Different growth performance, lipid deposition, and nutrient utilization in in-season (S1) Atlantic salmon post-smolt fed isoenergetic diets differing in protein-to-lipid ratio

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Highlights:

- High dietary protein-to-lipid ratio significantly increased feed consumption and growth rates during the autumn
- Low dietary lipid levels did not negatively affect feed conversion or nutrient retention
- Reduced feed intake among fish fed high lipid levels during autumn coincided with increased visceral mass and lipid levels
- Condition factor, carcass yield and body protein significantly differed between the dietary groups at trial termination

Statement of relevance

The present study confirms the importance of balanced dietary lipid-to-protein ratios for optimal production efficiency and nutrient utilization, and the significant effects of dietary and seasonal interaction on lipid deposition and production related parameters. To our knowledge, few have investigated the effect of isoenergetic diets differing in protein-to-lipid ratio on growth performance and nutrient utilization of juvenile Atlantic salmon reared in seawater under natural conditions. The experiment used feed formulations, fish breed and rearing conditions relevant for current commercial salmon farming practices.

Considering the current increase in the cost of lipid sources, it would be beneficial for the aquaculture industry if dietary lipid content could be reduced without compromising growth and feed utilization of the fish. We believe our findings will provide useful and relevant information regarding dietary formulations and nutritional knowledge for the global fish feed industry and salmon producers.

Abstract

The aim of the present study was to evaluate how isoenergetic diets with different protein-to-lipid ratio affects feed intake, growth performance, lipid deposition, feed and nutrient utilization in Atlantic salmon post-smolt. A 6-month's feeding trial was conducted with in-season (S1) Atlantic salmon post-smolt reared in the sea under natural conditions (May – September). Quadruple groups of salmon (initial weight 95 g) were fed two isoenergetic diet series formulated to contain a high (HP) and low (LP) protein-to-lipid ratio designed to resemble upper and lower levels of ratios used in commercial feeds. The group fed the HP diet had a significantly ($P \leq 0.05$) lower muscle fat content (HP = 4.7 %, LP = 5.7 %), whole body lipid (HP = 9.0 %, LP = 9.6 %) and energy content (HP = 7.7 MJ kg^{-1}, LP = 8.0 MJ kg^{-1}) than the group fed the LP diet after the period June-July. These differences were mainly due to significantly lower absolute apparent lipid retention in the summer period for post-smolt fed HP diet. In the subsequent experimental period (July-September), a significantly higher specific feed intake (HP = 1.38 %, LP = 1.33 %), thermal growth coefficient (HP = 3.82, LP = 3.46) and weight gain (HP = 658 g, LP = 552 g) were observed for fish fed the HP diet. The period from July – September was associated with higher water temperatures and declining day length. The reduced feed intake in the LP group coincide with increased visceral mass and lipid deposition, indicating a possible involvement of lipostatic regulation. The retention efficiency of nutrients increased with the up-regulation in feed consumption. The HP fed fish had a significantly higher whole body lipid retention (HP = 74.4 %, LP = 67.2 %), but significantly reduced visceral mass compared to LP fed fish during the autumn. The overall improved growth, good protein utilization and reduced visceral adiposity among the HP fed fish resulted in significantly improved final condition factor (HP = 1.46, LP = 1.40), carcass yield (HP = 86.0 %, LP = 84.1 %), feed conversion based on gutted weight (HP = 0.98, LP = 0.93) and whole body protein (HP = 17.6 %, LP = 16.9 %). The present study reveals that low dietary protein-to-lipid ratios for salmon post-smolt may negatively affect production parameters, although digestible energy contents in the diets are similar.

1. Introduction

The majority of Atlantic salmon (*Salmo salar* L.) is farmed in open sea pens that are exposed to seasonal variations in environmental conditions. Important production parameters such as appetite, feed utilization and growth rate are modulated by temperature and photoperiod, and by a wide range of other internal and external factors such as genetics, health status, adiposity, water quality, fish size, dietary composition and feeding regime (Austreng et al. 1987; Bendiksen et al. 2003a; Bendiksen et al. 2003b; Einen & Roem 1997; Gjedrem 2000; Gjøen & Bentsen 1997; Hillestad et al. 1998; Jobling & Johansen 1999; Johansen & Jobling 1998; Sveier & Lied 1998; Thodesen et al. 1999; Thorarensen & Farrell 2011). Farmed salmon in the mid-west part of Norway encounter periods with low feed intake, decreased growth rate, low lipid retention and the depletion of energy stores during their first spring in the sea (Alne et al. 2011). In contrast, the salmon experience high feed intake, rapid growth, and altered deposition and retention of lipids during the late summer and early autumn (Alne et al. 2011; Hemre & Sandnes 2008; Mørkøre & Rørvik 2001; Måsøval et al. 1994; Oehme et al. 2010). This phenomena seems to occur both for smolt transferred to the sea during the autumn and for those transferred during the spring (Alne et al. 2011), which suggests that salmon have a seasonal growth pattern that is triggered by external photoperiodic information. Thus, season-specific signals and internal factors induce metabolic changes in salmon that significantly affect the production efficiency in natural environments.

