were the other main

URN:NBN:no-6435

feed ingredients, and an inert marker ($Y_2O_3$, 0.01%) was added to all feeds for nutrient digestibility assessment (Table 1). The feeds were designated LFFO, LFVO, HFFO and HFVO according to fat level (LF—low fat; HF—high fat) and oil source (FO—fish oil; VO—vegetable oil). The feeds were bagged in 25-kg bags and stored in the dark at − 22 °C.

Feed dry matter contents were determined by drying at 105 °C for 24 h, crude protein contents were estimated by Kjeldahl analyses (nitrogen × 6.25, Kjeltec Autoanalyser, Tecator, Sweden), crude fat was estimated on acid hydrolysed samples (3 M HCl) using the Soxhlet method with petroleum ether extraction, and ash was determined by combustion at 550 °C for 16 h. Feed energy content was determined by bomb calorimetry (Parr adiabatic bomb calorimeter). Lipids were also extracted using chloroform/methanol/water (Bligh and Dyer, 1959) and methyl esters were prepared according to the method described by Metcalfe et al. (1966). Methyl esters, extracted in isooctane, were separated by gas chromatography using a Perkin Elmer Auto System XL gas chromatograph equipped with a split/splitless injector fitted to a fused silica capillary column (CP Wax 52CB, Chrompak, 25 m × 0.25 mm i.d.) and a flame-ionisation detector. Helium was used as the mobile phase. Temperature was increased at 30 °C/min from 90 to 150 °C, and thereafter at 3 °C/min to 225 °C; the total running time was 35 min. Injector and detector temperatures were set at 250 and 280 °C, respectively. The fatty acids were identified

Table 1
Feed ingredients and analysed compositions of test feeds

| | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| *Ingredients, g kg$^{-1}$* | | | | |
| Fish meal[a] | 638 | 638 | 486 | 486 |
| Wheat | 190 | 190 | 178 | 178 |
| Sandeel oil | 140 | | 270 | |
| Rapeseed oil[b] | | 104 | | 200 |
| Linseed oil[b] | | 36 | | 70 |
| Monosodium phosphate | 10 | 10 | 25 | 25 |
| Vitamin and mineral premixes | 12 | 12 | 12 | 12 |
| Fat absorber[c] | 10 | 10 | 30 | 30 |
| Yttrium oxide | 0.1 | 0.1 | 0.1 | 0.1 |
| *Analysed composition* | | | | |
| Dry matter (%) | 94.5 | 94.1 | 96.4 | 96.3 |
| Crude protein (N × 6.25)(%) | 50.2 | 50.4 | 40.3 | 40.2 |
| Crude fat (%) | 20.7 | 21.4 | 33.5 | 33.9 |
| Ash (%) | 9.1 | 9.3 | 10.3 | 10.4 |
| Residue (%) | 14.5 | 13 | 12.3 | 11.7 |
| Σ Saturated fatty acids, g kg$^{-1}$ | 37.7 | 21.4 | 67.0 | 29.8 |
| Σ Monoenic fatty acids, g kg$^{-1}$ | 75.8 | 90.7 | 135.3 | 153.2 |
| Σ PUFAs, g kg$^{-1}$ | 62.5 | 71.9 | 103.4 | 119.6 |
| Gross energy, MJ kg$^{-1}$ | 22.5 | 22.5 | 24.8 | 24.5 |
| Calculated P/E ratio | 22.3 | 22.4 | 16.3 | 16.4 |

Feed codes are as follows: LF, low fat; HF, high fat; FO, fish oil; VO, vegetable oil.

[a] Ultra Flash fish meal purchased from Fiskernes Fiskeindustri A.M.B.A., Denmark.

[b] The vegetable oils were purchased from Superfos Agro, Denmark.

[c] Diatomaceous earth purchased from Damolin, Denmark.

URN:NBN:no-6435

using Turbochrom software by reference to fatty acid ester standards (68D, Nu-Chek-Prep., Minnesota, USA). Sums of fatty acid esters in crude fat were determined by adding a C17:0 standard to the crude fat, followed by extraction and analysis of methyl esters. Concentrations of fatty acids in feeds were estimated by combining information about the proportion of fatty acids in extracted fat (85–91%), with that of corresponding fat contents (Table 1).

The 6-month feeding trial was conducted at the Aquaculture Research Station, Kårvika, Tromsø, Norway (70°N), from November 1999 to May 2000. Atlantic salmon (*Salmo salar* L.) alevins of the AquaGen strain (Aqua Gen, Kyrksæterøra, Norway) were first fed in mid-February 1999 under continuous light and at a water temperature of ∼ 12 °C, and from mid-March until August water temperature was ambient. During August–October, the photoperiod and water temperature were gradually reduced to simulate the onset of winter, and in the second week of October the photoperiod was set to 12 h light:12 h dark (‘lights-on’ between 0900 and 2100 h without twilight). On 23 and 24 September, about 1500 fish were tagged (FTF-69, Floy Tag and Manufacturing, Seattle, WA), and in mid-October 160 fish, i.e. 100 untagged fish and 60 tagged fish, were stocked into each of 24 tanks (225 l) supplied with freshwater. Flow rates were 8–10 l $min^{-1}$ and current speeds were 8–10 cm $s^{-1}$ in all tanks. Fish were then held for 4 weeks, during which time water temperature fell to 4–5 °C. The fish were fed a commercial dry pellet feed (Ecostart 2 mm, BioMar AS; declared composition: protein 49%, fat 23%, gross energy 23 kJ $g^{-1}$) prior to the experiment.

On November 10 and 11, the fish were anaesthetised in aerated benzocaine solution (*p*-aminobenzoic acid ethyl ester, 50 mg $l^{-1}$) and weighed individually to the nearest 0.5 g. Groups of 150 fish (19.3 g ( ± 4.3 g); overall mean ± S.D.) were established to give stocking densities of 11–12 kg $m^{-3}$. Water temperature was adjusted to 8 °C in half of the tanks and to 2 °C in the remaining 12 tanks. Stable temperatures were maintained throughout the study by mixing the stock supply with heated or chilled water. Dissolved oxygen (11.9 ± 1.5 mg $l^{-1}$; overall mean ± S.D.) was measured regularly in outlet water from each tank, and never fell below 8.4 mg $l^{-1}$.

Provision of the test feeds commenced 1 day after initial weighing, and each feed was given to triplicate groups of fish at each temperature for 6 months (176 days) as described by Bendiksen et al. (2002). Uneaten feed was collected in a feed waste collector and feed intake was estimated on dry matter basis (Bendiksen et al., 2002). Fish were deprived of feed for 48 h prior to weight and length measurement after approximately 2 months (62 days) and 4 months (114 days). Fish in tanks within the same temperature treatment were weighed on the same day, and all tanks on two subsequent days. During weighing, 20 fish from each tank (30 fish from the initial population) were killed with a sharp blow to the head and sampled for body composition analysis. Samples of three body compartments (muscle, viscera and carcass) from 10 untagged fish were taken, while 10 tagged fish were frozen for additional analyses and back-up. Each fish was dissected, the viscera removed, and any feed remains removed from the gut. The muscle sample was obtained as de-skinned fillets, and the carcass sample comprised the remaining head, skin, fins and bones, and included the kidney. Each body compartment was weighed, and a pooled sample of each compartment was then prepared from the fish in each tank. Condition factor $[K=(WL^{-3})\times 100]$ and visceral-somatic index (VSI=[visceral mass $W^{-1}]\times 100$) were

URN:NBN:no-6435

calculated, where $W$ is the body mass in grams and $L$ is fork length in cm, respectively. The tissue samples were minced, transferred to brown glass vials and flushed with nitrogen to limit oxidation, and the samples were then kept frozen at $-22$ °C until analysed for proximate composition.

At the sampling undertaken after 6 months, faecal samples were collected by stripping (Austreng, 1978), and the faecal material was frozen at $-22$ °C. After an additional week of feeding, the collection of faeces was repeated. Faeces sampled from fish in the same tank were pooled to yield sufficient material for chemical analysis. One faeces sample from the LFFO fish held at the higher temperature was lost during storage, giving only two replicates for this treatment.

At the time of analysis, the tissue homogenates were placed in a refrigerator overnight, reground in a half-thawed condition, and analysed for proximate composition as described by Johansen et al. (2001). Samples (5–10 g) were transferred to pre-weighed aluminium boats and dried at 105 °C for 24 h. Fat was extracted in petroleum ether (40–60 °C, 90 min) using a Behrotest TRS 200 (Behr Labor-technik, Düsseldorf, Germany) fitted with sintered glass extraction thimbles (pores: 40–100 μm). Ash content was determined by combustion (500 °C, 12 h), and protein was estimated by difference. Data for the masses and proximate composition of each compartment were combined to obtain estimates of whole body proximate composition (Jørgensen et al., 1997). Estimates of body energy content were obtained using caloric values of 39.5 and 23.6 kJ $g^{-1}$ for fat and protein, respectively (Blaxter, 1989).

For nutrient digestibility analyses, the faeces and feed samples were freeze-dried at $-40$ °C for 48 h (Heto freezedryer CD13) and then finely ground in a porcelain mortar. Fat determination was performed using supercritical fluid extraction (LECO FA-100, LECO, St. Joseph, MI). Protein (Protein = nitrogen × 6.25) was calculated from nitrogen determined using a nitrogen analyser (LECO FP 2000, LECO, Henderson, NV). Yttrium was quantified using inductively coupled plasma mass spectrometry (ICP MS) as described by Refstie et al. (1997) and the yttrium oxide concentration was subsequently calculated.

Specific growth rates (SGR, % body weight $day^{-1}$) were calculated as $[(\ln W_1 - \ln W_0)/(T-t)] \times 100$, where $W_0$ and $W_1$ are weights in grams at the start and at the end of the growth period, respectively, and $T-t$ is the time in days between weighing (Jobling, 1994).

Feed efficiency ratio (FER, gain $feed^{-1}$) was calculated according to the formula: [(g final biomass + g dead fish) − g initial biomass] × cumulative feed $intake^{-1}$, where cumulative feed intake was determined in grams on a dry matter basis.

Protein retention efficiency (PRE, g protein gain g protein $ingested^{-1}$) was calculated as: PRE (%) = $100 \times [(P_1 W_1 - P_0 W_0)(P_F \times \text{cumulative feed intake})^{-1}]$, where $P_0$ and $P_1$ are the initial and final protein concentrations of the fish, $W_0$ and $W_1$ are the initial and the final fish weights in grams, $P_F$ is the protein concentration of the feed on a dry matter basis, and cumulative feed intake was determined in grams on a dry matter basis.

Energy retention efficiency (ERE, kJ gain kJ $ingested^{-1}$) was calculated as: ERE (%) = $100 \times [(GE_1 W_1 - GE_0 W_0)(GE_F \times \text{cumulative feed intake})^{-1}]$, where $GE_0$ and $GE_1$ are the initial and final gross energy concentrations of the fish, $W_0$ and $W_1$ are the initial and the final fish weights in grams, $GE_F$ is the gross energy of the feed on a dry matter basis, and cumulative feed intake was determined in grams on a dry matter basis.

URN:NBN:no-6435

Apparent digestibility coefficients (ADCs) for fat and protein were calculated from the measurements of the nutrient-to-indicator ratios in the feeds and faeces: ADC (%) = 100 − 100 × [(marker in feed (%)/marker in faeces (%)) × (nutrient in faeces (%)/ nutrient in feed (%))].

Data are presented as the treatment means ± S.E. The data were analysed by the General Linear Model (GLM) procedure in the SPSS for Windows (Version 10.0) statistical package, using tank as the experimental unit in the tests. Initially, the data were analysed by a three-factor ANOVA model with temperature, feed fat content and feed oil source as the three fixed factors investigated, and mean fish weight for each tank included as co-variate. Subsequently, a two-factor ANOVA model within temperature treatment, with feed fat content and oil source as the fixed factors, was investigated. The results of the ANOVAs are presented as the proportion of total variance explained by each of the factors and their interactions, calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total sum of squares. In addition, weight gain and digestibility data were examined with one-way ANOVA followed by Tukey's HSD multiple range test to rank the four feed treatments within each temperature. Levene's test was used to test whether error variances of dependent variables were equal across groups. Data presented as percentages were arcsine-transformed (Zar, 1984) prior to the statistical tests. In addition, individual weights obtained from tagged fish in each tank followed throughout the experiment ($n = 32–42$) were examined using a repeated measure ANOVA model with diet as treatment factor and tank replicate nested within diet at each temperature. Fish that had failed to grow were removed from the data set prior to growth analysis of individual fish. In all tests, statistical significance was set at $P = 0.05$.

## 3. Results

Water temperature remained at target (8.0 ± 0.2 and 2.0 ± 0.2 °C, respectively (overall mean ± S.D.)) throughout the experiment, and six fish died over the 6-month study. The feeds were well accepted by the fish at both temperatures. Feed intake at 2 °C was approximately 20% of that at 8 °C (Table 2), and while fish weights doubled at the lower temperature they increased fivefold at 8 °C (Fig. 1). Water temperature influenced feed intake ($P < 0.001$), and at 8 °C feed intake was significantly higher for the fish fed the low fat feeds than for those fed the high fat feeds (Tables 2 and 3; $P < 0.001$).

Accordingly, final weights and SGRs were significantly influenced by temperature ($P < 0.001$) and feed fat content ($P < 0.01$), and a significant interaction effect was found between temperature and fat content (Table 3; $P < 0.01$). At the higher temperature, the fish fed the low fat feeds were heavier than those fed the high fat feeds after 2, 4 and 6 months, and by the end of the experiment the mean weight differences between groups fed low fat and high fat feeds were 14.3 g (98.6 ± 1.7 vs. 112.9 ± 2.3 g; tank mean ± S.E.) (Fig. 1; Table 2). The corresponding specific growth rates (SGR) obtained over the total period for the low- and high-fat feeds were 0.99 ± 0.01 and 0.93 ± 0.01% day$^{-1}$ (tank mean ± S.E.) (Table 2). The feeds containing vegetable oil seemed to give higher final weights than the feeds containing fish oil (Fig. 1; Table 2), but this trend was not statistically significant. In

URN:NBN:no-6435

Table 2
Feed intake, growth, feed utilisation and nutrient retention in Atlantic salmon parr fed four diets at two temperatures for 6 months

| | Temperature—8 °C | | | | | | | Temperature—2 °C | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Feed[a] | | | | ANOVA | | | Feed[a] | | | | ANOVA | | |
| | LFFO | LFVO | HFFO | HFVO | Fat | Oil | F × O | LFFO | LFVO | HFFO | HFVO | Fat | Oil | F × O |
| Initial weight, g | 19.8 | 19.7 | 18.9 | 19.6 | | | | 19.1 | 19.3 | 19.0 | 19.0 | | | |
| Feed intake, g DM $fish^{-1}$ | 68.5 | 66.1 | 62.4 | 58.2 | 0.72*** | ns | ns | 13.6 | 14.3 | 12.1 | 13.0 | ns | ns | ns |
| Weight gain, g | 90.7 | 95.7 | 78.4 | 80.3 | 0.72*** | ns | ns | 19.8 | 19.5 | 18.9 | 18.8 | ns | ns | ns |
| Specific growth rate, SGR[b] | 0.98 | 1.01 | 0.93 | 0.92 | 0.64** | ns | ns | 0.41 | 0.40 | 0.39 | 0.39 | ns | ns | ns |
| $ADC_{fat}$ | 98.9 | 97.9 | 99.1 | 98.5 | 0.21* | 0.57** | ns | 95.4 | 94.5 | 97.2 | 98.1 | 0.72*** | ns | ns |
| $ADC_{protein}$ | 91.5 | 91.7 | 90.3 | 91.4 | 0.38** | 0.37** | 0.11* | 90.3 | 91.9 | 89.3 | 91.7 | ns | 0.78*** | ns |
| Feed efficiency ratio, FER[c] | 1.31 | 1.44 | 1.24 | 1.36 | ns | 0.43* | ns | 1.47 | 1.38 | 1.56 | 1.44 | ns | ns | ns |
| Protein efficiency ratio, PRE[d] | 48 | 51 | 51 | 59 | 0.35** | 0.39** | ns | 46 | 42 | 57 | 52 | 0.69** | ns | ns |
| Energy efficiency ratio, ERE[e] | 46 | 50 | 50 | 54 | ns | ns | ns | 54 | 48 | 59 | 58 | 0.66*** | 0.12* | ns |

Results from two-factor analysis of variance (ANOVA) run within each temperature treatment, and with feed fat content (F) and oil source (O) as fixed factors are shown. The proportion of total variance explained by each of the significant factors and their interaction is given, and was calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total of squares. Significance levels are indicated as follows; ns, nonsignificant effect ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data are presented as means ($n = 3$ per treatment).

[a] Feed codes as follows: LF, low fat (21%); HF, high fat (34%); FO, 100% fish oil; VO, 100% vegetable oil. See Table 1 for feed composition.

[b] % $day^{-1}$.

[c] g gain × g DM $feed^{-1}$.

[d] (g prot. gain × g prot. $ingested^{-1}$) × 100.

[e] (kJ gain × kJ $ingested^{-1}$) × 100.

URN:NBN:no-6435

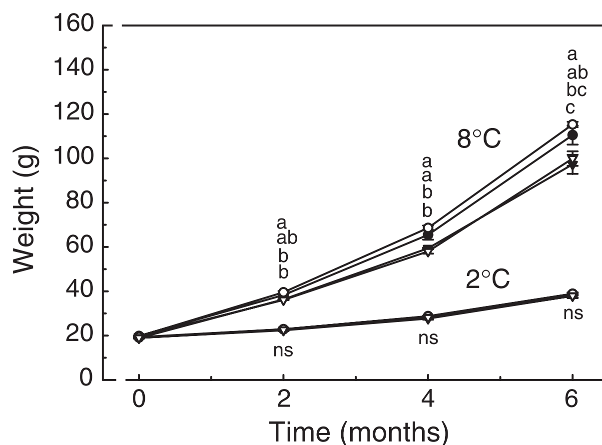


Fig. 1. Growth of salmon parr held at 2 and 8 °C while being fed one of four feeds (see Table 1 for feed composition). Feed codes are as follows; LF, low fat (circles); HF, high fat (triangles); FO, fish oil (filled); VO, vegetable oil (open). Data are presented as mean ± S.E. ($n=3$ per treatment). Different letters indicate significant differences between dietary treatments within sampling times.

accord with this, the repeated measures ANOVA test of individual weights revealed a highly significant effect of feed fat content on weight ($P<0.001$), but failed to reveal any significant effect of feed oil source. Similar final weights (range of tank mean; 37.9–38.9 g) were achieved by fish in all groups at the lower temperature (SGR; 0.40 ± 0.01% day$^{-1}$;

Table 3
ANOVA table showing the effect of temperature, feed fat content and feed oil source, and the interaction effect between the main treatment factors on feed intake, growth and feed utilisation

| | Feed intake, g DM fish$^{-1}$ | Weight gain, g | SGR, % day$^{-1}$ | ADC$_{fat}$, % | ADC$_{protein}$, % | FER, gain feed$^{-1}$ | PRE, G g$^{-1}$ | ERE, kJ kJ$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Weight[a] | | | | ns | 0.06* | 0.22** | ns | 0.22*** |
| Main effects | | | | | | | | |
| Temperature (T) | 0.98*** | 0.97*** | 0.98*** | 0.51*** | 0.06* | 0.23** | 0.04* | ns |
| Fat content (F) | 0.01*** | 0.01*** | < 0.01** | 0.23** | 0.05* | 0.12* | 0.59*** | 0.53*** |
| Oil source (O) | ns | ns | ns | ns | 0.56*** | ns | ns | ns |
| Interaction effects | | | | | | | | |
| T × F | < 0.01** | 0.01*** | < 0.01** | 0.04** | ns | ns | 0.04* | ns |
| T × O | < 0.01* | ns | ns | 0.04* | 0.09** | 0.15** | 0.14** | 0.06* |
| F × O | ns | ns | ns | 0.03* | 0.04* | ns | ns | ns |
| T × F × O | ns | ns | ns | ns | ns | ns | ns | ns |

The proportion of total variance explained by each of the significant factors and their interaction is given, and was calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total of squares. Digestibility data were arcsine transformed prior to analysis. Significance levels are indicated as follows; ns, nonsignificant effect ($P>0.05$); *$P<0.05$; **$P<0.01$; ***$P<0.001$.