The minimum requirements of salmonids for protein, amino acids and energy have been partly established (NRC 2011; Wilson 2002). Juvenile salmonids undergoing rapid body growth require a higher portion of digestible protein than larger salmonids (Cho & Kaushik 1990; Einen & Roem 1997; Grisdale-Helland et al. 2013b), which use large amounts of the dietary energy for maintenance (Azevedo et al. 2004; Jobling 1994). However, sufficient dietary energy is important to ensure optimal feed utilization (Hillestad & Johnsen 1994; Hillestad et al. 1998). Several studies do not detect significant differences in growth performance between groups of salmon fed diets varying in protein/lipid ratio (Azevedo et al. 2004b; Hillestad & Johnsen 1994; Hillestad et al. 1998; Karalazos et al. 2007; Karalazos et al. 2011). In particular, studies using isoenergetic grower diets identified no negative influence of low protein/lipid ratio on growth performance or feed utilization, but a favorable protein sparing effect (Karalazos et al. 2007; Karalazos et al. 2011). These observations imply that salmon have high ability to utilize large amounts of lipids in high-energy diets efficiently for growth. The above mentioned factors together with the fact that lipid has historically been a cheap source of energy compared to protein, have lead the industry to reduce the amount of protein and increase the lipid content in the diets (Torrissen et al. 2011). Consequently, the dietary protein/lipid ratio in modern diets are thus lower compared with the traditional diets for salmonids. However, high demand of lipids and competitive pressure from competing industries, including direct human consumption, has increased the cost of lipids. Nutritional knowledge, raw material availability and world markets are under constant change and development, and thus, cost-effective and sustainable salmon production requires optimal utilization of both protein and lipids.

Most studies examining different dietary protein-to-lipid concentrations for salmon use non-isoenergetic diets (Einen & Roem 1997; Grisdale-Helland & Helland 1997), although several adjusted the dietary ration level so that the diets tested were fed isonitrogenously or isoenergetically (Hillestad & Johnsen 1994; Hillestad et al. 1998). In addition, some studies indicate that salmonids are able to adjust their feed consumption according to the dietary energy level (Bendiksen et al. 2002; Boujard & Medale 1994). As a result, this may complicate the direct comparisons among studies. To our knowledge, few have investigated the effect of isoenergetic diets differing in protein-to-lipid ratio fed ad-libitum on growth performance of juvenile salmon (0.1 – 1 kg) reared in seawater under natural conditions. In-house laboratory studies with constant light and temperature or short-term experiments may disregard the vital impact of seasonal environmental variations that influence the growth pattern.

Salmon increase the deposition of muscle fat and visceral adiposity as the fat content in the feed increases (Bendiksen et al. 2003; Einen & Roem 1997; Hillestad et al. 1998; Jobling et al. 2002a). The carcass yield consequently decreases (Hillestad et al. 1998). Increased amount of lipid deposition correlates with decreased feed intake in salmonids (Jobling & Johansen 1999; Jobling et al. 2002b; Johansen et al. 2002; Johansen et al. 2003; Shearer et al. 1997a; Shearer et al. 1997b; Silverstein et al. 1999). This finding is consistent with the lipostatic regulatory hypothesis (Jobling & Johansen 1999; Keesey & Corbett 1984; Kennedy 1953; Schwartz & Seeley 1997), which suggests that the amount of stored fat is an important regulator of energy intake and the homeostasis of adiposity. The hypothesis suggests that adipose tissue exerts a negative feedback control on appetite and feed consumption in fish. There is, thus, a risk of impaired growth as lipid deposition become excessive.

In view of the above-mentioned studies, it can be assumed that a diet with a low lipid level but with sufficient energy content, (i.e. increasing the dietary protein/lipid ratio), is an effective approach to reduce the deposition of lipids and enhance feed intake. This may be especially prominent for S1 juvenile salmon the first autumn in sea, since this period is associated with rapid growth and, elevated deposition and retention of lipids (Alne et al. 2011; Hemre & Sandnes 2008; Mørkøre & Rørvik 2001; Måsøval et al. 1994). However, excessive dietary protein or lipids, may lead to increased catabolism of the dietary nutrients and reduce the retention efficiency of protein and lipids, respectively (Kacaznowski & Beamish 1996; Refstie et al. 2001; Walton et al. 1984).

During a five month period after sea transfer, the present study test the hypothesis that increased dietary protein-to-lipid ratio improves the feed intake and growth of S1 Atlantic salmon, compared to lower dietary protein-to-lipid ratio (using commercially formulated ratios). The dietary and seasonal effects on lipid deposition, feed conversion, whole body composition, nutrient retention, body shape and carcass yield were assessed.

2. Materials and methods

2.1 Experimental diets

The diets used in the study were based on commercial formulations and manufactured by Havsbrún (Fuglafjørður, Faroe Islands) by extrusion and vacuum coating with oil. Two diets series that differed in protein/lipid ratio, but were isoenergetic with respect to digestible energy (DE), were formulated. Diets were produced as 3, 4 and 6 mm pellets according to fish size. The ingredients used and the compositions of macronutrients in diets for pellets of each given size are shown in Table 1. The approximate chemical compositions of the diets are shown in Table 2. The high-protein diet series (HP) had a higher content of protein and a lower content of lipid than the low-protein diet series (LP). The formulations were designed to resemble high and low protein-to-lipid ratios of commercial feeds used for salmon. The level of protein was decreased whereas the level of lipid was increased with the increase in pellet size, in accordance with commercial feed formulations. This upregulated the total energy level in order to account for the increase in fish weight. The difference in crude protein content (~ 40 g kg^{-1}) between the experimental diets was kept constant within all the pellet sizes, and the lipid level was adjusted to obtain equal levels of DE. The feed batches were stored in a refrigerated room (4 °C) and the amounts of feed corresponding to on-week consumption was taken out and kept in boxes at room temperature. Feed samples were taken on arrival from the manufacturer and stored frozen (-20°C) until they were analyzed as described below. The diets were formulated to meet the NRC nutritional recommendations for salmonid fish (NRC, 2011).

2.2 Fish, rearing conditions and experimental design

On the 29 March 2012, 8000 S1 Atlantic salmon smolt from the Rauma strain (Rauma Broodstock AS, Sjøholt, Norway) were sorted out, weighed in bulk and distributed among eight tanks with 1000 fish in each, on a truck at the Straumsnes Settefisk AS hatchery at Tingvoll, Norway. The smolts were visually examinated and individuals with similar size were selected and weighted in bulk. Fish with obvious signs of wounds, parr-marks or runts were removed. The fish were then transferred to Marine Harvest research station at Ekkilsøy (63° 03' N, 7° 35' E) on the west coast of Norway during the same day. On arrival, fish from each tank on the truck were allocated to one of eight pens (5 x 5 x 5 m, 125 m^3 volume). The smoltification status was checked by conducting a seawater challenge test, followed by determination of plasma osmolality, chloride content and gill Na^+,K^+-ATPase activity (Clarke et al. 1996), before the fish were exposed to seawater. The mean initial body weight of the smolt was 95.1 ± 0.2 g (mean ± st.dev). Each pen was assigned to one of two dietary groups in a randomized block design of quadruple net pens.

The eight pens were initially illuminated by four submerged 400 W light sources, 24 h day^{-1}. This was done in order to promote schooling behavior and avoid physical contact with the net wall in the pens. The light bulbs were removed on 29 May, and the salmon were subsequently exposed to the natural photoperiod until the feeding trial ended on 25 September 2012 (Figure 1A). Daylight hours were defined as the period from twilight in the morning until the center of the sun was 6° below the horizon in the evening, referred to as civil twilight (data obtained from the website; www.timeanddate.no). The experiment was divided into three periods: April-June (spring), June-July (summer) and July-September (autumn) (Table 2). The periods were adjusted to fit with the guidelines of the feed manufacturer with respect to pellet sizes, which have been determined according to the weight of fish (Table 3). The ambient seawater temperature and oxygen level were recorded daily at a depth of 3 m. The seawater temperature at transfer was 6 °C, and it increased to a maximum of 15 °C in late August. The average for the complete trial was 9.8 °C (Figure 1A). The average temperatures for the three periods were: 7.5 °C in April-June, 11.5 °C in June-July and 13.6 °C in July-September. The oxygen level decreased with increasing water temperature, and ranged from 12.8 to 7.2 mg l^{-1}, with an average of 9.8 mg l^{-1} (Figure 1B).

2.3 Feed-monitoring system and feed administration

The feed-monitoring system used in the trial was established by combining the methods described by Einen et al. (1999) and Helland et al. (1996). Feed was administered by automatic feeders (Betten Maskinstasjon AS, Vågland, Norway) and uneaten pellets were collected in a plastic funnel at the bottom of each net pen. The uneaten feed was pumped up into wire mesh sieves through a plastic pipe using pressurized air. The uneaten feed was collected after each meal and quantified each day, in order to determine the daily feed intake and feed conversion ratio accurately. The daily feed intake was calculated as described by Helland et al. (1996). All feeds were tested for the recovery of dry matter in empty net pens after the trial. The fish were fed to satiation (four times a day), and the feed ration was set such that they received approximately 10-20% more than the estimated daily feed intake. Adjustments of the feed ration was done according to the amount of uneaten feed collected.