[a] Final weight (tank mean) was included as a co-variate in the three-factor ANOVA when the total variation of the dependent variable related significantly to weight (this is indicated by asterisks in the first row).

URN:NBN:no-6435

tank mean ± S.E.), and there were no significant effects of either feed fat content or feed oil source on growth at this temperature (Fig. 1; Table 2).

Both fat and protein digestibilities were high ($ADC_{fat}$; ~ 94–99%, $ADC_{protein}$; ~ 89–93%), and fat digestibility was significantly ($P < 0.001$) lower at the lower temperature ($ADC_{fat}$; 96.3 ± 0.5% vs. 98.2 ± 0.4%; tank mean ± S.E.) (Fig. 2; Tables 2 and 3). Fat digestibility was higher for high fat feeds (Table 2; $P < 0.01$), with the most significant differences being found at the lower temperature. Protein digestibility was significantly ($P < 0.05$) enhanced at the higher temperature ($ADC_{protein}$; 91.2 ± 0.2% vs. 90.8 ± 0.4%; tank mean ± S.E.), and was also influenced by feed fat content ($P < 0.05$) and feed oil

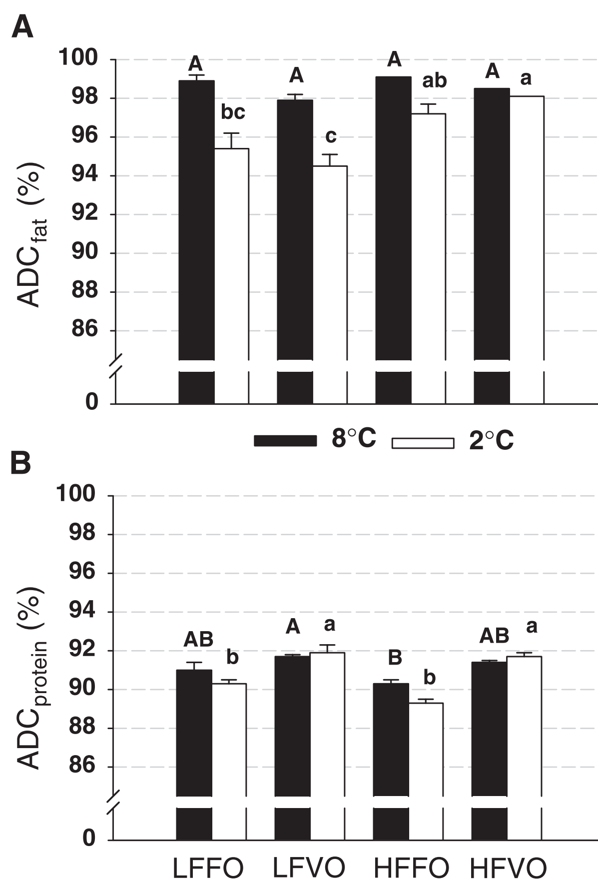


Fig. 2. Apparent digestibility coefficients for fat (A) and protein (B) of salmon parr held at 8 °C (shaded columns) and 2 °C (open columns) while being fed one of four feeds (see Table 1 for feed composition). Feed codes are as follows; LF, low fat; HF, high fat; FO, fish oil; VO, vegetable oil. Data are presented as mean ± S.E. ($n = 3$ per treatment). Different upper and lower case letters indicate significant differences between dietary treatments at 8 and 2 °C, respectively.

URN:NBN:no-6435

Table 4
Body composition (% wet weight), condition factor (*K*-factor) and visceral-somatic index (VSI) of Atlantic salmon parr fed four diets at two temperatures for 6 months

| | | Temperature—8 °C | | | | | | | Temperature—2 °C | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Feeds[a] | | | | ANOVA | | | Feeds[a] | | | | ANOVA | | |
| | Initial | LFFO | LFVO | HFFO | HFVO | Fat | Oil | F × O | LFFO | LFVO | HFFO | HFVO | Fat | Oil | F × O |
| Dry matter, % | 29.4 | 29.6 | 29.3 | 32.9 | 32.5 | 0.91*** | ns | ns | 29.5 | 28.5 | 30.4 | 30.8 | 0.71*** | ns | ns |
| Protein, % | 17.8 | 18.2 | 17.8 | 16.7 | 17.4 | 0.70*** | ns | 0.17** | 16.8 | 16.7 | 16.5 | 16.3 | 0.36* | ns | ns |
| Fat, % | 9.5 | 9.1 | 9.1 | 14.1 | 13.0 | 0.92*** | ns | ns | 10.4 | 9.9 | 12.0 | 12.6 | 0.87*** | ns | ns |
| Ash, % | 2.1 | 2.3 | 2.2 | 2.1 | 2.2 | ns | ns | ns | 2.0 | 2.0 | 2.0 | 1.9 | ns | ns | ns |
| *K*-factor, g $cm^3$ | 1.12 | 1.12 | 1.13 | 1.16 | 1.17 | 0.77*** | ns | ns | 1.21 | 1.21 | 1.24 | 1.25 | 0.62** | ns | ns |
| VSI, % | 8.8 | 6.3 | 6.5 | 8.3 | 8.6 | 0.74*** | ns | ns | 9.3 | 9.6 | 11.1 | 11.1 | 0.70** | ns | ns |

Results from two-factor analysis of variance (ANOVA) run within each temperature treatment, and with feed fat content (F) and oil source (O) as fixed factors are shown. The proportion of total variance explained by each of the significant factors and their interaction is given, and was calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total of squares. Significance levels are indicated as follows; ns, nonsignificant effect ($P > 0.05$); $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Data are presented as means ($n = 3$ per treatment).

[a] Feed codes as follows: LF, low fat (21%); HF, high fat (34%); FO, 100% fish oil; VO, 100% vegetable oil. See Table 1 for feed composition.

URN:NBN:no-6435

source (Tables 2 and 3; $P < 0.001$). The interaction effects (Table 3) relate to protein digestibility being higher when vegetable oils were used in formulating the feeds (Table 2), and this effect was more pronounced at the lower temperature (Fig. 2). Fat digestibility was significantly influenced by feed fat content at the lower temperature ($P < 0.001$), while feed oil source had a significant effect on protein digestibility ($P < 0.001$) at this temperature (Table 2).

Initial and final proximate body compositions of fish exposed to the different temperature and feed treatments are shown in Table 4. Body protein content was significantly influenced by the weight of the fish (Tables 4 and 5; $P < 0.001$). At the end of the experiment, the fish fed the high fat feeds had higher body fat concentrations than did those fed the low fat feeds ($P < 0.001$), and there were several interaction effects (Tables 4 and 5). Protein concentrations were significantly influenced by temperature ($P < 0.01$) and feed fat content (Tables 4 and 5; $P < 0.01$). Protein concentrations were higher in fish raised at the higher temperature, and at 8 °C fish on the low fat feeds tended to have higher concentrations of body protein (Table 4). Ash concentrations were not affected by feed type (Table 4), although an effect of fish weight was found (Table 5; $P < 0.001$). Both $K$ and VSI were significantly influenced by fish weight ($P < 0.001$) and by feed fat content (Tables 4 and 5; $P < 0.001$). $K$ and VSI tended to be higher in the fish given the high fat feeds, and were also higher at 2 °C than at 8 °C (Table 4).

Feed efficiency ratio (FER) was higher in fish reared at 2 °C than in those held at 8 °C (1.46 ± 0.03 vs. 1.34 ± 0.03; tank mean ± S.E.) (Table 2), and FER was also influenced by feed fat content and by temperature and feed oil interactions (Tables 2 and 3). Protein retention efficiency (PRE) was significantly ($P < 0.05$) higher for fish reared at 8 °C than for those held at 2 °C (PRE: 52 ± 1 vs. 49 ± 2; tank mean ± S.E.) (Table 2). In addition, a

Table 5
ANOVA table showing the effect of temperature, feed fat content and feed oil source (three-factor analysis), and the interaction effect between the main treatment factors on proximate body composition

| | Dry matter, % | Protein, % | Fat, % | Ash, % | $K$-factor, g cm$^{-3}$ | VSI, % |
|---|---|---|---|---|---|---|
| Weight[a] | 0.11*** | 0.57*** | 0.01* | 0.53*** | 0.77*** | 0.68*** |
| Main effects | | | | | | |
| Temperature (T) | 0.43*** | 0.07** | 0.47*** | ns | ns | 0.03* |
| Fat content (F) | 0.31*** | 0.12** | 0.36*** | ns | 0.15*** | 0.17*** |
| Oil source (O) | ns | ns | ns | ns | ns | ns |
| Interaction effects | | | | | | |
| T × F | 0.04* | ns | 0.08*** | ns | 0.02* | 0.03* |
| T × O | ns | ns | 0.02** | ns | ns | ns |
| F × O | ns | ns | ns | ns | ns | ns |
| T × F × O | ns | 0.06* | 0.02** | ns | ns | ns |

The proportion of total variance explained by each of the significant factors and their interaction is given, and was calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total of squares. Data on proximate body composition and VSI were arcsine transformed prior to analysis. Significance levels are indicated as follows; ns, nonsignificant effect ($P$>0.05); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[a] Final weight (tank mean) was included as a co-variate in the three-factor ANOVA when the total variation of the dependent variable related significantly to weight (this is indicated by asterisks in the first row).

URN:NBN:no-6435

higher feed fat content improved PRE significantly ($P < 0.001$; Tables 2 and 3), and both temperature and feed fat content and temperature and feed oil source interacted to influence PRE (Table 3). Although not significant, energy retention efficiency (ERE) tended to be higher for the fish reared at the lower temperature (ERE: $55 \pm 2$ vs. $50 \pm 1$; tank mean ± S.E.) (Table 2). ERE was significantly higher for fish fed the high fat feeds (Tables 2 and 3; $P < 0.001$), and there was a significant interaction between temperature and feed oil source that influenced ERE (Table 3).

## 4. Discussion

In ectotherms, low temperature restricts the amount of energy available for anabolic processes by reducing rates of energy intake (Elliott, 1982; Jobling, 1994). Salmonids have evolved within seasonally varying environments, and seasonal cycles in feed intake, growth and energy partitioning may confound the study of temperature effects per se. In the present study, the fish were subjected to decreases in photoperiod and water temperature over the months prior to the start of the experiment to simulate the onset of winter at high latitude. It was hoped that this pre-treatment would allow realistic assessments to be made of the production potential of salmon exposed to low water temperature during winter months.

Feed intake at 2 °C was approximately 20% of that at 8 °C, and growth was much slower at the lower temperature (Table 2; Fig. 1). Consequently, a 4 months longer feeding period was required at 2 °C for fish weights to double from ∼ 20 to ∼ 40 g. In addition to illustrating the rate-limiting effect of lowered temperature on feed intake and growth, the results show that the juvenile Atlantic salmon were able to feed and grow at temperatures close to zero. Our results are in line with previous findings that several salmonid species are capable of maintaining feeding and growth at low temperatures (Brännäs and Wicklund, 1992; Heggenes et al., 1993; Fraser et al., 1993; Koskela et al., 1997a,b; Jobling et al., 1998).

The Atlantic salmon parr had higher FERs at 2 °C than at the higher temperature (Table 2). This differs from findings in several previous investigations on salmonids (Alanärä, 1994; Azevedo et al., 1998; Larsson and Berglund, 1998), but Alanärä (1992) reported a linear decrease in feed efficiency in rainbow trout as temperature increased. Production periods with poor feed utilisation at low temperature during winter have been reported (e.g. Costello et al., 1996; Mørkøre and Rørvik, 2001) but this may be indicative of sub-optimal feeding routines rather than reflecting the effects of water temperature per se. In the present study, the fish were fed excess rations provided continuously throughout the 12-h light period. Whether a continuous feeding regime rather than providing feed in a few large meals is favourable in order to optimise feed utilisation during low temperature periods is uncertain. In accord with improved growth rates at the higher temperature feeds with vegetable oils seemed to improve the FERs at this temperature, while no such effect of oil source was found at the lower temperature.

Growth and efficient feed utilisation (Fig. 1; Table 2) were achieved at both temperatures as a result of high nutrient digestibilities across rearing temperatures and dietary treatments (Fig. 2). Both PRE and ERE were high under all treatment conditions (Table 2), and such high efficiencies could only have been achieved on feeds that had nutrients that

URN:NBN:no-6435

were readily available to the fish. There is some controversy regarding the effects of temperature on nutrient digestibility in salmonids, although most studies have been carried out on rainbow trout. In the rainbow trout, higher protein and energy digestibility has been reported at 15 °C than at 6 °C (Azevedo et al., 1998). Other data also indicate that exposure to reduced temperature may lead to reduced nutrient digestibility in rainbow trout (Watanabe et al., 1996a,b) and Arctic charr (Olsen and Ringø, 1998). However, Cho and Kaushik (1990) and Médale et al. (1991) found no effect of temperature on protein, fat or energy digestibility in the rainbow trout. Data from the present study are in accord with the former results, in that protein and fat digestibilities were significantly reduced at the lower rearing temperature irrespective of feed treatment. Reduced activity of digestive enzymes at low temperature would be expected, but a temperature-induced expression of trypsin isozymes has been reported in Atlantic salmon (Torrissen and Shearer, 1992; Rungruangsak-Torrissen et al., 1998). This may reduce the potential negative impact of low temperature on protein digestion.

Austreng et al. (1979) found that fat digestibility did not differ significantly in rainbow trout reared at 3 and 11 °C, but digestibility was influenced by the degree of hydrogenation of the feed fatty acids. Saturated fatty acids have higher melting points than unsaturated fatty acids of the same chain-length and are less easily digested and absorbed by coldwater fish (Austreng et al., 1979; Cho and Kaushik, 1990; Olsen and Ringø, 1998; Torstensen et al., 2000). In the present study, oil source influenced fat digestibility only at the higher temperature and the fat digestibility was improved for fish fed marine oil based diets. No such effect was found at the lower temperature. However, a higher fat digestibility was found when dietary fat levels were increased at the lower temperature. This could be related to a co-operative effect of dietary fat and temperature on gastric evacuation, which is slowed both with increased dietary fat and decreasing temperature. A slowing of gastric emptying may provide more time for lipase enzymes to catalyse hydrolysis of fats, leading to improved digestion and absorption.

Increased feed fat resulted in reduced weight gain at the higher temperature, whereas no such effect was seen at the lower temperature (Fig. 1; Table 2). The protein requirement for fish is probably not influenced by water temperature (NRC, 1993; Wilson, 2002), but lipid β-oxidation capacity may be enhanced during cold acclimatization (Cordiner and Egginton, 1997; Thibault et al., 1997). The protein-to-energy ratios of the high fat feeds used in the present trial were low compared to those in commercial feeds for pre-smolt salmon (Table 1), and it is possible that this contributed to the poorer growth of the fish at the higher temperature. Fish fed the high fat feeds accumulated more body fat than those fed the low fat feeds (Table 4). This may have had a suppressive effect on appetite thereby reducing the amounts of nutrients available for growth (cf. Silverstein et al., 1997; Regost et al., 2001; Jobling et al., 2002; Johansen et al., 2002). According to the lipostatic theory of energy regulation, signals that relate to the size of the body fat stores impose a negative feedback on feed intake (Kennedy, 1953; Jobling and Johansen, 1999; Woods and Seeley, 2000), and this may have consequences for growth (Jobling et al., 2002). At the lower temperature, no significant differences in growth were observed between fish given the high fat and low fat feeds, but the trends in weight gain between fish fed low- and high-fat feeds were similar to those seen at 8 °C (Table 2). The differences in body fat concentrations

URN:NBN:no-6435

between fish fed the low- and high-fat feeds were also much less pronounced at the lower temperature.

The VSIs of the fish fed the high fat feeds were higher than in those given the low fat feeds (Table 4). This may be a reflection of an increased deposition of visceral fat among the fish given the high fat feeds, as body fat concentrations were also higher in these fish (Table 4). Similar findings have been recorded in several other studies carried out on salmonids (e.g. Hillestad et al., 1998; Jobling et al., 1998, 2002). On the other hand, the VSIs of the fish held at 2 °C were higher than those of the fish reared at 8 °C, even though the salmon exposed to the higher temperature had higher body fat concentrations (Table 4). It is possible that these differences were the result of differences in final body size between the fish exposed to the two thermal regimes (2 °C, ca. 40 g; 8 °C, ca. 100 g). In Atlantic salmon, fat deposition tends to increase with increasing fish size (Wathne, 1995; Jobling and Johansen, 2003), and there may also be ontogenetic changes that lead to the gastrointestinal tract representing a lower proportion of the body mass as fish increase in size.

A consistent pattern of improved protein retention was observed when the fish were provided with the high fat feeds. This effect was seen at both temperatures and with both oil sources (Tables 2 and 3). These data are suggestive of a protein-sparing effect, in line with the results of several previous studies on the influence of feed fat concentrations on nutrient partitioning in fish (Lee and Putman, 1973; Medland and Beamish, 1985; Cho and Kaushik, 1990; Einen and Roem, 1997; Hillestad et al., 1998). Nevertheless, even though protein catabolism appears to have been reduced, the feeding of the high fat feeds resulted in some reduction in weight gain (Fig. 1; Table 2), and was accompanied by increased body fat deposition (Table 4). In accord with the effects on protein digestibility, feeds with vegetable oils seemed to give a greater protein sparing effect and improved feed efficiency than formulations with fish oil at the higher temperature, while the opposite trend was seen at the lower temperature (Table 2). The possibility of temperature-dependent effects of dietary oil on protein utilisation deserves further investigation.

In summary, the Atlantic salmon parr were able to maintain growth at 2 °C, even though a rate-controlling effect of low temperature on ingestion and growth was observed. Feed utilisation and nutrient retention efficiencies (FER, PRE and ERE) were high at both temperatures and with all feed treatments, indicating that the feeds were highly digestible and their nutrients were readily available to the fish. Both protein and fat digestibility were slightly lower at 2 °C than at 8 °C after 6 months of feeding. There was no evidence that the vegetable oils were inferior to the fish oil as a source of fat and energy, and the use of vegetable oil as the fat source even seemed to result in a slight enhancement of protein digestibility, especially at the lower temperature.

**Acknowledgements**

This work was carried out with support from BioMar AS and the Norwegian Research Council grant no. 130690/120. We would like to thank the staff of HiT, Tromsø, for technical assistance and husbandry of the fish.

URN:NBN:no-6435

## References

Alanärä, A., 1992. Demand feeding as a self-regulating feeding system for rainbow trout (*Oncorhynchus mykiss*) in net-pens. Aquaculture 108, 347–356.

Alanärä, A., 1994. The effect of temperature, dietary energy content and reward level on the demand feeding activity of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 126, 349–359.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analyses of contents from different segments of the gastrointestinal tract. Aquaculture 13, 265–272.

Austreng, E., Skrede, A., Eldegard, Å., 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agric. Scand. 29, 119–126.

Azevedo, P.A., Cho, C.Y., Leeson, S., Bureau, D.P., 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquat. Living Resour. 11, 227–238.

Bell, G.J., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rape seed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535–1543.

Bendiksen, E.Å., Jobling, M., Arnesen, A.M., 2002. Feed intake of Atlantic salmon parr *Salmo salar* L. in relation to temperature and feed composition. Aquac. Res. 33, 525–532.