2.4 Weighing and sampling procedures

All fish were counted and weighed in bulk at the end of each feeding period. The fish were collected from each experimental pen using a fish-landing net and anesthetized in batches with MS-222 (Metacaine 0.1 g l^{-1}; Alpharma, Animal Health Ltd., Hampshire, UK) in a 1000-liter tank of fresh seawater. All fish with obvious signs of wounds, runts or sexual maturation were removed and killed during the weighing procedure (the weights and numbers of such fish were recorded). An initial sample of 30 fish (three pooled samples with 10 fish in each) was taken before sea transfer, and 10 fish from each pen were sampled (sampled fish presented a mean body weight corresponding to the mean weight of all fish in the net pen) at the end of each feeding period. Sampled fish were anaesthetized in MS-222 and then killed by a blow to the head. The gill arches were cut and the fish were bled out in ice water. The fish were subsequently transported to the processing hall nearby, where individual weights and fork lengths were measured. The fish were then cut open, sex was determined by inspection of the gonads, and visceral fat was assessed visually on a score from 1 to 5 according to the visibility of the pyloric caeca (1 = clearly visible, 2 = visible, 3 = visible through cracks 4 = visible through the fat, 5 = not visible). The viscera (including the spleen) and the liver were dissected and weighed, in order to calculate the viscerosomatic index (VSI) and the hepatosomatic index (HSI). The heart and kidney were then removed before the fish was rinsed with water and the gutted weight recorded. Finally, muscle samples (Norwegian Quality Cut, NQC, NS 9401, 1994) were taken for analysis of lipid content. In addition, 30 fish (3 x 10) were taken at the start of the experiment, and 10 fish per pen on each sampling point, for the analysis of the whole-body proximate composition. These selected fish presented a mean body weight corresponding to the mean weight of all fish in the pen, then exposed to a lethal concentration of MS-222, before being frozen at -20°C. The fish were not starved before the sampling occasions in June and July, so feed matter was removed from the esophagus, stomach and intestines of all fish taken for analysis at these samplings. At the final sampling in September, samples were taken 48 h after the last meal and no feed matter was observed in the gastrointestinal system.

The pens were checked for mortalities daily and all the dead fish, were collected and weighed. During period 1, 3 and 2 fish died in the HP and LP group, respectively. During period 2, the average morality rate was 1.0 % for the HP group and 1.6 % for the LP group. During period 3, the average morality rate was 1.4 % for the HP group and 0.6 % for the LP group. There were no significant differences in mortality.

2.5 Analysis

Feces and diets were analyzed gravimetrically for dry matter (DM) after drying at 105 °C until constant weight, and for ash by flame combustion and incineration at 550 °C. Nitrogen was analyzed using the semi-automated Kjeldahl method (Kjetec Auto System, Foss Tecator, Höganäs, Sweden) and crude protein calculated as N x 6.25. The amount of crude lipid after hydrolysis with hydrochloric acid (HCl) and petroleum ether extraction was determined using the Soxtec HT6 system and a Soxtec1047 hydrolyzing unit (Foss Tecator, Höganäs, Sweden). The gross energy content was determined by adiabatic bomb calorimetry (Parr 6400 oxygen bomb calorimeter, Parr Instrument Company, Moline, IL, USA). Starch was analyzed as glucose, after enzymatic hydrolysis using a Megazyme K-TSTA 05/06 total starch assay kit (Megazyme International Ltd., Wicklow, Ireland) according to the Association of Analytical

Communities (AOAC) method, number 996.11. The amount of crude fiber was determined using a modified version of ISO 5498, by means of a Fibertec system (Foss Tecator, Höganäs, Sweden).

The amounts of crude protein and energy in homogenates of whole-fish body samples were determined as described for feeds. Whole-body fat was analyzed using a semi-automatic Soxhlet extractor (Soxtec Avanti 2055 apparatus, Foss Tecator, Höganäs, Sweden) with petroleum ether as the extracting solvent. The total fat content in muscle (NQC) was determined by extraction with ethyl acetate as described in NS 9402 (1994). The chemical analyses of muscle fat were conducted on pooled homogenized NQC samples from 10 fish per pen.

2.6 Calculations

The growth rates of the fish are presented as the thermal growth coefficients (TGC), calculated as described by Cho (1992).

$TGC = (W_1^{1/3} – W_0^{1/3}) \text{ x } (\Sigma T)^{-1} \text{ x } 1000$

where W_0 and W_1 are the initial and final weights, respectively, and ΣT is the sum of day degrees during the period (feeding days x average temperature, °C).

The biological feed conversion ratio (FCRb) was calculated as: feed intake (kg) x (biomass increase + biomass of dead fish (kg))$^{-1}$.

The feed conversion ratio on gutted weight basis (FCRg) was calculated as FCRg = FCRb x carcass yield^{-1}

The specific feeding rate (SFR) was calculated as:

(feed intake during the time period (kg) x average biomass weight during the time period (kg)) x 100^{-1}.