Blaxter, K., 1989. Energy Metabolism in Animals and Man. Cambridge Univ. Press, Cambridge. 336 pp.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Brännäs, E., Wicklund, B.-S., 1992. Low temperature growth potential of Arctic charr and rainbow trout. Nord. J. Freshw. Res. 67, 77–81.

Brett, J.R., 1971. Energetic responses of salmon to temperature. A study of some thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Am. Zool. 11, 99–113.

Cho, C.Y., Kaushik, S.J., 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev. Nutr. Diet. 61, 132–172.

Cordiner, S., Egginton, S., 1997. Effects of seasonal temperature acclimatization on muscle metabolism in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 16, 333–342.

Costello, M.J., Quigley, D.T.G., Dempsey, S., 1996. Seasonal changes in food conversion ratio as an indicator of fish feeding management. Bull. Aquac. Assoc. Can. 96, 58–60.

Einen, O., Roem, A.J., 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquac. Nutr. 3, 115–126.

Elliott, J.M., 1976. The energetics of feeding metabolism and growth of brown trout (*Salmo trutta* L.) in relation to body weight, water temperature and ration size. Freshw. Biol. Assoc. 45, 923–948.

Elliott, J.M., 1982. The effects of temperature and ration size on the growth and energetics of salmonids in captivity. Comp. Biochem. Physiol. 73B, 81–91.

Farkas, T., Fodor, E., Kitajka, K., Halver, J.E., 2001. Response of fish membranes to environmental temperature. Aquac. Res. 32, 645–655.

Farmer, G.J., Ashfield, D., Goff, T.R., 1983. A feeding guide for juvenile Atlantic salmon. Can. Rep. Fish. Aqua. Sci. 1718, 1–13.

Fodor, E., Jones, R.H., Buda, C., Kitajka, K., Dey, I., Farkas, T., 1995. Molecular architecture and biophysical properties of phospholipids during thermal adaptation in fish: an experimental and model study. Lipids 30, 1119–1126.

Fraser, N.H.C., Metcalfe, N.B., Thorpe, J.E., 1993. Temperature-dependent switch between diurnal and nocturnal foraging in salmon. Proc. R. Soc. Lond., B 252, 135–139.

Grisdale-Helland, B., Ruyter, B., Rosenlund, G., Obach, A., Helland, S.J., Sandberg, M.G., Standal, H., Røsjø, C., 2002. Influence of high contents of dietary soybean oil on growth, feed utilization, tissue fatty acid composition, heart histology and standard oxygen consumption of Atlantic salmon (*Salmo salar*) raised at two temperatures. Aquaculture 207, 311–329.

Hardy, R.W., Scott, T.M., Harrell, L.W., 1987. Replacement of herring oil with menhaden oil, soybean oil or tallow in the diets for Atlantic salmon raised in marine net-pens. Aquaculture 65, 267–277.

URN:NBN:no-6435

Hazel, J.R., 1984. Effects of temperature on the structure and metabolism of cell membranes in fish. Am. J. Physiol. 246, R460–R470.

Heggenes, J., Krog, O.M.W., Lindås, O.R., Dokk, J.G., Bremnes, T., 1993. Homeostatic behavioural responses in a changing environment: brown trout (*Salmo trutta*) become nocturnal during winter. J. Anim. Ecol. 62, 295–308.

Hillestad, M., Johnsen, F., Austreng, E., Åsgård, T., 1998. Long-term effects of dietary fat level and feeding rate on growth, feed utilization and carcass quality of Atlantic salmon. Aquac. Nutr. 4, 89–97.

Jobling, M., 1994. Fish Bioenergetics. Chapman & Hall, London. 309 pp.

Jobling, M., Johansen, S.J.S., 1999. The lipostat, hyperphagia and catch-up growth. Aquac. Res. 30, 473–478.

Jobling, M., Johansen, S.J.S., 2003. Fat distribution in Atlantic salmon, *Salmo salar* L., in relation to body size and feeding regime. Aquac. Res. 34, 311–316.

Jobling, M., Koskela, J., Pirhonen, J., 1998. Feeding time, feed intake and growth of Baltic salmon, *Salmo salar*, and brown trout, *Salmo trutta*, reared in monoculture and duoculture at constant low temperature. Aquaculture 163, 73–84.

Jobling, M., Larsen, A.V., Andreassen, B., Olsen, R.L., Sigholt, T., 2002. Influence of a dietary shift on temporal changes in fat deposition and fatty acid composition of Atlantic salmon post-smolt during the early phase of seawater rearing. Aquac. Res. 33, 875–889.

Johansen, S.J.S., Ekli, M., Stangnes, B., Jobling, M., 2001. Weight gain and lipid deposition in Atlantic salmon, *Salmo salar*, during compensatory growth: evidence for lipostatic regulation? Aquac. Res. 32, 963–974.

Johansen, S.J.S., Ekli, M., Jobling, M., 2002. Is there lipostatic regulation of feed intake in Atlantic salmon *Salmo salar* L.? Aquac. Res. 33, 515–524.

Jørgensen, E.H., Johansen, S.J.S., Jobling, M., 1997. Seasonal patterns of growth, lipid deposition and lipid depletion in anadromous Arctic charr. J. Fish Biol. 51, 312–326.

Kennedy, G.C., 1953. The role of depot fat in hypothalamic control of food intake in the rat. Proc. R. Soc. Lond., B 140, 578–592.

Kestemont, P., Baras, E., 2001. Environmental factors and feed intake: mechanisms and interactions. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), Food Intake in Fish. Blackwell, Oxford, pp. 131–156.

Koskela, J., Pirhonen, J., Jobling, M., 1997a. Feed intake, growth rate and body composition of juvenile Baltic salmon exposed to different constant temperatures. Aquac. Int. 5, 351–360.

Koskela, J., Pirhonen, J., Jobling, M., 1997b. Effect of low temperature on feed intake, growth rate and body composition of juvenile Baltic salmon. Aquac. Int. 5, 479–488.

Larsson, S., Berglund, I., 1998. Growth and food consumption of 0+ Arctic charr fed pelleted or natural food at six different temperatures. J. Fish Biol. 52, 230–242.

Lee, D.J., Putman, G.B., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr. 103, 34–39.

Médale, F., Aguirre, P., Kaushik, S.J., 1991. Utilization of dietary carbohydrates by rainbow trout at two water temperatures. In: Wenk, C., Boessinger, M. (Eds.), Energy Metabolism in Farmed Animals. EAAP Publication, vol. 58, pp. 392–395.

Medland, T.E., Beamish, F.W.H., 1985. Influence of diet and fish density on apparent heat increment in rainbow trout, *Salmo gairdneri*. Aquaculture 47, 1–10.

Metcalfe, L.D., Schimtz, A.A., Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.

Mørkøre, T., Rørvik, K.-A., 2001. Seasonal variations in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater as 0+ smolts or 1+ smolts. Aquaculture 199, 145–157.

NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington, DC. 114 pp.

Olsen, R.E., Ringø, E., 1998. The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. Aquac. Res. 29, 695–701.

Refstie, S., Helland, S.J., Storebakken, T., 1997. Adaptation to soybean meal in diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture 153, 263–272.

Regost, C., Arzel, J., Cardinal, M., Laroche, M., Kaushik, S.J., 2001. Fat deposition and flesh quality in seawater

URN:NBN:no-6435

reared, triploid brown trout (*Salmo trutta*) as affected by dietary fat levels and starvation. Aquaculture 193, 325–345.

Rungruangsak-Torrissen, K., Pringle, G.M., Moss, R., Houlihan, D.F., 1998. Effects of varying rearing temperatures on gene expression of different trypsin isozymes, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 19, 247–255.

Silverstein, J., Shearer, K.D., Dickhoff, W.W., Plisetskaya, E.M., 1997. Regulation of nutrient intake and energy balance in salmon. Aquaculture 177, 161–169.

Skonberg, D., Rasco, B.A., Dong, F.M., 1993. Effects of feeding high monounsaturated sunflower oil diets on sensory attributes of salmonid fillets. J. Aquat. Food Prod. Technol. 2, 117–133.

Thibault, M., Blier, P.U., Guderley, H., 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 16, 139–155.

Torrissen, K.R., Shearer, K.D., 1992. Protein digestion, growth and food conversion in Atlantic salmon and Arctic charr with different trypsin-like isozyme patterns. J. Fish Biol. 41, 409–415.

Torstensen, B.E., Lie, Ø., Frøyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids 35, 653–664.

Wallaert, C., Babin, P.J., 1994. Thermal adaptation affects the fatty acid composition of plasma phospholipids in trout. Lipids 29, 373–376.

Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996a. Digestible energy: methodological influences and the mode of calculation. Fish. Sci. 62, 288–292.

Watanabe, T., Takeuchi, T., Satoh, S., Kiron, V., 1996b. Digestible crude protein contents of various feedstuffs determined with four freshwater fish species. Fish. Sci. 62, 278–282.

Wathne, E., 1995. Strategies for directing slaughter quality of farmed Atlantic salmon (*Salmo salar*) with emphasis on diet composition and fat deposition. Dr. scient thesis, Agricultural University of Norway, Ås, Norway. 230 pp.

Wilson, R.P., 2002. Amino acids and protein. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, pp. 143–179.

Woods, S.C., Seeley, R.J., 2000. Adiposity signals and the control of energy homeostasis. Nutrition 16, 894–902.

Zar, J.H., 1984. Biostatistical Analysis. Prentice Hall International, London. 718 pp.

URN:NBN:no-6435

URN:NBN:no-6435

# Paper III

URN:NBN:no-6435

URN:NBN:no-6435

1 **Effects of temperature and feed composition on essential fatty acid (n-3 and n-6)**
2 **retention in Atlantic salmon (*Salmo salar* L.) parr**
3
4 E. Å. Bendiksen[1,2,*] and M. Jobling[3]
5
6 [1]Department of Biology, Norwegian University of Science and Technology (NTNU),
7 N-7491 Trondheim, Norway
8 [2]BioMar AS, Kjøpmannsgata 50, N-7484 Trondheim, Norway
9 [3]NFH, University of Tromsø, N-9037 Tromsø, Norway
10
11
12 [*]To whom correspondence should be addressed at BioMar AS, Kjøpmannsgata 50, N-
13 7484 Trondheim, Norway
14 Telf. +47 73871116; Fax +47 73871119; e-mail eldar.bendiksen@biomar.no
15
16
17
18
19
20
21
22
23
24 Keywords: body composition, feed oils, HUFA, lipids, salmonids, thermal biology
25

URN:NBN:no-6435

26 **Abstract**

27 Retentions of n-3 and n-6 essential fatty acids (EFAs) were assessed in Atlantic salmon
28 (*Salmo salar* L.) parr held at 8ºC and 2ºC until they increased in weight from ca. 19 g to
29 38 g. Feeds contained sandeel oil or a rapeseed:linseed oil blend at 21 and 34% dietary
30 fat. EFA retention efficiencies [(g EFA gained g EFA ingested$^{-1}$) × 100] were estimated
31 from feed intake and change in biomass for each tank of fish, and fatty acid composition
32 of feeds and the fish. The n-3 EFA retentions were higher (overall mean 71%) across
33 feed treatments and temperatures than the n-6 EFA retentions (overall mean 63%).
34 Retentions of the n-3 fatty acids were higher in the fish given the feeds with the lower
35 fat content (77% vs. 65%), implying improved retention with reduced n-3 EFA
36 availability. n-3 EFA retention tended to be higher at 2ºC than at 8ºC, although this was
37 not consistent across feeds. At low temperature there was very high retention of the n-3
38 EFAs in feeds containing sandeel oil (80%). Such high retention may represent an
39 adaptation response to low temperature. Lower n-6 EFA retentions imply that more n-6
40 fatty acids were metabolized than n-3 EFAs. Feed oil influenced retention of the n-6
41 fatty acids, retention being lower for the salmon parr given the feeds containing sandeel
42 oil (56% vs. 71%). This could indicate a higher tissue deposition of n-6 fatty acids when
43 they are freely available via the diet.

44

45

46 **Abbreviations**: AA, arachidonic acid (C20:4 n-6); DHA, docosahexaenoic acid (C22:6
47 n-3); EFA, essential fatty acid; EPA, eicosapentaenoic acid (C20:5 n-3); HUFA, highly-
48 unsaturated fatty acid (≥4 double bonds); MUFA, monounsaturated fatty acid; PL,
49 phospholipid; SFA, saturated fatty acid; TAG, triacylglycerol.

URN:NBN:no-6435

50 **Introduction**

51 Fish have a dietary requirement for n-3 and n-6 PUFAs, so these are termed essential
52 fatty acids (EFAs). The C18, C20 and C22 n-3 and n-6 fatty acids all have the potential
53 to meet EFA requirements, but the ability to convert the C18 n-3 and n-6 fatty acids to
54 the biologically active forms (EPA, 20:5 n-3; DHA, 22:6 n-3; AA, 20:4 n-6; and 22:5 n-
55 6) varies widely among species and life stages (reviewed by Henderson and Tocher
56 1987; Sargent et al. 1989, 2002; Henderson 1996; Higgs and Dong 2000). For
57 salmonids in fresh water 18:3 n-3 seems able to fulfil the requirement for dietary n-3
58 EFAs (Higgs and Dong 2000; Ruyter et al. 2000).

59 There are links between dietary fat composition and whole-animal physiology
60 (Sargent et al. 1989, 2002; Craig et al. 1995; Higgs and Dong 2000; Simandle et al.
61 2001; Hochachka and Somero 2002), but the possible links between thermal
62 environment and the EFA requirements of fish have been little studied. The body
63 temperatures of fish are usually within 1ºC of that of the surrounding water (Hochachka
64 and Somero 2002) and compensatory mechanisms exist to keep cell membranes in a
65 fluid state irrespective of prevailing temperature (i.e. homeoviscous adaptation). Several
66 complementary mechanisms are known (Hazel and Williams 1990; Hazel 1995; Farkas
67 et al. 2001; Hochachka and Somero 2002), and the role of the highly-unsaturated fatty
68 acids (HUFAs), and in particular DHA, is often highlighted (Hazel and Williams 1990;
69 Fodor et al. 1995; Logue et al. 2000).

70 Salmonids are coldwater stenotherms, and the positive relationship between
71 concentrations of EPA and DHA in Atlantic salmon, *Salmo salar* L., lipids and latitude
72 (Olsen and Skjervold 1991, 1995; Pickova et al. 1998) may be a reflection of thermal
73 adaptation mechanisms. Given the putative role of n-3 HUFAs in low-temperature

URN:NBN:no-6435

74 adaptation it might be expected that Atlantic salmon given feeds containing low
75 concentrations of n-3 EFAs would exhibit high retention efficiencies for these fatty
76 acids at low temperature. This hypothesis was tested in an experiment in which feeds
77 containing either low or high concentrations of n-3 HUFAs were fed to Atlantic salmon
78 parr held at two temperatures (8ºC and 2ºC).

79

80 **Material and methods**

81 Four extruded feeds (2.5 mm) were produced at Biomar TechCenter, Brande, Denmark.
82 The ingredients and proximate compositions of the feeds are shown in Table 1. The
83 feeds contained 340 g $kg^{-1}$ fat and 400 g $kg^{-1}$ protein or 210 g $kg^{-1}$ fat and 500 g $kg^{-1}$
84 protein, and were designated LFFO, LFVO, HFFO and HFVO according to fat level
85 (LF - low fat; HF - high fat) and oil source (FO - fish oil; VO - vegetable oil). The oil
86 sources were sandeel, *Ammodytes* spp., oil or a blend of rapeseed, *Brassica* sp., oil and
87 linseed, *Linum* sp., oil (ratio 7:3 by weight). This gave differences in concentrations and
88 contents of n-3 and n-6 fatty acids in the feeds (Tables 1 & 2), but all feeds fulfilled the
89 minimum known requirement of juvenile Atlantic salmon for EFAs (Ruyter et al. 2000).

90 The feeding experiment, conducted at Tromsø Aquaculture Research Station,
91 Kårvika, northern Norway, was started in November 1999 using Atlantic salmon, *Salmo*
92 *salar*, parr of the AquaGen strain (Aqua Gen AS, Kyrksæterøra, Norway). Alevins that
93 had been held at ambient temperature under continuous light until August were
94 subjected to a gradual reduction in day-length (LD24:0→LD12:12) and water
95 temperature until mid-September when the photoperiod was fixed at LD12:12. Prior to
96 the start of the experiment in November the water temperature gradually fell to 4-5ºC.

URN:NBN:no-6435

97 On November 10 and 11, the fish were anaesthetized in aerated benzocaine
98 solution (*p*-aminobenzoic acid ethyl ester, 50 mg $l^{-1}$) and weighed. A sample of 30 fish
99 was taken for body composition analysis. Groups of 150 fish (initial weight 19.3±4.3 g;
100 mean±SD) were established in each of 24 fiberglass tanks (260 l) giving densities of
101 11.6±0.1 kg $m^{-3}$ (mean±SE). Water temperature was set at 2°C in 12 tanks, and at 8°C
102 in the remaining 12 tanks. Water flows (8-10 l $min^{-1}$) and current speeds (8-10 cm $s^{-1}$)
103 were similar for all tanks. Water temperature was monitored daily (8.0±0.3ºC and
104 2.0±0.3ºC; mean±SD) and was maintained by mixing the stock supply with heated or
105 chilled water. Oxygen concentrations were measured twice a week, and never fell below
106 8.4 mg $l^{-1}$ during the six months study period.

107 Feeding with the test feeds was established the day after initial weighing,
108 according to the protocols described by Bendiksen et al. (2002). The four feeds were
109 provided to triplicate groups of fish at both temperatures until live weight had doubled.
110 This took approximately two and six months at 8ºC and 2ºC, respectively. On
111 termination, 20 fish were collected from each tank and killed with a sharp blow to the
112 head. The fish were dissected and feed remains removed from the intestine. Tissue
113 homogenates were prepared from de-skinned muscle, the viscera and 'carcass' (head,
114 skin, fins and bones including heart and kidney). Samples were pooled by tank,
115 transferred to brown glass vials, flushed with nitrogen and stored at -22ºC until
116 analyzed.

117 Lipids were extracted from 20 g half-thawed samples of muscle and carcass
118 homogenates, and from 3-5 g samples of viscera, using methanol:chloroform:water as
119 described by Bligh and Dyer (1959). The chloroform:water phase was retained and
120 solvents evaporated under a nitrogen atmosphere at 30ºC. Total lipids were determined

URN:NBN:no-6435

121 gravimetrically (precision; ±0.001 g). Polar and non-polar lipids were separated using
122 pre-packed solid phase silica columns (Sep-Pak$^{TM}$, Water Associates, Milford, MA,
123 USA) according to the method described by Hamilton and Comai (1988), and lipid
124 extracts were stored under a nitrogen atmosphere prior to preparation for fatty acid
125 analysis. All chemicals used were HPLC grade solvents from Merck, Darmstadt,
126 Germany.

127 Methyl esters were prepared by alkali transesterification with 0.5 M NaOH in
128 methanol (100ºC, 15 min), followed by methylation of free fatty acids in 12 % boron-
129 trifluoride-methanol (Metcalfe et al. 1966). Methyl esters, extracted in isooctane, were
130 separated by gas chromatography using a Perkin Elmer Auto System XL gas
131 chromatograph equipped with a split/splitless injector fitted to a fused silica capillary
132 column (CP Wax 52CB, Chrompak, 25 m × 0.25 mm i.d.) and a flame-ionisation
133 detector. Helium was used as the mobile phase. Temperature was increased at 30ºC per
134 min from 90ºC to 150ºC, and thereafter at 3ºC per min to 225ºC; the total running time
135 was 35 min. Operating temperatures for the injector and the detector were to 250ºC and
136 280ºC, respectively. The fatty acids were identified automatically using Turbochrom
137 software.

138 Identification of fatty acids of the n-3 (18:3 n-3, 18:4 n-3, 20:4 n-3, 20:5 n-3 and
139 22:6 n-3) and n-6 (18:2 n-6, 18:3 n-3, 20:3 n-6, 20:4 n-6 and 22:5 n-6) series was
140 conducted by reference to fatty acid ester standards (68D, Nu-Chek-Prep. Inc.,
141 Minnesota, USA). The amounts of n-3 and n-6 fatty acids present in the tissues were
142 estimated by combining information about the proportions of fatty acids in the extracted
143 lipids with that of the fat contents of the corresponding tissue. In making the
144 calculations it was assumed that fatty acids make up 75 and 95% of the mass of polar

URN:NBN:no-6435

145 and non-polar lipids, respectively (Arts et al. 2001). Concentrations of fatty acids in
146 feeds (Tables 1 & 2) were estimated by combining information about the proportion of
147 fatty acids in extracted fat (85-91%), with that of corresponding fat contents. The
148 consumption of n-3 and n-6 fatty acids during the course of the growth period was
149 estimated by combining the feed composition data with information about the amounts
150 of feed ingested by each tank of fish (Bendiksen et al. 2002).

151 EFA retention efficiencies for the n-3 and n-6 series fatty acids [(g gained g
152 ingested$^{-1}$) $\times$ 100] were estimated on a tank basis from data relating to feed intake,
153 changes in biomass and changes in whole body contents of n-3 and n-6 fatty acids:
154 EFA retention efficiency = 100 $\times$ (final mass of EFA in fish – initial mass of EFA in
155 fish) (mass EFA ingested)$^{-1}$.

156 Tank means were used as the observational units in the statistical tests. The data
157 were subjected to a three-factor ANOVA model using the GLM module of the SPSS for
158 Windows Version 10.0 statistical package. Temperature, feed fat content and oil source
159 were used as the fixed factors. The results of the ANOVA are presented as the
160 proportion of total variance explained by each of the factors and their interactions. Data
161 expressed as proportions or percentages were arcsine-transformed (Zar 1996) prior to
162 the statistical tests. Levene's test was used to check for homogeneity of variance across
163 groups within each temperature. Planned pair-wise comparisons between temperatures
164 within each feed treatment were conducted using the Mann-Whitney U test. In all
165 statistical tests the significance level was set to $P$<0.05.

166

167

168

URN:NBN:no-6435

169 **Results**

170 The data presented in Table 3 show that retention efficiencies of the n-3 fatty acids were
171 high across temperatures and feed treatments. The retention efficiency of the n-3 fatty
172 acids was significantly influenced by feed fat content ($P$<0.001), but not by oil source,
173 although there were interaction effects between temperature and oil source ($P$<0.001),
174 feed fat content and oil source ($P$<0.01), and all three main factors ($P$<0.001)(Table 4).
175 The ANOVA analysis revealed no significant effect of temperature, but the statistical
176 power of the test was low (test power; 0.20). The retention efficiencies for n-3 fatty
177 acids were higher in the fish given the feeds with the lower (21%) feed fat concentration
178 (Table 3). At the lower temperature ($2^oC$) retention of the n-3 fatty acids was
179 particularly high for the fish fed the feeds that contained sandeel oil (Table 3). The
180 retention of n-3 fatty acids was significantly higher ($P$<0.05) than retention of n-6 fatty
181 acids (Table 5; overall means 71% vs. 63%). Retention of n-6 fatty acids was
182 significantly influenced by oil source ($P$<0.001), with retention efficiencies being
183 highest for the fish given the feeds containing vegetable oils (Table 3). There were also
184 significant interactions between feed fat content and oil source ($P$<0.05), and
185 interactions between all three main factors influenced the retention of the n-6 EFAs
186 (Table 4).

187 Pair-wise comparisons of retention efficiencies between temperatures, but within
188 feed treatments, revealed significantly higher retention efficiencies of the n-3 fatty acids
189 at the lower temperature for three of the four feeds (Table 5). In the LFVO treatment
190 higher n-3 EFA retention was found at the higher temperature. With the exception of the
191 HFVO treatment, pair-wise comparisons of n-6 EFA retention efficiencies within feed
192 treatments revealed no differences between the salmon parr held at the two

URN:NBN:no-6435

193 temperatures. In the fish given the HFVO feed, retention of n-6 fatty acids was higher at
194 the lower of the two temperatures (Table 5).
195

196 **Discussion**

197 The results of the present study show that the n-3 and n-6 series fatty acids (n-3 and n-6
198 EFAs) were efficiently retained within the body of the salmon parr at both temperatures
199 and across all feed treatments (Table 3). Retention efficiencies in excess of 50% must
200 be considered high given the role that fatty acids play in fuelling oxidative metabolism
201 in fish tissues. However, monoenes (MUFAs) seem to be the preferred fatty acid
202 substrates for catabolism, and saturated fatty acids (SFAs) are also preferred over
203 polyenes. Of the MUFAs, 18:1 and 16:1 appear to be those most readily catabolised via
204 the β oxidation pathway, and 16:0 seems to be most preferred amongst the SFAs
205 (Henderson and Sargent 1985; Kiessling and Kiessling 1993; Siddell et al. 1995;
206 Egginton 1996; Henderson 1996).

207 Efficient retention of n-3 fatty acids should not be unexpected given the
208 important structural role of the n-3 HUFAs in cell membrane lipids. The n-3 HUFAs,
209 primarily DHA and EPA, are preferentially incorporated at the sn-2 position of the
210 glycerol backbone in phosphatidylcholines and phosphatidylethanolamines, the two
211 major membrane phospholipids (PLs) (Henderson and Tocher 1987; Sargent et al. 1989,
212 2002; Arts et al. 2001). However, the structural PLs usually make up a small proportion
213 of the total lipids in fish tissues, with the neutral, storage lipids, such as triacylglycerols
214 (TAGs), tending to dominate (reviewed by Henderson and Tocher 1987; Sargent et al.
215 1989, 2002; Higgs and Dong 2000; Jobling 2001). The Atlantic salmon parr used in the
216 present study had 9-14% total body lipids (Bendiksen et al. 2003), and deposited 2-3 g

URN:NBN:no-6435

217 lipid during the period in which they doubled in body weight. As such, quite large
218 quantities of n-3 fatty acids were incorporated into the neutral lipids, and following
219 deposition must have been preferentially conserved, rather than being metabolised.
220 Although 18:3 n-3 and EPA may be catabolised via β oxidation, they are not preferred
221 substrates, and DHA appears to be very resistant to catabolic degradation via β
222 oxidation (Henderson and Sargent 1985; Henderson 1996; Sargent et al. 2002).
223 Consequently, n-3 fatty acids might accumulate in the neutral lipids over time.
224 Preferential retention of n-3 fatty acids in the neutral lipids could represent a
225 physiological buffering mechanism, enabling n-3 EFAs to be mobilized to meet
226 essential functions under conditions of food limitation.

227 Although the n-3 fatty acids were retained efficiently by the salmon parr given
228 all four feed types, feed fat content had a significant effect on retention efficiency
229 (Table 4). A slightly higher retention of n-3 fatty acids by the fish fed the low-fat (21%)
230 feeds (Table 3) was not unexpected given the reduced concentrations of n-3 EFAs in
231 these feeds (Table 1). The feeds contained different amounts of n-3 fatty acids (Table
232 1), but another major difference was in the chain lengths and degree of unsaturation of
233 the n-3 EFAs present (Table 2). The feeds containing sandeel oil, had most of the n-3
234 fatty acids present as HUFAs, whereas the dominant n-3 fatty acid in the vegetable oil
235 feeds was 18:3 n-3, from linseed oil (Table 2). The n-3 HUFAs, such as DHA and EPA,
236 can be incorporated directly into PLs, whereas 18:3 n-3 must undergo chain elongation
237 and desaturation to form either EPA or DHA (Sargent et al. 1989, 2002; Henderson
238 1996; Higgs and Dong 2000; Arts et al. 2001). Despite these differences, oil source was
239 not found to have a significant effect on the efficiency with which the n-3 fatty acids
240 were retained by the salmon parr.

URN:NBN:no-6435

241 There was a general tendency for retention of the n-3 EFAs to be higher in the
242 fish held at the lower temperature, this being observed for the groups of salmon parr
243 given three of the four feeds (Table 5). The increased retention of n-3 fatty acids at low
244 temperature may be a reflection of a thermal acclimation response. When fish are
245 exposed to low temperatures a usual biochemical response is an increase in the
246 unsaturation of the fatty acids incorporated into both the cell membrane lipids and the
247 storage TAGs (Cossins and Lee 1985; Hazel and Williams 1990; Fodor et al. 1995;
248 Logue et al. 2000; Hsieh et al. 2003). There is consistently a reduction in the proportion
249 of SFAs and a corresponding increase in unsaturated fatty acids, but the SFAs may be
250 replaced by either MUFAs or polyenes (Cossins and Lee 1985; Hazel and Williams
251 1990; Fodor et al. 1995; Hsieh et al. 2003). In line with this, exposure of fish to low
252 temperature leads to changes in the enzyme systems of lipid biosynthesis. For example,
253 a common observation is the depression of production of SFAs relative to unsaturated
254 fatty acids (Hazel and Williams 1990). In addition to depressing rates of production of
255 SFAs exposure to low temperature may also lead to adjustments in the capacity for the
256 synthesis of unsaturated fatty acids. This may arise from an induction and up-regulation
257 of desaturase and elongase enzymes. These enzymes are required for the synthesis of
258 MUFAs from SFAs, and for the conversion of C18 precursor n-3 and n-6 fatty acids to
259 HUFAs (Henderson and Tocher 1987; Sargent et al. 1989; Hazel and Williams 1990;
260 Tiku et al. 1996; Trueman et al. 2000; Hsieh et al. 2003). Frequently the change in lipid
261 unsaturation that occurs during acclimation to low temperature results, at least in part,
262 from increased incorporation of n-3 HUFAs, particularly DHA, into both the polar and
263 non-polar lipids (Cossins and Lee 1985; Malak et al. 1989; Ingemansson et al. 1993;
264 Wallaert and Babin 1993, 1994; Fodor et al. 1995; Fracalossi and Lovell 1995).

URN:NBN:no-6435

265 The retention efficiencies for the n-6 EFAs were lower than those of the n-3
266 fatty acids (Table 3), and were little influenced by either temperature or the fat
267 concentrations in the feeds. Retention of the n-6 fatty acids was, however, higher
268 amongst the fish given the feeds containing vegetable oils than in those provided with
269 the feeds that contained sandeel oil (Table 3). Thus, retention appeared to be directly
270 related to the quantities of n-6 EFAs present in the feeds, since both of the feeds
271 formulated with vegetable oils contained more n-6 EFAs than did the feeds formulated
272 with sandeel oil (Tables 1 & 2). This implies that catabolic degradation of n-6 EFAs
273 was relatively less when they were supplied in larger amounts via the vegetable oils.
274 This might be a reflection of the low preference for polyenes as substrates for β
275 oxidation (Egginton 1996; Henderson 1996).

276 Nevertheless, the lower retention of n-6 fatty acids in comparison with n-3 EFAs
277 may be indicative of higher rates of oxidation of the n-6 fatty acids relative to n-3 fatty
278 acids. In keeping with this suggestion, there are very low rates of oxidation of DHA in
279 fish tissues, whereas 18:2n-6 is oxidized more readily (Henderson and Sargent 1985;
280 Kiessling and Kiessling 1993; Henderson 1996). In addition, lipids that contain HUFAs
281 are generally considered to be more easily digested and absorbed than those containing
282 less-saturated fatty acids (Henderson and Tocher 1987; Sargent et al. 1989; Higgs and
283 Dong 2000; Johnsen et al. 2000). As such, it is possible that the lower retention
284 efficiency of the n-6 EFAs could also have resulted from reduced digestion and
285 absorption in comparison with n-3 EFAs. However, Bendiksen et al. (2003) did not find
286 any oil-related differences in absorption efficiencies of the total lipids present in the
287 same feeds as used in the present study.

URN:NBN:no-6435

288 Amongst the EFAs the n-3 fatty acids tend to predominate in fish tissues, with
289 DHA and EPA being far more prevalent in cell membrane lipids than the n-6 HUFA
290 AA. The n-3 HUFAs are present in phosphatidylcholines and
291 phosphatidylethanolamines, the two major membrane PLs, whereas AA is located
292 almost exclusively in the sn-2 position of the glycerol backbone of the phosphoinositols
293 (Sargent et al. 1989, 2002; Bell and Sargent 2003). Phosphoinositols generally make up
294 only a small proportion of the cell membrane lipids, but they have important roles in
295 cellular signal transduction. In addition to being a component of the phosphoinositols
296 AA is a primary precursor for the synthesis of eicosanoids, which are known to have a
297 range of regulatory functions in fish tissues (Sargent et al. 2002; Bell and Sargent
298 2003). Despite the important physiological role of AA in the regulation of cellular
299 physiology and metabolism, the incorporation of AA into phosphoinositols would be
300 insufficient to explain the relatively high retention efficiencies of n-6 fatty acids.
301 However, n-6 fatty acids, such as 18:2 n-6, can also be sequestered from the diet and
302 incorporated into the storage lipids, such as TAGs (Sargent et al. 1989; Higgs and Dong
303 2000; Jobling 2001). Once incorporated into the storage lipids n-6 fatty acids may be
304 conserved, due to the apparent preference for MUFAs as $\beta$ oxidation substrates in
305 tissues of Atlantic salmon (Egginton 1996).

306 In summary, the results indicated that both temperature and feed composition
307 influence the deposition and retention of n-3 and n-6 EFAs by Atlantic salmon parr. A
308 large proportion of the n-3 EFAs was retained in the body of the fish irrespective of
309 temperature and feed type, whereas incorporation of n-6 fatty acids was lower. High
310 retention of n-3 fatty acids at low temperature is interpreted as a thermal acclimation

URN:NBN:no-6435

311 response, and the lower retention of n-6 fatty acids is taken to imply greater catabolic
312 degradation of the n-6 EFAs than the n-3 EFAs.
313
314 **Acknowledgements**

315 This work was carried out with support from BioMar AS and the Norwegian Research
316 Council grant no. 130690/120. We would like to thank the staff of HiT, Tromsø, for
317 technical assistance and husbandry of the fish, and the Departments of Biology and
318 Chemistry, Norwegian University of Science and Technology (NTNU) for providing
319 laboratory assistance and facilities.

320
321
322
323
324
325
326
327
328
329
330
331
332
333
334

URN:NBN:no-6435

335 **Literature**

336 Arts, M.T., Ackman, R.G. and Holub, B.J. 2001. 'Essential fatty acids' in aquatic
337 ecosystems: A crucial link between diet and human health and evolution. Can. J.
338 Fish. Aquat. Sci. 58: 122–137.
339 Bell, J.G. and Sargent, J.R. 2003. Arachidonic acid in aquaculture feeds: Current status
340 and future opportunities. Aquaculture 218: 491–499.
341 Bendiksen, E.Å., Jobling, M. and Arnesen, A.M. 2002. Feed intake of Atlantic salmon
342 parr *Salmo salar* L. in relation to temperature and feed composition. Aqua. Res.
343 33: 525–532.
344 Bendiksen, E.Å., Berg, O.K., Jobling, M., Arnesen, A.M. and Måsøval, K. 2003.
345 Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo*
346 *salar* L.) in relation to temperature, feed fat content and oil source. Aquaculture
347 in press.
348 Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and
349 purification. Can. J. Biochem. Physiol. 37: 911–917.
350 Cossins, A.R. and Lee, J.A.C. 1985. The adaptation of membrane structure and lipid
351 composition to cold. In: Circulation, Respiration and Metabolism. Pp. 543–552.
352 Edited by R. Gilles. Springer-Verlag, Berlin.
353 Craig, S.R., Neill, W.H. and Gatlin, D.M. III. 1995. Effects of dietary lipid and
354 environmental salinity on growth, body composition, and cold tolerance of
355 juvenile red drum (*Sciaenops ocellatus*). Fish Physiol. Biochem. 14: 49–61.
356 Egginton, S. 1996. Effect of temperature on the optimal substrate for β-oxidation. J.
357 Fish Biol. 49: 753–758.
358 Farkas, T., Fodor, E., Kitajka, K. and Halver, J.E. 2001. Response of fish membranes to

URN:NBN:no-6435

359 environmental temperature. Aquac. Res. 32: 645–655.

360 Fodor, E., Jones, R.H., Buda, C., Kitajka, K., Dey, I. and Farkas, T. 1995. Molecular
361 architecture and biophysical properties of phospholipids during thermal
362 adaptation in fish: An experimental and model study. Lipids 30: 1119–1126.

363 Fracalossi, D.M. and Lovell, R.T. 1995. Growth and liver polar fatty acid composition
364 of year-1 channel catfish fed various lipid sources at two water temperatures.
365 Prog. Fish. Cult. 57: 107–113.

366 Hamilton, J.G. and Comai, K. 1988. Rapid separation of neutral lipids, free fatty acids
367 and polar lipids using prepacked silica Sep-Pak columns. Lipids 23: 1146–1149.

368 Hazel, J.R. 1995. Thermal adaptation in biological membranes: Is homeoviscous
369 adaptation the explanation? Ann. Rev. Physiol. 57: 19–42.

370 Hazel, J.R. and Williams, E.E. 1990. The role of alterations in membrane lipid
371 composition in enabling physiological adaptation of organisms to their physical
372 environment. Prog. Lip. Res. 29: 167–227.

373 Henderson, R.J. 1996. Fatty acid metabolism in freshwater fish with particular reference
374 to polyunsaturated fatty acids. Arch. Ani. Nutr. 49: 5–22.

375 Henderson, R.J. and Sargent, J.R. 1985. Chain length specificities of mitochondrial and
376 peroxisomal β-oxidation of fatty acids in livers of rainbow trout. Comp.
377 Biochem. Physiol. 82B: 79–85.

378 Henderson, R.J. and Tocher, D.R. 1987. The lipid composition and biochemistry of
379 freshwater fish. Prog. Lip. Res. 26: 281–347.

380 Higgs, D.A. and Dong, F.M. 2000. Lipids and fatty acids. In: Encyclopedia of
381 Aquaculture. Pp. 476–496. Edited by R.R. Stickney. John Wiley and Sons, New
382 York.

URN:NBN:no-6435

383 Hochachka, P.W. and Somero, G.N. 2002. Biochemical Adaptation. Oxford University
384 Press, Oxford.
385 Hsieh, S.L., Chen, Y.N. and Kuo, C.M. 2003. Physiological responses, desaturase
386 activity, and fatty acid composition in milkfish (*Chanos chanos*) under cold
387 acclimation. Aquaculture 220: 903–918.
388 Ingemansson, T., Olsson, N.U. and Kaufmann, P. 1993. Lipid composition of light and
389 dark muscle of rainbow trout (*Oncorhynchus mykiss*) after thermal acclimation:
390 A multivariate approach. Aquaculture 113: 153–165.
391 Jobling, M. 2001. Nutrient partitioning and the influence of feed composition on body
392 composition. In: Food Intake in Fish. Pp. 354–375. Edited by D. Houlihan, T.
393 Boujard and M. Jobling. Blackwell Science, Oxford.
394 Johnsen, R.I., Grahl-Nielsen, O. and Roem, A. 2000. Relative absorption of fatty acids
395 by Atlantic salmon *Salmo salar* from different diets, as evaluated by multivariate
396 statistics. Aquacult. Nutr. 6: 255–261.
397 Kiessling, K.-H. and Kiessling, A. 1993. Selective utilization of fatty acids in rainbow
398 trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. Can. J. Zool.
399 71: 248–251.
400 Logue, J.A., DeVries, A.L., Fodor, E. and Cossins, A.R. 2000. Lipid compositional
401 correlates of temperature-adaptive interspecific differences in membrane
402 physical structure. J. Exp. Biol. 203: 2105–2115.
403 Malak, N.A., Brichon, G., Meister, R. and Zwingelstein, G. 1989. Environmental
404 temperature and metabolism of the molecular species of phosphatidylcholine in
405 the tissues of the rainbow trout. Lipids 24: 318–324.