The retention of nutrients were estimated on pen basis, using the values of cumulative feed intake, the chemical composition of the diets, and changes in the biomass and whole-body content of the nutrient: Relative nutrient retention (% of ingested) = 100 x (final mass of nutrient in fish – initial mass of nutrient in fish) (mass nutrient ingested)$^{-1}$.

Absolute amount of nutrient retained in whole body from the feed (g 100 g^{-1} feed) was calculated as: Absolute nutrient retention (g 100 g^{-1} feed): ((nutrient in the diet x percentage of nutrient retention) x 100^{-1}). For absolute nutrient retention of energy, MJ kg^{-1} feed was used.

The authors acknowledges that the relative and absolute lipid retention is apparent as the fish have the ability to synthesize this nutrient *de novo*. However, in the text the term apparent is not used.

The body weight (BW) of bled fish was estimated by adding 3% to the bled weight (BW = bled weight x 1.03) (Einen et al. 1998).

Viscerosomatic index (VSI) and carcass yield were calculated as:

Y (g) x body weight $(g)^{-1}$ x 100, where Y is the weight of the measured visceral or carcass mass.

The condition factor (CF) was defined as:

100 x total body weight with blood (g) x length^{-3}.

The CF and carcass yield on gutted weight basis were calculated by applying the same formulas, but with gutted weight instead of the body weight.

2.7 Statistical analysis

The trial was conducted using a randomized block design and all data were analyzed using the GLM procedure in the SAS 9.3 computer software (SAS Institute Inc., Cary, NC, USA). Diet and block were used as class variables. If differences based on the block variable were not significant, the data were analyzed using diet as the only experimental factor. Net pen was used as the experimental unit. Percentage data were subjected to arcsine square root transformation before the statistical analysis. Homogeneity of variances was tested using Bartlett's test, and for data with heterogeneous variances, Welch's test for differences among groups was performed. Non-parametric data (visual score) were tested using the Kruskal-Wallis test. The Pearson product-moment correlation coefficient was used to describe the association between two variables. The level of significance was chosen at $P \leq 0.05$, and the results are presented as mean ± standard error of mean (SEM), unless stated otherwise.

3. Results

3.1 Feed intake, growth performance and feed utilization

The feed intake was low after sea transfer and throughout the first feeding period from April-June. It then increased gradually during the experiment. The feed intake did not differ between the dietary groups in April-June and June-July. The duration of daylight decreased in the period

July-September and the water temperature was high (Figure 1A and B). During this period, the fish fed the HP diet had significantly higher feed intake than those fed the LP diet (Table 4).

The growth rate reflected the feed intake, and TGC, FCRb and BW did not differ significantly between the dietary treatments in April-June or June-July (Figure 3 and Table 4). The highest growth for both groups was observed during July-September (Figure 3). In addition, during this period fish fed the HP diet presented a significantly higher TGC compared to fish fish fed the LP diet (HP = 3.82 ± 0.00, LP = 3.46 ± 0.03, $P < 0.001$). FCRb did not differ between the two groups (Table 4). Thus, the final body weight of fish in the HP group (945 ± 4 g) was significantly ($P < 0.0001$) higher than that of fish in the LP group (836 ± 11 g). Consequently, the weight gain (corrected for differences in start weight) for the HP group was 106 grams higher (i.e. almost 20 % higher weight gain) than the LP group. Fish given the HP diet had a significantly lower FCR on gutted weight basis (FCRg) than fish given the LP diet during the period July-September (Table 4).

3.2 Fat deposition, proportional visceral weight and body shape

The developments in muscle fat content and VSI for the two diets are shown in figure 2. The amount of muscle fat was the same in both dietary groups until the second sampling in July, when the group fed the HP diet had lower muscle fat content than the LP group (HP = 4.7 ± 0.3%, LP = 5.7 ± 0.1%, $P = 0.03$). Muscle fat content of both groups increased substantially (P < 0.001) from July to September (6.5 %-units on average) and no significant differences in muscle fat content were detected between the two dietary groups in September (Figure 3). The VSI of the group fed the LP diet increased steadily during the trial, whereas the VSI of the group fed the HP diet remained almost constant. At the final sampling in September (Figure 3), the VSI of the HP group was lower than that of the LP group (HP = 12.6 ± 0.1, LP = 14.3 ± 0.2, $P < 0.001$), and thus the final carcass yield was significantly higher (Table 5). The CF and CFg followed a similar pattern throughout the trail as that from the lipid level: they did not increase during the two first periods, but then increased sharply in the period July-September. At the final sampling in September, the length, CF, CFg, and gutted weight were all significantly higher for salmon fed the HP diet compared to those fed the LP diet (Table 5).