URN:NBN:no-6435

406 Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. 1966. Rapid preparation of fatty acid
407 esters from lipids for gas chromatographic analysis. Anal. Chem. 38: 514–515.
408 Olsen, Y. and Skjervold, H. 1991. Impact of latitude on n-3 fatty acids in wild Atlantic
409 salmon. Omega 3 News 6: 1–4.
410 Olsen, Y. and Skjervold, H. 1995. Variation in content of ω3 fatty acids in farmed
411 Atlantic salmon, with special emphasis on effects of non-dietary factors. Aquac.
412 Int. 3: 22–35.
413 Pickova, J., Kiessling, A., Pettersson, A. and Dutta, P.C. 1998. Comparison of fatty acid
414 composition and astaxanthin content in healthy and by M74 affected salmon
415 eggs from three Swedish river stocks. Comp. Biochem. Physiol. 120B: 265–271.
416 Ruyter, B., Røsjø, C., Einen, O. and Thomassen, M.S. 2000. Essential fatty acids in
417 Atlantic salmon: Effects of increasing dietary doses of n-6 and n-3 fatty acids on
418 growth, survival and fatty acid composition of liver, blood and carcass.
419 Aquacult. Nutr. 6: 119–127.
420 Sargent, J.R., Henderson, R.J. and Tocher, D.R. 1989. The Lipids. In: Fish Nutrition.
421 Second edition, pp. 153–218. Edited by J.E. Halver. Academic Press, San Diego.
422 Sargent, J.R., Tocher, D.R. and Bell, J.G. 2002. The Lipids. In: Fish Nutrition. Third
423 edition, pp. 181–257. Edited by J.E. Halver and R.W. Hardy. Academic Press,
424 San Diego.
425 Siddell, B.D., Crockett, E.L. and Driedzic, W.R. 1995. Antarctic fish tissues
426 preferentially catabolize monoenoic fatty acids. J. Exp. Zool. 271: 73–81.
427 Simandle, E.T., Espinoza, R.E., Nussear, K.E. and Tracy, C.R. 2001. Lizards, lipids,
428 and dietary links to animal function. Physiol. Biochem. Zool. 74: 625–640.

URN:NBN:no-6435

429 Tiku, P.E., Gracey, A.Y., Macartney, A.I., Beynon, R.J. and Cossins, A.R. 1996. Cold-
430 induced expression of $\Delta^9$-desaturase in carp by transcriptional and
431 posttranslational mechanisms. Science 271: 815–818.
432 Trueman, R.J., Tiku, P.E., Caddick, M.X. and Cossins, A.R. 2000. Thermal thresholds
433 of lipid restructuring and $\Delta^9$-desaturase expression in the liver of carp (*Cyprinus*
434 *carpio* L.). J. Exp. Biol. 203: 641–650.
435 Wallaert, C. and Babin, P.J. 1993. Circannual variation in the fatty acid composition of
436 high-density lipoprotein phospholipids during acclimatization in trout. Biochi.
437 Biophys. Acta 1210: 23–26.
438 Wallaert, C. and Babin, P.J. 1994. Thermal adaptation affects the fatty acid composition
439 of plasma phospholipids in trout. Lipids 29: 373–376.
440 Zar, J.H. 1996. Biostatistical Analysis. Prentice-Hall International, London.

URN:NBN:no-6435

441 Table 1. Feed ingredients and analysed compositions of test feeds.
442 Feed codes are as follows: LF, low fat; HF, high fat; FO, fish oil; VO, vegetable oil.

| | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| Ingredients, g $kg^{-1}$ | | | | |
| Fish meal[1] | 638 | 638 | 486 | 486 |
| Wheat | 190 | 190 | 178 | 178 |
| Sandeel oil | 140 | | 270 | |
| Rapeseed oil[2] | | 104 | | 200 |
| Linseed oil[2] | | 36 | | 70 |
| Mono-sodium phosphate | 10 | 10 | 25 | 25 |
| Vitamin and mineral premixes | 12 | 12 | 12 | 12 |
| Fat absorber[3] | 10 | 10 | 30 | 30 |
| Analysed composition | | | | |
| Dry matter (%)[4] | 94.5 | 94.1 | 96.4 | 96.3 |
| Crude protein (%)[5] | 50.2 | 50.4 | 40.3 | 40.2 |
| Crude fat (%)[6] | 20.7 | 21.4 | 33.5 | 33.9 |
| Ash (%)[7] | 9.1 | 9.3 | 10.3 | 10.4 |
| Residue (%) | 14.5 | 13 | 12.3 | 11.7 |
| Gross energy[8], MJ $kg^{-1}$ | 22.5 | 22.5 | 24.8 | 24.5 |
| Calculated P/E-ratio | 22.3 | 22.4 | 16.3 | 16.4 |
| Σ n-3 EFAs, g $kg^{-1}$ | 53 | 43 | 91 | 68 |
| Σ n-6 EFAs, g $kg^{-1}$ | 9 | 29 | 12 | 52 |

443 [1]Ultra Flash fish meal purchased from Fiskernes Fiskeindustri A.M.B.A., Denmark.
444 [2]Neutralised, bleached and de-odorised vegetable oils purchased from Superfos Agro, Denmark.
445 [3]Diatomaceous earth purchased from Damolin AS, Denmark.
446 [4]Drying at 105ºC for 24 hours.
447 [5]Kjeldahl analysis (N×6.25) using a Kjeltec Autoanalyser (Tecator, Sweden).
448 [6]Acid hydrolysed samples (3M HCl) using Soxhlet method with petroleum ether extraction.
449 [7]Ashed at 550ºC for 16 hours.
450 [8]Bomb calorimetry.

URN:NBN:no-6435

451 Table 2. Relative content (%) of fatty acid classes and selected fatty acids in the total lipids of the test feeds. Estimates of concentrations (g $kg^{-1}$)
452 are given in parentheses. Feed codes are given in Table 1.

| Fatty acids | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| Σ SFAs | 21.4 (37.7) | 11.6 (21.4) | 21.9 (67.0) | 9.9 (29.8) |
| Σ MUFAs | 43.1 (75.8) | 49.3 (90.7) | 44.3 (135.3) | 50.6 (153.2) |
| Σ n-6 polyenes | 5.3 (9.3) | 15.6 (28.8) | 4.0 (12.1) | 17.2 (52.0) |
| 18:2n-6 | 3.1 (5.5) | 15.1 (27.8) | 2.4 (7.3) | 16.8 (50.7) |
| 18:3n-6 | 0.3 (0.5) | 0.1 (0.1) | 0.3 (0.9) | 0.1 (0.2) |
| 20:3n-6 | 0.3 (0.6) | 0.2 (0.3) | 0.3 (1.0) | 0.1 (0.4) |
| 20:4n-6 | 0.6 (1.0) | 0.2 (0.3) | 0.5 (1.7) | 0.1 (0.2) |
| 22:5n-6 | 1.0 (1.8) | 0.1 (0.2) | 0.4 (1.2) | 0.2 (0.5) |
| Σ n-3 polyenes | 30.2 (53.1) | 23.5 (43.2) | 29.9 (91.3) | 22.3 (67.6) |
| 18:3n-3 | 2.0 (3.6) | 15.7 (28.9) | 1.6 (5.0) | 18.3 (55.5) |
| 18:4n-3 | 4.2 (7.4) | 0.9 (1.7) | 4.4 (13.5) | 0.5 (1.4) |
| 20:4n-3 | 0.8 (1.4) | 0.2 (0.4) | 0.8 (2.3) | 0.1 (0.3) |
| 20:5n-3 | 10.1 (17.7) | 2.1 (3.9) | 10.8 (32.9) | 1.1 (3.4) |
| 22:6n-3 | 12.6 (22.1) | 4.3 (7.9) | 11.8 (36.0) | 2.2 (6.6) |
| n-3:n-6 ratio | 5.7 | 1.5 | 7.5 | 1.3 |

453
454
455
456
457
458
459
460
461
462
463
464
465

URN:NBN:no-6435

URN:NBN:no-6435



466 Table 3. The effects of temperature (2 °C vs. 8°C), feed fat content (21% vs. 34%) and feed oil source (fish (sandeel) oil vs. vegetable oils) on
467 feed intake, n-3 and n-6 series fatty acid compositions, and n-3 and n-6 EFA retention efficiencies of Atlantic salmon, *Salmo salar,* parr reared at
468 2 or 8°C until they doubled in body weight. Data are given as treatment means±SE, n = 3. Feed codes are as follows: LF, low fat; HF, high fat;
469 FO, fish oil; VO, vegetable oil (see Table 1 for details of feed compositions).

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LFFO | | LFVO | | HFFO | | HFVO | |
| | 2°C | 8°C | 2°C | 8°C | 2°C | 8°C | 2°C | 8°C |
| Initial weight (g fish$^{-1}$) | 19.1±0.1 | 19.8±0.3 | 19.3±0.2 | 19.7±0.1 | 19.0±0.2 | 18.9±0.3 | 19.0±0.2 | 19.6±0.1 |
| n-3 EFA (g tank$^{-1}$) | 70±0.3 | 72±1.1 | 70±0.8 | 72±0.3 | 69±0.8 | 69±0.8 | 69±0.9 | 72±1.1 |
| n-6 EFA (g tank$^{-1}$) | 14±0.1 | 15±0.2 | 14±0.2 | 14±0.1 | 14±0.2 | 14±0.2 | 14±0.2 | 14±0.1 |
| Final weight (g fish$^{-1}$) | 38.9±0.6 | 38.3±0.8 | 38.8±0.6 | 39.5±0.6 | 37.9±1.0 | 36.3±0.2 | 37.9±0.6 | 36.1±0.1 |
| n-3 EFA (g tank$^{-1}$) | 144±3.3 | 150±1.5 | 118±4.7 | 151±1.2 | 173±2.3 | 184±6.2 | 143±2.1 | 132±1.5 |
| n-6 EFA (g tank$^{-1}$) | 23±0.8 | 24±0.6 | 50±1.8 | 61±3.1 | 24±0.7 | 28±0.5 | 77±1.5 | 66±2.3 |
| Feed intake (g fish$^{-1}$) | 12.1±0.5 | 14.2±0.2 | 13.0±0.7 | 13.1±0.4 | 13.6±0.2 | 12.7±0.5 | 14.3±0.5 | 11.8±0.3 |
| n-3 intake (g tank$^{-1}$) | 89±3.6 | 113±1.6 | 76±3.8 | 85±2.5 | 136±3.0 | 174±6.3 | 109±4.7 | 120±3.5 |
| n-6 intake (g tank$^{-1}$) | 16±0.6 | 20±0.3 | 51±2.5 | 57±1.7 | 18±0.4 | 23±0.8 | 84±3.6 | 92±2.7 |
| Feed:gain | 0.68±0.00 | 0.77±0.01 | 0.73±0.03 | 0.67±0.02 | 0.65±0.03 | 0.74±0.04 | 0.69±0.01 | 0.72±0.02 |
| EFA retention (%) | | | | | | | | |
| n-3 EFA | 83±0.6 | 69±3.2 | 63±5.0 | 94±3.8 | 76±1.7 | 66±3.2 | 68±2.5 | 51±0.3 |
| n-6 EFA | 59±3.2 | 50±5.1 | 71±5.0 | 82±4.6 | 54±4.2 | 59±4.7 | 75±1.7 | 56±3.0 |

470
471
472
473

Table 4. ANOVA (three factor analysis) table showing the effects of temperature (2 °C vs. 8°C), feed fat content (21% vs. 34%) and feed oil source (fish (sandeel) oil vs. vegetable oils), and interaction effects, on n-3 and n-6 EFA retention efficiencies of Atlantic salmon, *Salmo salar,* parr. The proportion of total variance explained by each of the significant factors and their interaction is given, and was calculated as the marginal contribution of the mean square of the parameter (Type I Sum of Square) as a proportion of the corrected total sum of squares. Retention data are expressed as percentages, and were arcsine transformed prior to analysis. Significance levels are indicated as follows: ns, non-significant effect ($P$>0.05); *, $P$<0.05; **, $P$<0.01; ***, $P$<0.001.

| Retention efficiency[1] | n-3 EFAs | n-6 EFAs |
|---|---|---|
| Main effects | | |
| Temperature (T) | ns | ns |
| Fat content (F) | 0.21*** | ns |
| Oil source (O) | ns | 0.40*** |
| Interaction effects | | |
| T × F | 0.17*** | ns |
| T × O | 0.16*** | ns |
| F × O | 0.09** | 0.07* |
| T × F × O | 0.25*** | 0.22** |

[1] Estimated as g EFA gained g EFA ingested$^{-1}$×100

URN:NBN:no-6435

URN:NBN:no-6435



495 Table 5. Summary of n-3 and n-6 fatty acid retention efficiencies (%; given as treatment means) in Atlantic salmon, *Salmo salar,* parr.
496 Significance levels are indicated as follows: ns, non-significant effect ($P$>0.05); *, $P$<0.05.
497

| | n-3 EFA retention, %[1] Feed[2] | | | | n-6 EFA retention, %[1] Feed[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | LFFO | LFVO | HFFO | HFVO | LFFO | LFVO | HFFO | HFVO |
| Higher temperature (8ºC) | 69 | 94 | 66 | 51 | 50 | 82 | 59 | 56 |
| Lower temperature (2ºC) | 83 | 63 | 76 | 68 | 59 | 71 | 54 | 75 |
| Pair-wise comparisons (8ºC vs. 2ºC)[3] | * | * | * | * | ns | ns | ns | * |
| Mean retention efficiency | 71 | | | | 63 | | | |

498 [1] Estimated as g EFA gained g EFA ingested$^{-1}$×100.
499 [2] Feed codes as follows: LF, low fat (21%); HF, high fat (34%); FO, fish oil; VO, vegetable oils. See Table 1 for feed composition.
500 [3] Planned pair-wise comparisons run within a feed treatment, but between temperatures, using the non-parametric Mann-Whitney U test (two-
501 tailed).
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517 p

# Paper IV

URN:NBN:no-6435

URN:NBN:no-6435

Jobling, M. & Bendiksen, E.Å. 2003. Dietary lipids and temperature interact to influence tissue fatty acid compositions of Atlantic salmon, *Salmo salar* L., parr. Aquaculture Research 34, 1-19.

Paper not included due to copyright restrictions.

# Paper V

URN:NBN:no-6435

URN:NBN:no-6435

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Aquaculture 225 (2003) 149–163

Aquaculture

www.elsevier.com/locate/aqua-online

# Effects of dietary fatty acid profile and fat content on smolting and seawater performance in Atlantic salmon (*Salmo salar* L.)

E.Å. Bendiksen[a,b,*], A.M. Arnesen[c], M. Jobling[c,d]

[a] *Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim N-7491, Norway*
[b] *BioMar AS, Kjøpmannsgata 50, Trondheim N-7484, Norway*
[c] *Norwegian Institute of Fisheries and Aquaculture Research, Tromsø N-9291, Norway*
[d] *NFH, University of Tromsø, Tromsø N-9037, Norway*

**Abstract**

An experiment was conducted to study the effects of dietary fat level and fatty acid composition on seawater acclimation and growth in Atlantic salmon. Marine fish oil or a blend of rapeseed and linseed oils were added to extruded pellets to produce four feeds differing in fat content (LF: 21% and HF: 34%) and fatty acid composition. The feeds were designated LFFO, LFVO, HFFO and HFVO according to fat level (LF—low fat; HF—high fat) and oil source (FO—fish oil; VO—vegetable oil). Each feed was fed to salmon parr ( ~ 19 g) held at 2 °C on a 12L:12D regime for 6 months. Parr–smolt transformation was then induced by increasing the photoperiod from 12L:12D to 24L:0D, and water temperature to 8 °C. Fish fed the four feeds grew at similar rates both during the parr stage (SGR; $0.40 \pm 0.01$) and during the smoltification period (SGR; $0.64 \pm 0.01$). Fish fed the high-fat feeds had a higher percentage body fat than fish fed low-fat feeds, and fatty acid profiles resembled those of the feed. Parr–smolt transformation was accomplished within 3 weeks after change in photoperiod in all groups, as assessed by gill $Na^+$,$K^+$-ATPase activity, muscle water and plasma chloride following 24-h seawater tests. During the 42-days seawater period the fish were fed either LFFO or HFFO feed. Groups of 50–60 fish were subjected to one of eight feed treatments: no dietary shift, shift in feed oil type, shift in feed fat (energy) content or shift both in feed oil type and feed fat content at the time of seawater transfer. Fish in all groups lost weight during the first 3 weeks in seawater, but fish fed the LFVO feed (i.e. low-fat vegetable oil) during freshwater rearing gained weight during the total 6-week seawater period. Significantly better growth and a significantly higher proportion of fish with positive growth rates than in other treatments was, however, only seen in the group in which a shift in both lipid source (from VO to FO) and feed fat content (from LF to HF) had been applied. Whether this was an effect of increased supply of $n-3$ HUFAs or dietary energy, or a combination of both factors, is not

* Corresponding author. BioMar AS, Kjøpmannsgata 50, Trondheim N-7484, Norway. Tel.: +47-7387-1116; fax: +47-7387-1119.
*E-mail address:* eldar.bendiksen@biomar.no (E.Å. Bendiksen).

0044-8486/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0044-8486(03)00286-2

URN:NBN:no-6435

clear. There were no significant differences in plasma chloride or plasma osmolality between groups during seawater residence or in gill $Na^{+}$,$K^{+}$-ATPase activity at the end of the seawater period.
© 2003 Elsevier Science B.V. All rights reserved.

*Keywords:* Salmonids; Smolt; Dietary oils; Growth; Seawater acclimation

## 1. Introduction

As a part of their life history, anadromous salmonids undergo a parr–smolt transformation (or smoltification) which primes them for entry into seawater. Parr–smolt transformation involves changes in physiology, morphology and behaviour (reviewed by McCormick and Saunders, 1987; Hoar, 1988; Boeuf, 1993; Clarke, 2000), and alterations in lipid metabolism are regarded as an integral part of the process (Sheridan, 1989; Bell et al., 1997; Tocher et al., 2000). Relatively little attention has been paid to qualitative and quantitative aspects of lipid nutrition in relation to parr–smolt transformation, even though farmed salmonids may differ from their wild counterparts in both fat content and fatty acid composition (Plotnikoff et al., 1984; Ackman and Takeuchi, 1986; Bergström, 1989). Wild salmon smolts are generally smaller and leaner than their farmed counterparts, and may contain much higher proportions of arachidonic acid (AA, 20:4$n-6$) in their total lipids (Ackman and Takeuchi, 1986; Bergström, 1989).

AA is a precursor for eicosanoids that are involved in regulation of ion and water fluxes in the gills and kidney (Mustafa and Srivastava, 1989). AA can be produced from C18 $n-6$ fatty acid precursors, and the enzymatic bioconversion of C18 precursors is increased as a pre-adaptation to seawater entry. The enzymatic bioconversion of C18 precursors to AA is antagonised by $n-3$ fatty acids, such as eicosapentaenoic acid (EPA, 20:5$n-3$) and its eicosanoid derivatives (Bell et al., 1989). Thus, complex interactions exist between fatty acids and the metabolic pathways that determine eicosanoid biosynthesis in regulatory tissues (Bell et al., 1997; Sargent et al., 1999; Tocher et al., 2000).

Vegetable oils have fatty acid profiles that more closely resemble those of the natural prey of freshwater fish than do marine fish oils. Thus, it has been proposed that it may be beneficial to use vegetable oils in feeds formulated for salmon parr held in fresh water (Bell et al., 1994, 1997). On the other hand, $n-3$ highly unsaturated fatty acids ($n-3$ HUFAs) are more commonly encountered by wild salmon in the marine environment (Higgs et al., 1995; Sargent et al., 2002). The effects of providing a dietary shift in fatty acid composition between the freshwater and seawater rearing phases of Atlantic salmon do not seem to have been investigated in detail. The purpose of the present study was to investigate how shifts in dietary fatty acids and fat contents affect the performance of Atlantic salmon smolts.

## 2. Materials and methods

Four dry extruded pellet feeds were produced at the BioMar Technology Center, Brande, Denmark. Sand eel (*Ammodytes* spp.) oil or a blend of rapeseed (*Brassica* sp.) oil

URN:NBN:no-6435

and linseed (*Linum* sp.) oil (ratio 7:3 by weight) were used as fat sources, and fish meal and wheat were the other main feed ingredients (Table 1). The feeds comprised 340 g $kg^{-1}$ fat and 400 g $kg^{-1}$ protein or 210 g $kg^{-1}$ fat and 500 g $kg^{-1}$ protein; they were designated LFFO, LFVO, HFFO and HFVO according to fat level (LF—low fat; HF—high fat) and oil source (FO—fish oil; VO—vegetable oil). Feeds containing vegetable oils had high concentrations of oleic (18:1$n-9$), linoleic (18:2$n-6$) and linolenic (18:3$n-3$) acids and lower concentrations of EPA and DHA (docosahexaenoic acid, 22:6$n-3$) compared to feeds containing fish oil. Due to differences in the oil contents, the dietary level of fatty acids differed between the low-fat and high-fat feeds formulated with the same oil source, as indicated in Table 2.

Feed dry matter contents were determined by drying at 105 °C for 24 h. Crude protein contents were estimated by Kjeldahl analyses (N×6.25, Kjeltec Autoanalyser, Tecator, Sweden), crude fat was estimated on acid hydrolysed samples (3 M HCl) using the Soxhlet method with petroleum ether extraction, and ash was determined by combustion at 550 °C for 16 h. Feed energy contents were determined by bomb calorimetry (Parr adiabatic bomb calorimeter).

Lipids were also extracted using chloroform:methanol:water (Bligh and Dyer, 1959). Methyl esters were prepared by alkali transesterification with 0.5 M NaOH in methanol (100 °C, 15 min), followed by methylation of free fatty acids in 12 % boron–trifluoride–methanol (Metcalfe et al., 1966). Methyl esters, extracted in isooctane, were separated by gas chromatography using a Perkin Elmer Auto System XL gas chromatograph equipped with a split/splitless injector fitted to a fused silica capillary column (CP Wax 52CB,

Table 1
Ingredient and analysed composition of the test feeds

| | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| *Ingredients, percent of recipe* | | | | |
| Fish meal[a] | 63.8 | 63.8 | 48.6 | 48.6 |
| Wheat | 19.0 | 19.0 | 17.8 | 17.8 |
| Sand eel oil | 14.0 | | 27.0 | |
| Rapeseed oil[b] | | 10.4 | | 20.0 |
| Linseed oil[b] | | 3.6 | | 7.0 |
| Monosodium phosphate | 1.0 | 1.0 | 2.5 | 2.5 |
| Premixes | 1.2 | 1.2 | 1.2 | 1.2 |
| Fat absorber[c] | 1.0 | 1.0 | 3.0 | 3.0 |
| *Analysed composition, percent DM* | | | | |
| Dry matter | 94.5 | 94.1 | 96.4 | 96.3 |
| Crude protein | 50.2 | 50.4 | 40.3 | 40.2 |
| Crude fat | 20.7 | 21.4 | 33.5 | 33.9 |
| Ash | 9.1 | 9.3 | 10.3 | 10.4 |
| Residue | 14.5 | 13.0 | 12.3 | 11.7 |
| Gross energy, MJ $kg^{-1}$ | 22.5 | 22.5 | 24.8 | 24.5 |
| Calculated P/E ratio | 22.3 | 22.4 | 16.3 | 16.4 |

Codes are as follows: HF, high fat; LF, low fat; FO, fish oil; and VO, vegetable oil.
[a] Ultra Flash fish meal purchased from Fiskernes Fiskeindustri A.M.B.A., Denmark.
[b] The vegetable oils were neutralised, bleached and deodorised oils purchased from Superfos Agro, Denmark.
[c] Diatomaceous earth purchased from Damolin AS, Denmark.

URN:NBN:no-6435

Table 2
Relative content (%) of fatty acid classes and selected fatty acids of total fat in the test feeds. Estimates of corresponding concentrations (g kg$^{-1}$) are given in italics within parentheses. Feed codes are given in Table 1

| Fatty acids | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| 14:0 | 5.7 *(10.1)* | 1.5 *(2.8)* | 6.0 *(18.5)* | 0.8 *(2.5)* |
| 16:0 | 13.4 *(23.5)* | 7.0 *(12.9)* | 13.6 *(41.6)* | 5.7 *(17.3)* |
| 18:0 | 2.0 *(3.4)* | 2.3 *(4.3)* | 1.9 *(5.7)* | 2.3 *(7.0)* |
| ΣSAFAs | 21.4 *(37.7)* | 11.6 *(21.4)* | 21.9 *(67.0)* | 9.9 *(29.8)* |
| 16:1 | 5.1 *(8.9)* | 1.2 *(2.3)* | 5.4 *(16.5)* | 0.7 *(2.2)* |
| 18:1*n*−9 | 8.2 *(14.4)* | 38.4 *(70.7)* | 7.3 *(22.3)* | 43.3 *(130.9)* |
| 20:1 | 10.8 *(18.9)* | 3.1 *(5.7)* | 11.4 *(34.9)* | 2.2 *(6.8)* |
| 22:1 | 15.9 *(28.0)* | 3.8 *(7.0)* | 17.1 *(52.1)* | 2.0 *(6.0)* |
| ΣMUFAs | 43.1 *(75.8)* | 49.3 *(90.7)* | 44.3 *(135.3)* | 50.6 *(153.2)* |
| 18:2*n*−6 | 3.1 *(5.5)* | 15.1 *(27.8)* | 2.4 *(7.3)* | 16.8 *(50.7)* |
| 18:3*n*−3 | 2.0 *(3.6)* | 15.7 *(28.9)* | 1.6 *(5.0)* | 18.3 *(55.5)* |
| 18:4 | 4.2 *(7.4)* | 0.9 *(1.7)* | 4.4 *(13.5)* | 0.5 *(1.4)* |
| 20:4*n*−6 | 0.6 *(1.0)* | 0.2 *(0.3)* | 0.5 *(1.7)* | 0.1 *(0.2)* |
| 20:5*n*−3 | 10.1 *(17.7)* | 2.1 *(3.9)* | 10.8 *(32.9)* | 1.1 *(3.4)* |
| 22:6*n*−3 | 12.6 *(22.1)* | 4.3 *(7.9)* | 11.8 *(36.0)* | 2.2 *(6.6)* |
| ΣPUFAs | 35.5 *(62.5)* | 39.1 *(71.9)* | 33.8 *(103.4)* | 39.5 *(119.6)* |
| *n*−3/*n*−6 ratio | 6.7 | 1.4 | 8.2 | 1.3 |

Chrompak, 25 m×0.25 mm i.d.) and a flame-ionisation detector. Helium was used as the mobile phase. Temperature was increased at 30 °C min$^{-1}$ from 90 to 150 °C, and thereafter at 3 °C min$^{-1}$ to 225 °C; the total running time was 35 min. Operating temperatures for the injector and the detector were set at 250 and 280 °C, respectively. The fatty acids were identified automatically using Turbochrom software by reference to fatty acid ester standards (68D, Nu-Chek-Prep., Minnesota, USA). Sums of fatty acid esters in crude fat were determined by adding C17:0 internal standard to the crude fat followed by extraction of methyl esters in petroleum ether. Concentrations of fatty acids in feeds (Table 2) were estimated by combining information about the proportion of fatty acids in extracted fat (85–91%) with that of corresponding fat contents (Table 1).

The experiment was carried out with 1+ Atlantic salmon (*Salmo salar* L.) smolts of the AquaGen strain (AquaGen AS, Kyrksæterøra, Norway). Fish with an initial weight 19.1 g (±4.3 g; overall mean±S.D.) were reared in fresh water (2 °C; 12L:12D photoperiod) for 6 months (October 1999–May 2000) as described by Bendiksen et al. (2002). During this time there were triplicate circular fibreglass tanks (260 l), each stocked with 150 fish, for each feed treatment. The water flows (8–10 l min$^{-1}$) and current speeds (8–10 cm s$^{-1}$) were maintained the same in all tanks. On 10 May 2000, fish with external signs of sexual maturation were discarded, and 60–70 fish from each tank were then individually tagged using a colour code to indicate feeding history (FTF-69, Floy Tag and Manufacturing, Seattle, USA). On 29 May 2000 (about 8 months after introduction of 12L:12D) the fish were exposed to continuous light (24L:0D), and water temperature was increased to 8 °C. No further changes in photoperiod and water temperature were made thereafter. The square-wave photoperiod regime used here has been shown to induce parr–smolt transformation (e.g. Duston and Saunders, 1992; Sigholt et al., 1995; Handeland and Stefansson, 2001; Nordgarden et al., 2002). Smolt status was assessed by monitoring

URN:NBN:no-6435

fish survival and plasma chloride concentrations in seawater challenge tests (Blackburn and Clarke, 1987; Clarke, 2000). Seawater challenge tests were conducted by transferring 12 fish, taken at random from each feed group, directly from fresh water to circular tanks (260 l) supplied with running seawater (6–8 l $min^{-1}$, 32.7–33.1 ‰ salinity, 8 °C). After 24 h, mortality was recorded, and blood was sampled from the fish for plasma chloride and osmolality analysis. Fish were weighed, samples of gill tissue were taken for analysis of $Na^+$,$K^+$-ATPase activity, and muscle samples were taken for determination of water content. On 20 June 2000, the results of the seawater challenge test indicated that the fish had plasma chloride values characteristic for smolts. Additional samples were also taken on 24 June, 1 day prior to the transfer of the fish to seawater. One fish died during the freshwater period.

On 25 June the fish were transferred to eight circular tanks (260 l) supplied with running seawater (32.7–33.1 ‰ salinity, 8 °C). There were 50–60 fish per tank, derived from two different feed treatment groups. These fish were fed either LFFO (four tanks) or HFFO (four tanks) giving replicated groups for all eight combinations of freshwater and seawater feeds, as indicated in Table 3. Consequently, groups of 50–60 fish were subjected to one of eight feed treatments: no dietary shift, shift in feed oil type, shift in feed fat (energy) content or shift both in feed oil type and feed fat at the time of seawater transfer (Table 3).

Feed was supplied between 1000 and 2100 each day by means of automatic disc feeders. The feed requirement was calculated from expected specific growth rates (Austreng et al., 1987) assuming a conversion ratio of 1:1, and to ensure excess feeding the supply exceeded the estimated requirement by 20%.

Fish weights were monitored 3, 21 and 42 days after transfer to seawater, and samples were taken for analysis of plasma chloride and osmolality, and gill $Na^+$,$K^+$-ATPase activity (Day 42 only). Fish ($n$=12 per treatment) taken for sampling of blood and gill tissue were the first to be netted from each tank. These fish were killed by anaesthesia (benzocaine, *p*-aminobenzoic acid ethyl ester, 300 mg $l^{-1}$), weight measured, and blood was collected from the caudal vessels within 90 s using a syringe-vacuum tube system (Venoject tubes with Li-heparin added, Terumo, Leuven, Belgium). Blood samples were stored on ice for

Table 3
Experimental set-up indicating feed treatment during rearing in fresh water (FW—four feeds) and during the subsequent period of seawater (SW—two feeds) rearing. The change of feed type between freshwater and seawater rearing is indicated. Feed codes are given in Table 1

| Treatment | | Type of change (FW →SW) | |
|---|---|---|---|
| FW | SW | Feed fat content | Feed oil source |
| LFFO | LFFO | No change | |
| LFVO | LFFO | | VO→FO |
| HFFO | LFFO | HF→LF | |
| HFVO | LFFO | HF→LF | VO→FO |
| LFFO | HFFO | LF→HF | |
| LFVO | HFFO | LF→HF | VO→FO |
| HFFO | HFFO | No change | |
| HFVO | HFFO | | VO→FO |

URN:NBN:no-6435

max 15 min, centrifuged (2780 rpm, 8 min), and plasma was removed and stored at −80 °C until analysed for chloride (Corning 925, Ciba Corning Diagnostics, Essex, England) and osmolality (Fiske One-Ten Osmometer, Fiske Associates, Massachusetts, USA). Samples for analysis of gill $Na^+,K^+$-ATPase activity were taken immediately after blood sampling. Tissue, sampled from the second gill arch on the left side of each fish, was immediately placed into plastic tubes with ice-cold isotonic SEI-solution (0.3 M sucrose, 0.02 M $Na_2EDTA$, and 0.1 M imidazole), held on ice for max 10 min, and then stored at −80 °C until analysis. Gill $Na^+$-$K^+$-ATPase activity was determined according to the procedure of McCormick (1993), and expressed as μmol ADP mg $protein^{-1}$ $h^{-1}$. Muscle tissue (2–3 g) was taken from the region between the dorsal fin and the lateral line, weighed to the nearest 0.01 g, and stored at −80 °C until being analysed for water content (determined as loss of weight after 24 h drying at 105 °C). All sampled fish were dissected to check for maturity status. Samples of whole fish were stored prior to proximate body composition analysis, carried out as described by Johansen et al. (2001). Fatty acids were analysed following the same procedure as described for analysis of feed samples (see above). Following the destructive sampling, the weights (nearest 0.1 g) of all remaining fish in each tank were measured following anaesthesia (benzocaine, 65 mg $l^{-1}$). There were two mortalities during the seawater period.

Specific growth rates (SGR, % body weight $day^{-1}$) were calculated as $[(\ln W_T - \ln W_t)/(T-t)] \times 100$, where $W_t$ and $W_T$ are weights in g at times $t$ (start of growth period) and $T$ (end of growth period) and $T-t$ is the time in days between weighing (Jobling, 1994).

The data relating to plasma osmolality and chloride concentration, muscle water and gill $Na^+,K^+$-ATPase activity were normally distributed (Lilliefors test) and values are given as arithmetic means (±standard error of the mean, replicates pooled). Plasma chloride, muscle water and gill $Na^+,K^+$-ATPase activity data for the period prior to transfer to seawater were examined using a two-way ANOVA with feed and time as fixed factors. Subsequently, the Tukey's HSD multiple range test was used to locate significant feed effects, and one-way ANOVAs (Bonferroni adjusted probability levels) were used to examine for temporal effects within feed treatments. Body composition data were expressed as percentages and were arcsine-transformed before carrying out the statistical tests. The strength of association between fatty acid composition of feed and fish was tested by Pearson's correlation test. Data from the seawater phase (plasma osmolality, plasma chloride, gill $Na^+,K^+$-ATPase activity and muscle water) were initially examined using a three-way ANOVA with freshwater feed, seawater feed, time and their interactions as factors, and body weight as a covariate. When appropriate, effects were investigated further using one- or two-way ANOVAs with tank replicates nested under feed treatment, and body weight used as a covariate. Tukey's HSD multiple range tests were used for pairwise comparisons to identify where significant differences occurred. Data on fish weights were initially analysed using a two-way repeated measures ANOVA, with freshwater and seawater feeds as factors. Data on specific growth rate in seawater were not normally distributed. Values are presented as medians, and Kruskal–Wallis ANOVA and Mann–Whitney $U$-tests with Bonferroni adjusted $P$-values (post hoc multiple pairwise comparisons) were used for statistical testing. A Pearson Chi-square test was used to test for differences in the proportions of fish with positive growth in relation to total fish number per feed treatment during the seawater period. The data were analysed by the

URN:NBN:no-6435

General Linear Model (GLM) procedure in the SPSS for Windows (Version 10.0) statistical package. In all tests, a probability level of <0.05 was considered significant.

## 3. Results

The fish grew at similar rates in fresh water both during the parr stage (SGR; 0.40±0.01; tank mean±S.E.), and following the increase in temperature (2 °C→8 °C) and photoperiod (12L:12D→24L:0D) used to induce parr–smolt transformation (SGR; 0.64±0.01; tank mean±S.E.). The fish weighed 51.5 g (±8.1 g; overall mean±S.D.) at seawater transfer, and there were no significant differences in weights between the dietary groups.

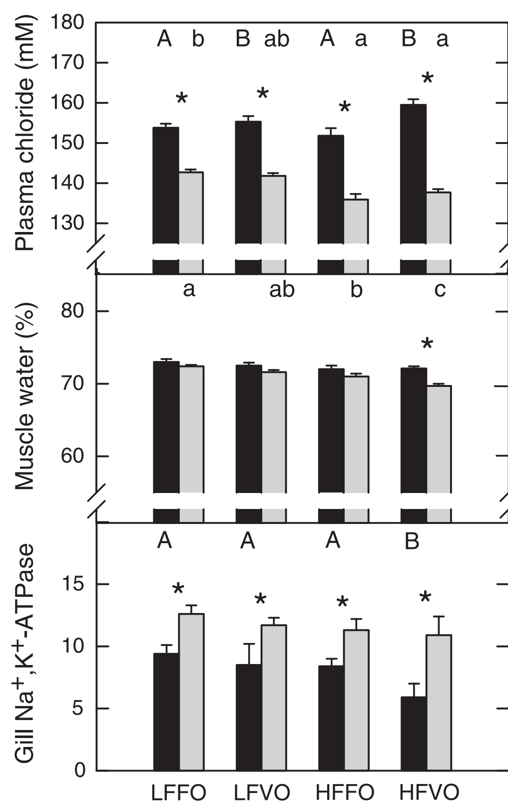


Fig. 1. Plasma chloride concentration (top panel), muscle water percent (middle panel) and gill $Na^+$,$K^+$-ATPase (μmol ADP mg protein$^{-1}$ h$^{-1}$) (bottom panel) of fish subjected to 24-h seawater challenge tests 3 weeks (black columns) and 5 days (grey columns) before seawater transfer. Data are given as means±S.E. ($n$=12). Different letters indicate significant differences between feed treatments, and an asterisk (*) significant differences between the test occasions.