3.3 Whole body analysis and nutrient retention

The fish fed the LP diet had significantly higher whole body lipid and energy content than the fish fed the HP diet at the sampling in July. The levels of whole body fat and energy were not different between the two groups at the final sampling in September. However, fish in the HP group had a significantly higher protein content than those in the LP group at the September sampling (Table 6). The relative retention of protein (% of ingested) did not differ between the dietary groups in the periods April-June or July-September. However, the absolute protein retention (g 100 g^{-1} feed) in the HP group was significantly higher than in the LP group during April-June (HP = 25.3 ± 0.6, LP = 23.6 ± 0.2, $P = 0.05$) and July-September (HP = 22.1 ± 0.3, LP = 20.4 ± 0.3, $P = 0.01$) (Figure 4A and B). The relative protein retention differed significantly between the two diets only during June-July, when the retention of the protein was lower in the HP group than in the LP group (HP = 45.8 ± 0.9%, LP = 51.2 ± 0.9%, $P = 0.01$ (Figure 4A). No differences in absolute protein retention during this period were detected. In line with the whole body lipid in July, the LP group showed a trend towards higher relative lipid retention and significantly higher absolute lipid retention (HP = 12.4 ± 1.0, LP = 16.9 ± 0.6, $P = 0.01$) compared to the group fed the HP diet during the period June-July (Figure 4C and D). In the period July-September, the HP group had a significantly higher relative lipid retention than the group fed the LP diet (HP = 74.4 ± 2.0%, LP = 67.2 ± 1.1%, $P = 0.02$, Figure 4C), but no differences in absolute retention were observed (Figure 4D). The relative retention of energy was not significantly different between the two dietary groups during the experiment (Figure 4E). However, the absolute energy retention (MJ kg^{-1} feed) coincided with the absolute lipid retention with a significant difference between the groups in June-July (HP = 10.3 ± 0.5, LP = 11.9 ± 0.3, $P = 0.03$) (Figure 4F).

3.4 Relationships between overall feed intake and other parameters

The overall daily feed intake was highly correlated with the temperature during the experiment ($r = 0.96$, $P < 0.001$). The relative lipid retention efficiency was positively correlated to the increase in feed intake ($r = 0.98$, $P < 0.001$). The SFR during the period July-September was negatively correlated with the level of muscle fat at the sampling in July ($r = -0.82$; $P = 0.01$).

4. Discussion

The feed intake and growth of salmon smolt are generally low during the first 4-8 weeks after seawater transfer (Alne et al. 2011; Jobling et al. 2002a; Oehme et al. 2010; Rørvik et al. 2007), and the manner by which feed intake and growth return to normal vary (Jobling et al. 2002a; Usher et al. 1991). After sea transfer, the fish need to adapt to new environmental conditions, a new feeding system and a new social hierarchy, and these are all factors that may influence feed intake and growth during the initial stages of a trial (Gilmour et al. 2005). In the present study, feed intake and growth improved as time progressed, and high SFRs (1.27-1.39) and TGCs (3.37-3.83) were observed during the latter stage of the trial in the period July-September. These corresponded to 120% of the growth predicted by Austreng (1987) compared to only 40% during the April-June period. Condition factor, body lipids and energy all increased markedly during this period. These parameters often increase during the autumn (Alne et al. 2011; Mørkøre & Rørvik 2001; Måsøval et al. 1994), which is a period when the duration of daylight declines rapidly and the water temperature is high. The changes by time in feed intake, growth, fat content and body shape are in line with those of previous studies of S1 smolt reared at the same site and under similar conditions (Alne et al. 2011; Mørkøre & Rørvik 2001; Oehme et al. 2010). As in most poikilothermic species, feed intake was highest during the period July-September, when the average water temperature was 14 °C. This is in agreement with a study done by Handeland et al. (2008), showing that the feed intake of Atlantic salmon post-smolt is higher for those reared at 14 °C than for those reared at other temperatures (6, 10 and 18 °C).

Our results differ from those from Karalazos et al. (2007 and 2011), in which the dietary protein/lipid level did not affect growth when kept at a normal temperature (11 °C) or at low a temperature (4.2 °C). However, fish fed a diet with a low protein/lipid ratio tended to have lower final weights than fish fed other diets (Karalazos et al. 2011). Karalazos et al. (2007 and 2011) studied larger salmon (with initial weights of 1168 and 2053 gram, respectively) and tested diets with a low inclusion of fishmeal and low protein/lipid ratios, ranging from 390/330 to 290/380 g kg^{-1}. Small salmonids require higher dietary proportions of digestible protein than larger salmonids (Cho & Kaushik 1990; Einen & Roem 1997), and this may explain why the results obtained in the previous studies differ from those presented here. Azevedo et al. (2004b) found no difference in weight gain or growth of rainbow trout or Atlantic salmon fed isoenergetic diets with different protein/lipid ratios. They used, however, a wild salmon strain, and both species were reared in freshwater with a constant temperature of 8 °C.