URN:NBN:no-6435

Table 4
Proximate body composition (% wet weight) of Atlantic salmon sampled one day before (Start) and 42 days after seawater transfer. The feed codes are given in Table 1, and the experimental set-up is shown in Table 3. The data are means and standard errors (S.E.) for analyses made on samples of fish from three (start) or two (Day 42) replicate tanks

| FW feed | LFFO | | LFVO | | HFFO | | HFVO | |
|---|---|---|---|---|---|---|---|---|
| | Mean | S.E. | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| *Start* | | | | | | | | |
| Fat | $10.5^{b}$ | (0.4) | $11.0^{b}$ | (0.2) | $13.7^{a}$ | (0.2) | $13.8^{a}$ | (0.4) |
| Water | $69.9^{a}$ | (0.4) | $69.5^{a}$ | (0.2) | $67.1^{b}$ | (0.1) | $67.5^{b}$ | (0.3) |
| Protein | $17.6^{b}$ | (0.1) | $17.5^{ab}$ | (0.1) | $17.2^{a}$ | (0.1) | $16.7^{c}$ | (0.1) |
| Ash | 1.9 | (0.0) | 2.0 | (0.0) | 2.0 | (0.1) | 1.9 | (0.1) |
| *Day 42* | | | | | | | | |
| LFFO | | | | | | | | |
| Fat | $8.7^{+b}$ | (0.2) | $8.4^{+*b}$ | (0.1) | $11.8^{+a}$ | (0.0) | $12.0^{a}$ | (0.4) |
| Water | $72.4^{+}$ | (0.1) | $72.6^{+*}$ | (0.2) | $69.7^{+}$ | (0.1) | $69.9^{a}$ | (0.4) |
| Protein | $16.8^{+}$ | (0.2) | 16.9 | (0.3) | $16.4^{+}$ | (0.1) | $16.1^{+}$ | (0.0) |
| Ash | $2.1^{+}$ | (0.0) | $2.2^{+}$ | (0.0) | 2.1 | (0.0) | 2.1 | (0.0) |
| HFFO | | | | | | | | |
| Fat | $9.0^{b}$ | (0.2) | $10.3^{b}$ | (0.0) | $12.0^{+a}$ | (0.5) | $12.6^{a}$ | (0.2) |
| Water | $72.2^{+a}$ | (0.4) | $70.8^{+ab}$ | (0.2) | $69.6^{+b}$ | (0.6) | $69.0^{+b}$ | (0.1) |
| Protein | $16.7^{+}$ | (0.3) | $16.8^{+}$ | (0.2) | $16.3^{+}$ | (0.0) | $16.3^{+}$ | (0.0) |
| Ash | 2.1 | (0.1) | $2.1^{+}$ | (0.0) | 2.1 | (0.0) | 2.1 | (0.0) |

Superscripts indicate significant differences between the groups; different lower case letters indicate significant freshwater feed effects within sampling times and seawater feeds; + indicates significant differences between sampling times for given treatments; * indicates significant differences between seawater feeds compared within freshwater feed treatments.

Table 5
Relative content (%) of fatty acid classes and selected fatty acids of total fat in whole fish sampled one day before seawater transfer. Feed codes are given in Table 1

| Fatty acids | LFFO | LFVO | HFFO | HFVO |
|---|---|---|---|---|
| 14:0 | 4.3 | 2.2 | 4.7 | 1.4 |
| 16:0 | 15.5 | 11.6 | 14.7 | 8.8 |
| 18:0 | 2.9 | 3.3 | 2.3 | 2.6 |
| ΣSAFAs | 22.8 | 17.2 | 21.9 | 13.1 |
| 16:1 | 5.4 | 2.7 | 6.1 | 1.7 |
| 18:1$n-9$ | 18.6 | 36.5 | 14.3 | 40.4 |
| 20:1 | 8.6 | 4.4 | 10.4 | 3.4 |
| 22:1 | 8.1 | 1.3 | 10.1 | 2.2 |
| ΣMUFAs | 43.3 | 47.5 | 43.4 | 50.3 |
| 18:2$n-6$ | 4.2 | 10.9 | 3.2 | 13.5 |
| 18:3$n-3$ | 1.9 | 7.8 | 1.4 | 10.1 |
| 18:4 | 2.6 | 1.9 | 3.1 | 2.5 |
| 20:4$n-6$ | 0.4 | 0.2 | 0.4 | 0.2 |
| 20:5$n-3$ | 5.6 | 2.3 | 7.0 | 1.8 |
| 22:6$n-3$ | 15.5 | 8.8 | 15.5 | 5.8 |
| ΣPUFAs | 33.9 | 35.2 | 34.7 | 36.6 |
| $n-3/n-6$ ratio | 6.0 | 2.0 | 7.9 | 1.6 |

URN:NBN:no-6435

At the time of the increase in temperature and photoperiod, mean plasma chloride concentrations were within the range of 152–158 mM following the seawater challenge test, and 16 days later mean plasma chloride concentrations were 136–143 mM. On the latter date, plasma chloride concentrations were higher in the LFFO group (ANOVA, $P<0.05$) than in fish on the high-fat feed treatments (Fig. 1, top panel), and muscle water content was significantly lower in the HFVO fed fish than in those from other treatments (Fig. 1, middle panel). Gill $Na^+,K^+$-ATPase activity was lowest (ANOVA, $P<0.05$) in fish fed the HFVO feed at the first sampling after exposure of the fish to long-day conditions (Fig. 1, bottom panel). Enzyme activity increased significantly (ANOVA, $P<0.05$) in all groups prior to seawater transfer (Fig. 1, bottom panel).

Fish fed the high-fat feeds had significantly higher proportions of body fat (ANOVA, $P<0.001$) than those fed low-fat feeds (Table 4). Percentage fat was inversely related to whole body water percentage. Protein was within the range of 16.7–17.6%, and was lower in fish fed the HFVO feed than in fish on the other feed treatments. The fatty acid profiles of the fish (Table 5) were highly correlated with those of the feeds (Table 2, $r$=0.95–0.97;

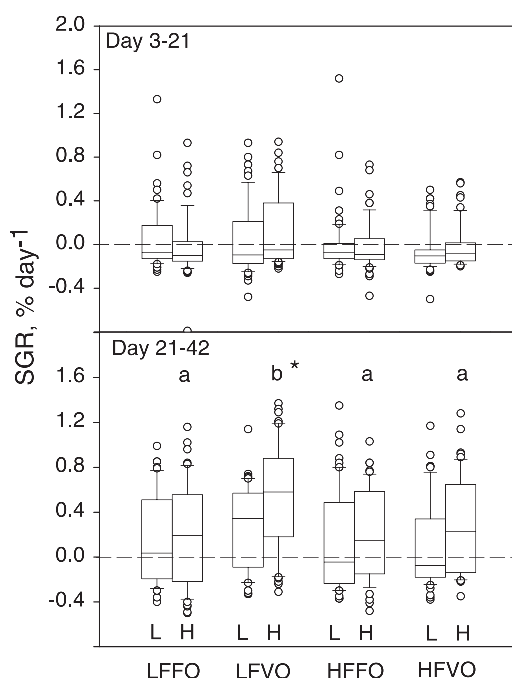


Fig. 2. Box-plots ($n$=50–60 in each plot) showing growth rates in seawater of fish fed four different feeds during rearing in fresh water (LFFO, LFVO, HFFO and HFVO), and subjected to new feeds at seawater entry (L=LFFO, H=HFFO). The box contains 50% of the data (90% of data when whiskers are included), while extreme values are indicated by circles. The horizontal line within each box indicates the median. An asterisk (*) indicates significant differences between L and H treatments, whereas different lower case letters indicate significant differences between freshwater feed groups within L or H treatments.

URN:NBN:no-6435

$P<0.01$). There were higher proportions of oleic, linoleic and linolenic acids in the fish fed vegetable oils whereas fish fed the fish oil-based feeds had higher proportions of EPA and DHA, and long-chain monoenoic fatty acids (Table 5).

Growth was poor during the first period in seawater (Day 3–21) irrespective of treatment, and there was weight loss during this period (Fig. 2). A subjective visual assessment based on treatment means seemed to indicate a larger proportion of fish with positive growth rates in fish previously fed the LFVO feed, but this effect was not significant (Table 6). Growth improved for all groups during the second period (Day 21–42), and the highest growth rate was seen in fish fed the LFVO feed in fresh water and the HFFO feed in seawater (Fig. 2). The growth rates of these fish were significantly higher than the growth rates of the fish fed the LFFO feed in seawater. The repeated measures ANOVA test of individual weights confirmed that the highest weights were achieved by the fish that experienced a shift in both lipid source and feed fat concentration (ANOVA, $P<0.001$). During the second period in seawater (Day 21–42) there were larger proportions of fish with positive growth rates amongst fish fed the LFVO feed in fresh water (Table 6). This was noted as a trend in the fish fed LFFO feed ($\chi^2$, $P=0.066$; test power; 0.60), while a significant effect was seen for fish fed the HFFO feed ($\chi^2$, $P<0.005$)(Table 6).

At the end of the trial, relative contents of fat and protein were reduced compared to the pre-transfer freshwater condition (Table 4). This was seen in fish exposed to all feed treatments. Nevertheless, the relative differences in whole body composition established during the freshwater phase of rearing were still discernible at the end of the seawater period.

There were significant differences in plasma osmolality (ANOVA, $P<0.001$) and plasma chloride concentrations (ANOVA, $P<0.001$) between samples taken at different times during the seawater period. However, the feed treatments applied during freshwater and seawater rearing did not affect either plasma chloride (Day 3; mean range; 145–150 mM; Day 21; mean range; 154–156 mM; Day 42; mean range; 150–154 mM) or plasma osmolality (Day 3: mean range, 374–389 mOsm; Day 21: mean range, 371–380 mosM kg$^{-1}$; Day 42: mean range, 348–354 mOsm) during the seawater phase. Gill $Na^+,K^+$-ATPase activity at the termination of the experiment (Day 42; mean range; 9–14 μmol

Table 6
Percentages of smolts with positive specific growth rates (+) and zero or negative growth (−) in seawater in relation to the feed treatments given during the freshwater and seawater rearing phases (n=50–60). Feed codes are given in Table 1

| | LFFO | | LFVO | | HFFO | | HFVO | | Sign. differences (Chi-square) |
|---|---|---|---|---|---|---|---|---|---|
| | + | − | + | − | + | − | + | − | |
| *Low-fat feed* | | | | | | | | | |
| Day 3–21 | 38 | 62 | 33 | 67 | 29 | 71 | 21 | 79 | $P=0.266$ |
| Day 21–42 | 54 | 46 | 68 | *32** | 48 | 52 | 42 | 58 | *$P=0.066$ |
| *High-fat feed* | | | | | | | | | |
| Day 3–21 | 28 | 72 | 38 | 62 | 34 | 66 | 27 | 73 | $P=0.599$ |
| Day 21–42 | 49 | 51 | 81 | *19** | 52 | 48 | 59 | 41 | *$P=0.005$ |

URN:NBN:no-6435

ADP mg protein$^{-1}$ h$^{-1}$) did not differ between fish subjected to the different feed treatments.

## 4. Discussion

Even though they differed in body composition the fish from all four freshwater feed treatment groups underwent parr–smolt transformation. Three weeks after exposing the fish to 24L:0D, plasma chloride was below 150 mM following the 24-h seawater challenge. This is a level regarded as normal for salmon smolt (e.g. Sigholt et al., 1995; Clarke et al., 1996). Gill $Na^{+}$,$K^{+}$-ATPase activity increased after long-day provision and reached a level considered characteristic for salmon smolt prior to seawater transfer (McCormick et al., 1995; Handeland and Stefansson, 2001).

The growth rates of the fish were low throughout the 42-day seawater period, and many fish lost weight during the first 3 weeks of seawater rearing. This is in accordance with several previous reports (Jørgensen and Jobling, 1994; Stead et al., 1996; Arnesen et al., 1998; Handeland et al., 2000). Seawater growth may also have been influenced by the temperature and light regime applied prior to seawater transfer (Sigholt et al., 1998), although the indices used to assess smolt status were within normal ranges (Fig. 1). The growth of the fish seemed to be affected by previous feeding history, although no growth differences had been observed when the fish were held in fresh water. Fish that had been fed the LFVO feed in fresh water grew best, when both feed oil source and fat content were changed (i.e. from LFVO to HFFO) on transfer to seawater. Thus, inclusion of vegetable oils in the feed given to the salmon parr combined with an increased supply of $n-3$ HUFAs and/or energy in the feed provided to the fish in seawater gave a positive growth effect.

The observed growth differences in seawater may be related to changes in lipid metabolism, which is regarded as being an integral part of parr–smolt transformation (Sheridan et al., 1983, 1985; Ackman and Takeuchi, 1986; Sheridan, 1989; Bell et al., 1997; Tocher et al., 2000). Freshwater fish appear to have the ability to elongate and desaturate C18 fatty acids to longer-chain HUFAs (Henderson and Tocher, 1987). Salmon parr also seem to have this capability, and Atlantic salmon fed vegetable oils have a greater capacity for enzymatic conversion of C18 ($n-3$) and ($n-6$) fatty acids during parr–smolt transformation than do conspecifics given feeds containing marine fish oils (Bell et al., 1997). Consequently, it has been suggested that the differences in fatty acid compositions between vegetable oils and marine fish oils may influence parr–smolt transformation of farmed salmon (Bell et al., 1994, 1997; Tocher et al., 2000). Post-smolts have a reduced $\Delta$5-desaturase enzyme activity compared to parr (Bell et al., 1997), which may imply that more $n-3$ HUFAs must be supplied in the feed during seawater rearing. It is possible that the growth differences that we observed in the fish following transfer to seawater relate to changes in their fatty acid requirements: the observation of best growth in the fish fed LFVO in fresh water and HFFO in seawater lends support to this. This may suggest that a high dietary level of $n-3$ HUFAs is required after seawater transfer, to provide the fish with the fatty acids typical for the marine environment (Higgs et al., 1995; Sargent et al., 2002).

URN:NBN:no-6435

It has been reported that fish fed a feed containing vegetable oil had lower plasma chloride concentrations following a seawater challenge than did those fed fish oils (Bell et al., 1997; Tocher et al., 2000). In the present study there were no differences in plasma chloride, plasma osmolality and gill $Na^{+}$,$K^{+}$-ATPase activity across feed treatments. Therefore, the observed growth differences did not seem to relate directly to differences in osmoregulatory capacity between fish on the different feed treatments.

Aerobic metabolism in fish is, in part, fuelled by fatty acid oxidation, and monoenoic fatty acids are the preferred substrate (Henderson and Sargent, 1985; Kiessling and Kiessling, 1993; Henderson, 1996; McKenzie, 2001). In the present study, oleic acid represented a higher proportion of the fatty acids in the feeds containing vegetable oils than those formulated with fish oil, while the fish oil-based feeds contained higher proportions of long-chain monoenoic fatty acids. Consequently, more monoenoic fatty acids accumulated in the fish fed the LFVO and HFVO feeds in fresh water than in fish fed the fish oil-based diets. Thus, the fat stores of the fish fed the vegetable oils may have contained a surplus of preferred energy substrate compared to fish fed feeds with marine fish oil. It is possible that these fat stores could have been readily mobilised for catabolism in the critical period following transfer of the fish to seawater, at a time when the appetite of the fish may be suppressed (Usher et al., 1991; Jørgensen and Jobling, 1994; Arnesen et al., 1998). The growth of the smolt fed the LFVO feed during freshwater residence was also better than that of the smolt previously fed the HFVO feed. This may be related to the higher fat accumulation in fish fed the high-fat feeds in the fresh water because increased body fat may have a negative impact on appetite (Jobling and Johansen, 1999). Due to the pooling of groups with different feed history at seawater transfer in the present trial, feed intake could not be measured during seawater rearing. However, data from the pre-smolt stage revealed a negative correlation between feed energy content and cumulative feed intake of the fish (Bendiksen et al., 2002), and this trend may have been sustained throughout seawater rearing.

Parr–smolt transformation is an energy demanding process, and there may also be increased maintenance costs in seawater (Boeuf and Payan, 2001). Smolts transferred to seawater have been reported to have a reduced lipid content compared to fish retained in fresh water (Woo et al., 1978; Sheridan et al., 1983; Usher et al., 1991). In addition, the transfer of smolt to seawater is often followed by a period with reduced feed intake and growth (Usher et al., 1991; Jørgensen and Jobling, 1994; Arnesen et al., 1998), leading to some depletion of fat stores (Jobling et al., 2002). The fish originating from the LFVO freshwater group grew better when given HFFO, rather than LFFO, feed in seawater: this could be related to the energy density differences between these two feeds. The higher energy density HFFO feed may have more readily fulfilled the energy needs of the fish during a period when they were feeding poorly.

In summary, the results show that feeding history may be important for the growth of Atlantic salmon in the period immediately following the transfer of smolt from fresh water to seawater. Best growth was seen when fish fed a low-fat vegetable oil-based feed in fresh water were provided with a high-fat (energy) feed containing marine fish oil following transfer to seawater. Whether this was an effect of increased supply of certain fatty acids from marine fish oil, increased feed energy supply per se, or a combination of both, remains to be elucidated.

URN:NBN:no-6435

## Acknowledgements

This work was carried out with support from BioMar AS and the Norwegian Research Council grant no. 130690/120. We would like to thank the staff of HiT, Tromsø, for husbandry of the fish. Jo Espen T. Strand and Siv Andreassen are acknowledged for their excellent technical assistance during sampling.

## References

Ackman, R.G., Takeuchi, T., 1986. Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon *Salmo salar*. Lipids 21, 117–120.

Arnesen, A.M., Johnsen, H.K., Mortensen, A., Jobling, M., 1998. Acclimation of Atlantic salmon (*Salmo salar* L.) smolts to 'cold' seawater following direct transfer from fresh water. Aquaculture 168, 351–367.

Austreng, E., Storebakken, T., Åsgård, T., 1987. Growth rate estimates for cultured Atlantic salmon and rainbow trout. Aquaculture 60, 157–160.

Bell, J.G., Youngson, A., Mitchell, A.I., Cowey, C.B., 1989. The effects of enhanced intake of linoleic acid on the fatty acid composition of tissue polar lipids of post-smolt Atlantic salmon (*Salmo salar*). Lipids 24, 240–242.

Bell, J.G., Ghioni, C., Sargent, J.R., 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. Aquaculture 128, 301–313.

Bell, J.G, Tocher, D.R., Farndale, B.M., Cox, D.I., McKinney, R.W., Sargent, J.R., 1997. The effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation. Lipids 32, 515–525.

Bendiksen, E.Å., Jobling, M., Arnesen, A.M., 2002. Feed intake of Atlantic salmon parr *Salmo salar* L. in relation to temperature and feed composition. Aquac. Res. 33, 525–532.

Bergström, E., 1989. Effect of natural and artificial diets on seasonal changes in fatty acid composition and total body lipid content of wild and hatchery-reared Atlantic salmon (*Salmo salar* L.) parr–smolt. Aquaculture 82, 205–217.

Blackburn, J., Clarke, W.E., 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515 (35 pp.).

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Boeuf, G., 1993. Salmonid smolting: a pre-adaptation to oceanic environment. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman & Hall, London, pp. 105–133.

Boeuf, G., Payan, P., 2001. How should salinity influence fish growth? Comp. Biochem. Physiol. 130C, 411–423.

Clarke, W.C., 2000. Smolting. In: Stickney, R.R. (Ed.), Encyclopedia of Aquaculture. Wiley, New York, pp. 879–884.

Clarke, W.C., Saunders, R.L., McCormick, S.D., 1996. Smolt production. In: Pennell, W., Barton, B.A. (Eds.), Principles of Salmonid Culture. Elsevier, Amsterdam, pp. 517–567.

Duston, J., Saunders, R.L., 1992. Effect of 6-, 12-, and 18-month photoperiod cycles on smolting and sexual maturation in juvenile Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 49, 2273–2279.

Handeland, S.O., Berge, Å., Björnsson, B.T., Lie, Ø., Stefansson, S.O., 2000. Seawater adaptation by out-of-season Atlantic salmon (*Salmo salar* L.) smolts at different temperatures. Aquaculture 181, 377–396.

Handeland, S.O., Stefansson, S.O., 2001. Photoperiod control and influence of body size on off-season parr–smolt transformation and post-smolt growth. Aquaculture 192, 291–307.

Henderson, R.J., 1996. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. Arch. Anim. Nutr. 49, 5–22.

Henderson, R.J., Sargent, J.R., 1985. Chain length specificities of mitochondrial and peroxisomal β-oxidation of fatty acids in livers of rainbow trout. Comp. Biochem. Physiol. 82B, 79–85.

Henderson, R.J., Tocher, D.R., 1987. The lipid composition and biochemistry of freshwater fish. Prog. Lipid Res. 26, 281–347.