Salmonids seem to adjust their feed intake according to the dietary energy level (Bendiksen et al. 2002; Boujard & Medale 1994), and this may be an influencing factor in trials in which feeds with different energy content are evaluated. Therefore, the use of isoenergetic diets eliminates this issue. Most studies that have investigated different protein/lipid levels for fish used diets with different total energy contents. Einen & Roem (1997) fed salmon reared from 1.0-2.9 kg in seawater diets that contained different protein/lipid levels and different energy levels. In this study, the TGC of a group fed a diet with a protein/lipid level of 480/308 g kg^{-1} (corresponding to a DP/DE ratio of 18.8 g MJ^{-1}) was significantly higher than that of a group fed a diet with a protein/lipid level of 425/364 g kg^{-1} (DP/DE of 16.4 g MJ^{-1}). The difference in growth observed in the latter study was only recorded during the last phase of the study, when the growth rates were high following a 60-day period with low appetite and growth. The results of Einen & Roem (1997) agree with those of the present study, and both indicate that a low ratio of dietary protein to lipids (below 16~17 g MJ kg^{-1} DP/DE) reduces feed consumption in salmon. This in turn affects the intake of protein and other nutrients and reduce the availability of essential nutrients for optimal growth (Bendiksen et al. 2003b; Johansen et al. 2002; Shearer et al. 1997a; Shearer et al. 1997b; Silverstein et al. 1999). Our findings confirm this line of results using feed formulations, fish breed and rearing conditions commonly used in commercial farming of salmon.

The observed negative relation between muscle fat in July and the subsequent feed intake in the period July-September suggest that the significantly higher lipid deposition in the LP group may have suppressed appetite and reduced feed consumption. This, together with a leaner HP diet, may have contributed to a higher feed intake among HP fed fish in latter stages of our trial. The lower feed intake in the LP group than in the HP group is consistent with the theory of lipostatic regulation (Jobling & Johansen 1999; Keesey & Corbett 1984; Kennedy 1953; Schwartz & Seeley 1997). In accordance with this, the VSI of the group fed the LP diet increased continuously, indicating increased adiposity. However, the pure effect of body fat content on feed intake cannot be separated in the present trail. To be able to elucidate this, the two groups should have received the same feed in the period after achieving differences in lipid content.

The VSI of fish in the HP group did not increase during the experiment, whereas that of fish in the LP group increased gradually to a high value. Normally, an increase in VSI reflects a higher deposition of visceral fat (Bendiksen et al. 2003b; Hillestad et al. 1998; Jobling et al. 1998; Jobling et al. 2002a). The VSI correlated with both the visual assessment of visceral fat and the level of whole body lipids. This indicates that the HP group stored dietary lipids preferentially in the muscle, whereas the LP group stored lipids in both muscle and viscera. The muscle is the major site of fat deposition and storage in salmonids, accounting for 60-65% of the body mass (Aursand et al. 1994, Jobling et al. 2002a; Polvi & Ackman 1992). The increase in VSI and consequent decrease in carcass yield of the LP group may suggests that dietary lipids were in excess, and the protein/lipid ratio unbalanced.

The increase in feed intake throughout the experiment (Table 4) correlated with the increased relative and absolute retention of energy and lipids (Figure 4). Increased energy and lipid retention with increased feed intake are in accordance with the results obtained by Grisdale-Helland et al. (2013b). Our results are also consistent with the observation from Alne et al. (2011), who showed that S1 smolt had low relative lipid retention (~20%) during the spring and high relative lipid retention (~60%) during the autumn. The absolute lipid retention was identical between the two dietary groups during the autumn period, due to a significant up-regulated relative lipid retention for the HP group. This shift in relative lipid retention indicate that fat deposition and storage during this period are a high priority. However, it is noteworthy that although the absolute lipid retention was equal between the groups during autumn, the VSI of HP group was significantly lower than that in the LP group in September. The relative retention of protein was reasonably stable (at approximately 50%) and far less dynamic than the retention of lipid, as previously reported (Alne et al. 2011). The significantly higher absolute protein retention of the HP group compared with LP group during April-June and July-September, suggests that dietary protein was efficiently incorporated to body protein in the fish fed the HP diet during these periods. For the period Jul-Sep, the increased absolute protein retention coincided with the high CF, carcass yield and body protein content among the HP fed fish. These factors are again interrelated with the improved feed intake and growth in the fish fed the HP diet. The lower protein retention in the fish fed the HP diet compared to that in the fish fed LP diet in June-July is in accordance with several trials showing a protein sparing effect of reduced protein-to-lipid ratio within certain ranges (Einen & Roem 1997; Grisdale-Helland & Helland 1997; Grisdale-Helland et al. 2013a).

FCRb did not change significantly during the experiment. However, FCRg was significantly higher in fish fed the HP diet than it was in fish fed the LP diet during the period July-September (Table 4). This indicates that less of the dietary nutrients were used to increase the visceral mass, and more nutrients were used for carcass growth. This is consistent with the observed nutrient retention and is an important observation, as the carcass is the primary edible product for sale and holds the most value (often referred to as head on gutted, HOG, in relation to sale and price estimations).