URN:NBN:no-6435

Higgs, D.A., MacDonald, J.S., Levings, C.D., Dosanjh, B.S., 1995. Nutrition and feeding habits in relation to life history stage. In: Groot, C., Margolis, L., Clarke, W.C. (Eds.), Physiological Ecology of Pacific Salmon. UBC Press, Vancouver, pp. 161–315.

Hoar, W.S., 1988. The physiology of smolting salmonids. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. XIB. Academic Press, New York, pp. 275–343.

Jobling, M., 1994. Fish Bioenergetics. Chapman & Hall, London. 309 pp.

Jobling, M., Johansen, S.J.S., 1999. The lipostat, hyperphagia and catch-up growth. Aquac. Res. 30, 473–478.

Jobling, M., Andreassen, B., Larsen, A.V., Olsen, R.L., 2002. Fat dynamics of Atlantic salmon, *Salmo salar* L., smolt during early seawater growth. Aquac. Res. 33, 739–745.

Johansen, S.J.S., Ekli, M., Stangnes, B., Jobling, M., 2001. Weight gain and lipid deposition in Atlantic salmon during compensatory growth: evidence for lipostatic regulation? Aquac. Res. 32, 963–974.

Jørgensen, E.H., Jobling, M., 1994. Feeding and growth of exercised and unexercised juvenile Atlantic salmon in freshwater, and performance after transfer to seawater. Aquac. Int. 2, 154–164.

Kiessling, K.-H., Kiessling, A., 1993. Selective utilization of fatty acids in rainbow trout (*Oncorhynchus mykiss* Walbaum) red muscle mitochondria. Can. J. Zool. 71, 248–251.

McCormick, S.D., 1993. Methods for non-lethal gill biopsy and measurements of $Na^+$, $K^+$-ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

McCormick, S.D., Saunders, R.L., 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am. Fish. Soc. Symp. 1, 211–229.

McCormick, S.D., Björnsson, B.Th., Sheridan, M., Eilertson, C., Carey, J.B., O'Dea, M., 1995. Increased daylength stimulates plasma growth hormone and gill $Na^+$, $K^+$-ATPase in Atlantic salmon (*Salmo salar*). J. Comp. Physiol., B 165, 245–254.

McKenzie, D.J., 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. Comp. Biochem. Physiol. 128A, 607–621.

Metcalfe, L.D., Schimtz, A.A., Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.

Mustafa, T., Srivastava, K.C., 1989. Prostaglandins (eicosanoids) and their role in ectothermic organisms. Adv. Comp. Environ. Physiol. 5, 157–207.

Nordgarden, U., Hemre, G.-I., Hansen, T., 2002. Growth and body composition of Atlantic salmon (*Salmo salar* L.) parr and smolt fed diets varying in protein and lipid contents. Aquaculture 207, 65–78.

Plotnikoff, M.D., Higgs, D.A., Markert, B.S., Dosanjh, B.S., McBride, J.R., Buckley, J.T., 1984. Nutrition and marine survival of chinook salmon (*Oncorhynchus tshawytscha*) II: potential role of smolt body composition. Can. Tech. Rep. Fish. Aquat. Sci. 1235, 1–17.

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.

Sargent, J., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.F., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, San Diego, pp. 153–218.

Sheridan, M.A., 1989. Alterations in lipid metabolism accompanying smoltification and seawater adaptation of salmonid fish. Aquaculture 82, 191–203.

Sheridan, M.A., Allen, W.V., Kerstetter, T.H., 1983. Seasonal variations in lipid composition of the steelhead trout, *Salmo gairdnerii* Richardson, associated with parr–smolt transformation. J. Fish Biol. 23, 125–134.

Sheridan, M.A., Allen, W.V., Kerstetter, T.H., 1985. Changes in the fatty acid composition of steelhead trout, *Salmo gairdnerii* Richardson, associated with parr–smolt transformation. Comp. Biochem. Physiol. 80B, 671–676.

Sigholt, T., Staurnes, M., Jakobsen, H.J., Åsgård, T., 1995. Effects of continuous light and short-day photoperiod on smolting, seawater survival and growth in Atlantic salmon (*Salmo salar*). Aquaculture 130, 373–388.

Sigholt, T., Åsgård, T., Staurnes, M., 1998. Timing of parr–smolt transformation in Atlantic salmon (*Salmo salar*): effects of changes in temperature and photoperiod. Aquaculture 160, 129–144.

Stead, S.M., Houlihan, D.F., McLay, H.A., Johnstone, R., 1996. Effect of ration and seawater transfer on food consumption and growth of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 53, 1030–1037.

Tocher, D.R., Bell, J.R., Dick, J.R., Henderson, R.J., McGhee, F., Michell, D., Morris, P.C., 2000. Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*) undergoing parr–smolt transformation and the effects of dietary linseed and rapeseed oils. Fish Physiol. Biochem. 23, 59–73.

URN:NBN:no-6435

Usher, M.L., Talbot, C., Eddy, F.B., 1991. Effects of transfer to seawater on growth and feeding in Atlantic salmon smolts (*Salmo salar* L.). Aquaculture 94, 309–326.
Woo, N.T.S., Bern, H.A., Nishioka, R.S., 1978. Changes in body composition associated with smoltification and premature transfer to seawater in coho salmon (*Oncorhynchus kisutch*) and king salmon (*O. tschawytscha*). J. Fish Biol. 13, 421–428.

URN:NBN:no-6435

URN:NBN:no-6435

**Doctoral theses in Biology**

**Norwegian University of Science and Technology**

| Year | Name | Degree | Title |
|---|---|---|---|
| 1974 | Tor-Henning Iversen | Dr. philos Botany | The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism |
| 1978 | Tore Slagsvold | Dr. philos. Zoology | Breeding events of birds in relation to spring temperature and environmental phenology. |
| 1980 | Arnfinn Langeland | Dr. philos. Zoology | Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake. |
| 1980 | Helge Reinertsen | Dr. philos Botany | The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton |
| 1982 | Gunn Mari Olsen | Dr. scient Botany | Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana* |
| 1982 | Dag Dolmen | Dr. philos. Zoology | Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation. |
| 1984 | Eivin Røskaft | Dr. philos. Zoology | Sociobiological studies of the rook *Corvus frugilegus.* |
| 1984 | Anne Margrethe Cameron | Dr. scient Botany | Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats |
| 1984 | | Dr. scient Botany | Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test |
| 1985 | Jarle Mork | Dr. philos. Zoology | Biochemical genetic studies in fish. |
| 1985 | John Solem | Dr. philos. Zoology | Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains. |
| 1985 | Randi E. Reinertsen | Dr. philos. Zoology | Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds. |
| 1986 | Bernt-Erik Sæther | Dr. philos. Zoology | Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach. |
| 1986 | Torleif Holthe | Dr. philos. Zoology | Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna. |
| 1987 | Helene Lampe | Dr. scient. Zoology | The function of bird song in mate attraction and territorial defence, and the importance of song repertoires. |
| 1987 | Olav Hogstad | Dr. philos. Zoology | Winter survival strategies of the Willow tit *Parus montanus*. |
| 1987 | Jarle Inge Holten | Dr. philos Bothany | Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway |

URN:NBN:no-6435

| | | |
|---|---|---|
| 1987 Rita Kumar | Dr. scient<br>Botany | Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium* |
| 1987 Bjørn Åge Tømmerås | Dr. scient.<br>Zoology | Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction. |
| 1988 Hans Christian Pedersen | Dr. philos.<br>Zoology | Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care. |
| 1988 Tor G. Heggberget | Dr. philos.<br>Zoology | Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure. |
| 1988 Marianne V. Nielsen | Dr. scient.<br>Zoology | The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*). |
| 1988 Ole Kristian Berg | Dr. scient.<br>Zoology | The formation of landlocked Atlantic salmon (*Salmo salar* L.). |
| 1989 John W. Jensen | Dr. philos.<br>Zoology | Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth. |
| 1989 Helga J. Vivås | Dr. scient.<br>Zoology | Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*. |
| 1989 Reidar Andersen | Dr. scient.<br>Zoology | Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation. |
| 1989 Kurt Ingar Draget | Dr. scient<br>Botany | Alginate gel media for plant tissue culture, |
| 1990 Bengt Finstad | Dr. scient.<br>Zoology | Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season. |
| 1990 Hege Johannesen | Dr. scient.<br>Zoology | Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung. |
| 1990 Åse Krøkje | Dr. scient<br>Botany | The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test |
| 1990 Arne Johan Jensen | Dr. philos.<br>Zoology | Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams. |
| 1990 Tor Jørgen Almaas | Dr. scient.<br>Zoology | Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues. |
| 1990 Magne Husby | Dr. scient.<br>Zoology | Breeding strategies in birds: Experiments with the Magpie *Pica pica*. |
| 1991 Tor Kvam | Dr. scient.<br>Zoology | Population biology of the European lynx (*Lynx lynx*) in Norway. |
| 1991 Jan Henning L'Abêe Lund | Dr. philos.<br>Zoology | Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular. |
| 1991 Asbjørn Moen | Dr. philos<br>Botany | The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands |

URN:NBN:no-6435

| | | |
|---|---|---|
| 1991 Else Marie Løbersli | Dr. scient Botany | Soil acidification and metal uptake in plants |
| 1991 Trond Nordtug | Dr. scient. Zoology | Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods. |
| 1991 Thyra Solem | Dr. scient Botany | Age, origin and development of blanket mires in Central Norway |
| 1991 Odd Terje Sandlund | Dr. philos. Zoology | The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism. |
| 1991 Nina Jonsson | Dr. philos. | Aspects of migration and spawning in salmonids. |
| 1991 Atle Bones | Dr. scient Botany | Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase) |
| 1992 Torgrim Breiehagen | Dr. scient. Zoology | Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher. |
| 1992 Anne Kjersti Bakken | Dr. scient Botany | The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.) |
| 1992 Tycho Anker-Nilssen | Dr. scient. Zoology | Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica* |
| 1992 Bjørn Munro Jenssen | Dr. philos. Zoology | Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks. |
| 1992 Arne Vollan Aarset | Dr. philos. Zoology | The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans. |
| 1993 Geir Slupphaug | Dr. scient Botany | Regulation and expression of uracil-DNA glycosylase and $O^6$-methylguanine-DNA methyltransferase in mammalian cells |
| 1993 Tor Fredrik Næsje | Dr. scient. Zoology | Habitat shifts in coregonids. |
| 1993 Yngvar Asbjørn Olsen | Dr. scient. Zoology | Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects. |
| 1993 Bård Pedersen | Dr. scient Botany | Theoretical studies of life history evolution in modular and clonal organisms |
| 1993 Ole Petter Thangstad | Dr. scient Botany | Molecular studies of myrosinase in Brassicaceae |
| 1993 Thrine L. M. Heggberget | Dr. scient. Zoology | Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*. |
| 1993 Kjetil Bevanger | Dr. scient. Zoology | Avian interactions with utility structures, a biological approach. |
| 1993 Kåre Haugan | Dr. scient Bothany | Mutations in the replication control gene trfA of the broad host-range plasmid RK2 |
| 1994 Peder Fiske | Dr. scient. Zoology | Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek. |
| 1994 Kjell Inge Reitan | Dr. scient Botany | Nutritional effects of algae in first-feeding of marine fish larvae |

URN:NBN:no-6435

| Year | Name | Degree | Title |
|---|---|---|---|
| 1994 | Nils Røv | Dr. scient. Zoology | Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*. |
| 1994 | Annette-Susanne Hoepfner | Dr. scient Botany | Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.) |
| 1994 | Inga Elise Bruteig | Dr. scient Bothany | Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers |
| 1994 | Geir Johnsen | Dr. scient Botany | Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses |
| 1994 | Morten Bakken | Dr. scient. Zoology | Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*. |
| 1994 | Arne Moksnes | Dr. philos. Zoology | Host adaptations towards brood parasitism by the Cockoo. |
| 1994 | Solveig Bakken | Dr. scient Bothany | Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply |
| 1995 | Olav Vadstein | Dr. philos Botany | The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions. |
| 1995 | Hanne Christensen | Dr. scient. Zoology | Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*. |
| 1995 | Svein Håkon Lorentsen | Dr. scient. Zoology | Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition. |
| 1995 | Chris Jørgen Jensen | Dr. scient. Zoology | The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity |
| 1995 | Martha Kold Bakkevig | Dr. scient. Zoology | The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport. |
| 1995 | Vidar Moen | Dr. scient. Zoology | Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations. |
| 1995 | Hans Haavardsholm Blom | Dr. philos Bothany | A revision of the *Schistidium apocarpum* complex in Norway and Sweden. |
| 1996 | Jorun Skjærmo | Dr. scient Botany | Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae. |
| 1996 | Ola Ugedal | Dr. scient. Zoology | Radiocesium turnover in freshwater fishes |
| 1996 | Ingibjørg Einarsdottir | Dr. scient. Zoology | Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines. |
| 1996 | Christina M. S. Pereira | Dr. scient. Zoology | Glucose metabolism in salmonids: Dietary effects and hormonal regulation. |
| 1996 | Jan Fredrik Børseth | Dr. scient. Zoology | The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics. |

URN:NBN:no-6435

| | | |
|---|---|---|
| 1996 Gunnar Henriksen | Dr. scient. Zoology | Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region. |
| 1997 Gunvor Øie | Dr. scient Bothany | Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae. |
| 1997 Håkon Holien | Dr. scient Botany | Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters. |
| 1997 Ole Reitan | Dr. scient. Zoology | Responses of birds to habitat disturbance due to damming. |
| 1997 Jon Arne Grøttum | Dr. scient. Zoology | Physiological effects of reduced water quality on fish in aquaculture. |
| 1997 Per Gustav Thingstad | Dr. scient. Zoology | Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher. |
| 1997 Torgeir Nygård | Dr. scient. Zoology | Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as<br>Biomonitors. |
| 1997 Signe Nybø | Dr. scient. Zoology | Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway. |
| 1997 Atle Wibe | Dr. scient. Zoology | Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry. |
| 1997 Rolv Lundheim | Dr. scient. Zoology | Adaptive and incidental biological ice nucleators. |
| 1997 Arild Magne Landa | Dr. scient. Zoology | Wolverines in Scandinavia: ecology, sheep depredation and conservation. |
| 1997 Kåre Magne Nielsen | Dr. scient Botany | An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*. |
| 1997 Jarle Tufto | Dr. scient. Zoology | Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models |
| 1997 Trygve Hesthagen | Dr. philos. Zoology | Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters |
| 1997 Trygve Sigholt | Dr. philos. Zoology | Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet |
| 1997 Jan Østnes | Dr. scient. Zoology | Cold sensation in adult and neonate birds |
| 1998 Seethaledsumy Visvalingam | Dr. scient Botany | Influence of environmental factors on myrosinases and myrosinase-binding proteins. |
| 1998 Thor Harald Ringsby | Dr. scient. Zoology | Variation in space and time: The biology of a House sparrow metapopulation |

URN:NBN:no-6435

| | | |
|---|---|---|
| 1998 Erling Johan Solberg | Dr. scient. Zoology | Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment |
| 1998 Sigurd Mjøen Saastad | Dr. scient Botany | Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity. |
| 1998 Bjarte Mortensen | Dr. scient Botany | Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro. |
| 1998 Gunnar Austrheim | Dr. scient Botany | Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach. |
| 1998 Bente Gunnveig Berg | Dr. scient. Zoology | Encoding of pheromone information in two related moth species |
| 1999 Kristian Overskaug | Dr. scient. Zoology | Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach |
| 1999 Hans Kristen Stenøien | Dr. scient Bothany | Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts) |
| 1999 Trond Arnesen | Dr. scient Botany | Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway. |
| 1999 Ingvar Stenberg | Dr. scient. Zoology | Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos* |
| 1999 Stein Olle Johansen | Dr. scient Botany | A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis. |
| 1999 Trina Falck Galloway | Dr. scient. Zoology | Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.) |
| 1999 Torbjørn Forseth | Dr. scient. Zoology | Bioenergetics in ecological and life history studies of fishes. |
| 1999 Marianne Giæver | Dr. scient. Zoology | Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic |
| 1999 Hans Martin Hanslin | Dr. scient Botany | The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*. |
| 1999 Ingrid Bysveen Mjølnerød | Dr. scient. Zoology | Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques |
| 1999 Else Berit Skagen | Dr. scient Botany | The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces |
| 1999 Stein-Are Sæther | Dr. philos. Zoology | Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe |

URN:NBN:no-6435

| | | |
|---|---|---|
| 1999 Katrine Wangen Rustad | Dr. scient. Zoology | Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease |
| 1999 Per Terje Smiseth | Dr. scient. Zoology | Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*) |
| 1999 Gunnbjørn Bremset | Dr. scient. Zoology | Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions |
| 1999 Frode Ødegaard | Dr. scient. Zoology | Host spesificity as parameter in estimates of arhrophod species richness |
| 1999 Sonja Andersen | Dr. scient Bothany | Expressional and functional analyses of human, secretory phospholipase A2 |
| 2000 Salvesen, Ingrid | Dr. scient Botany | Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture |
| 2000 Ingar Jostein Øien | Dr. scient. Zoology | The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race |
| 2000 Pavlos Makridis | Dr. scient Botany | Methods for the microbial econtrol of live food used for the rearing of marine fish larvae |
| 2000 Sigbjørn Stokke | Dr. scient. Zoology | Sexual segregation in the African elephant (*Loxodonta africana*) |
| 2000 Odd A. Gulseth | Dr. philos. Zoology | Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard |
| 2000 Pål A. Olsvik | Dr. scient. Zoology | Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway |
| 2000 Sigurd Einum | Dr. scient. Zoology | Maternal effects in fish: Implications for the evolution of breeding time and egg size |
| 2001 Jan Ove Evjemo | Dr. scient. Zoology | Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species |
| 2001 Hilmo, Olga | Dr. scient Botany | Lichen response to environmental changes in the managed boreal forset systems |
| 2001 Ingebrigt Uglem | Dr. scient. Zoology | Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.) |
| 2001 Bård Gunnar Stokke | Dr. scient. Zoology | Coevolutionary adaptations in avian brood parasites and their hosts |
| 2002 Ronny Aanes | Dr. scient | Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) |
| 2002 Mariann Sandsund | Dr. scient. Zoology | Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses |
| 2002 Dag-Inge Øien | Dr. scient Botany | Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway |
| 2002 Frank Rosell | Dr. scient. Zoology | The function of scent marking in beaver (*Castor fiber*) |
| 2002 Janne Østvang | Dr. scient Botany | The Role and Regulation of Phospholipase $A_2$ in Monocytes During Atherosclerosis Development |

| | | |
|---|---|---|
| 2002 Terje Thun | Dr. philos Biology | Dendrochronical constructions of Norwegian conifer chronologies providing dating of historical material |
| 2002 Birgit Hafjeld Borgen | Dr. scient Biology | Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth |
| 2002 Bård Øyvind Solberg | Dr. scient Biology | Effects of climatic change on the growth of dominating tree species along major environmental gradients |
| 2002 Per Winge | Dr. scient Biology | The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and |
| 2002 Henrik Jensen | Dr. scient Biology | Causes and consequenses of individual variation in fitness-related traits in house sparrows |
| 2003 Jens Rohloff | Dr. philos Biology | Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control |
| 2003 Åsa Maria O. Espmark Wibe | Dr. scient Biology | Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L. |
| 2003 Dagmar Hagen | Dr. scient Biology | Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach |
| 2003 Bjørn Dahle | Dr. scient Biology | Reproductive strategies in Scandinavian brown bears |
| 2003 Cyril Lebogang Taolo | Dr. scient Biology | Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana |
| 2003 Marit Stranden | Dr.scient Biology | Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*) |
| 2003 Kristian Hassel | Dr.scient Biology | Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum* |
| 2003 David Alexander Rae | Dr.scient Biology | Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments |
| 2003 Åsa A Borg | Dr.scient Biology | Sex roles and reproductive behaviour in gobies and guppies: a female perspective |

URN:NBN:no-6435