5. Conclusion

Muscle fat content in fish fed high dietary protein-to-lipid ratio (HP) was significantly reduced compared to that in fish fed low dietary protein-to-lipid ratio (LP) prior to first autumn in sea, without any negative effects on growth and feed conversion. In the subsequent autumn period, fish fed the HP diet showed a significantly higher feed intake, growth rate and weight gain (almost 20%). During this period, HP fed fish presented a significantly higher absolute protein retention and reduced the visceral mass compared to LP fed fish, resulting in significantly higher whole body protein, condition factor, improved carcass yield and feed conversion based on gutted weight. The present study shows that it is possible to modulate lipid deposition and growth by seasonal and dietary interaction.

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TABLES:

Table 1: Formulation (g kg^{-1}) of the experimental diets

Pellet size	3 mm		4 mm		6 mm	
Diet code	LP	HP	LP	HP	LP	HP
Formulation, (g kg^{-1})						
Micro ingredients[a]	25	25	25	25	15	15
Wheat	119	105	138	100	140	125
Wheat gluten	20	58	20	63	28	69
Soy protein concentrate	38	26	15	61	56	45
Fish meal	492	531	520	511	387	425
Krill meal	55	55	15	15	0.0	0.0
Porcine blood meal	00	00	00	00	45	30
Fish oil	110	95	127	116	151	136
Rapeseed oil	110	95	127	116	151	136
Pigment[b] (mg kg^{-1})	50	50	50	50	50	50

[a] Vitamin and mineral premixes

[b] Astaxanthin

Table 2: Approximate chemical compositions (g kg^{-1}) of the diets

Pellet size	3 mm		4 mm		6 mm	
Diet code	LP	HP	LP	HP	LP	HP
Chemical composition, (g kg^{-1})						
Crude protein (*N* x 6.25)	444	483	413	452	390	441
Crude lipid	286	260	328	285	347	316
Ash	89	94	85	90	55	58
Water	71	73	64	79	62	62
Crude fiber	1.6	1.2	0.8	0.7	1.1	1.0
Total starch	73	73	77	69	101	88
NFE[a]	108.4	88.8	109	93	145	122
Gross energy, (MJ kg^{-1})	23.8	23.3	24.4	23.4	25.2	24.9
Crude protein/lipid ratio	1.55	1.86	1.26	1.59	1.12	1.40
Digestibility calculations[b]						
Calculated DP, (g kg^{-1})	382	415	355	389	335	379
Calculated DE, (MJ kg^{-1})	20.6	20.3	21.5	20.6	22.1	21.8
Estimated DP/DE ratio (g MJ kg^{-1})	18.5	20.5	16.5	18.9	15.2	17.4

[a] NFE = Nitrogen-free extracts = 1000 – (protein+lipids+ash+fiber+water)

[b] The amounts of digestible protein (DP) and digestible energy (DE) were estimated assuming 23.7, 39.5 and 17.2 MJ kg^{-1} as the gross energy content of protein, lipids and carbohydrates, respectively. The apparent digestibility coefficients (ADCs) for protein and lipids used were 0.86 and 0.94, respectively (Einen & Roem 1997), whereas 0.50 was used for NFE (Arnesen & Krogdahl 1993).

Table 3: The experimental periods with duration, dates, pellet size used and sampling date. The preferred fish weight intervals of the different pellet sizes are also given.

Feeding period	Duration	Dates	Pellet size used	Preferred fish weight (g)	Samplings
Apr – Jun	11 weeks	29 Mar. - 11 Jun.	3 mm	100 ~ 150	1: 11 Jun.
Jun-Jul	6 weeks	11 Jun. - 23 Jul.	4 mm	150 ~ 300	2: 23 Jul.
Jul-Sept	9 weeks	23 Jul. - 24 Sep.	6 mm	300 ~ 800	3: 24 Sep.

Table 4: Weight gain, feed intake and feed utilization (mean ± SEM, n = 4)

Dietary group		LP	HP	Dietary effect (*P*-value)
April – June, 3 mm diet				
Weight gain, g		66 ± 1	67 ± 2	0.533
SFR		0.55 ± 0.01	0.54 ± 0.00	0.205
FI, g^{-1} $fish^{-1}$		52 ± 1	51 ± 1	0.487
FCRb		0.79 ± 0.01	0.76 ± 0.02	0.277
FCRg		0.93 ± 0.02	0.88 ± 0.01	0.087
June – July, 4 mm diet				
Weight gain, g		123 ± 2	126 ± 2	0.383
SFR		1.03 ± 0.02	1.05 ± 0.02	0.536
FI, g^{-1} $fish^{-1}$		92 ± 1	95 ± 2	0.280
FCRb		0.74 ± 0.00	0.75 ± 0.01	0.210
FCRg		0.89 ± 0.01	0.88 ± 0.01	0.372
July – September, 6 mm diet				
Weight gain, g		552 ± 9.3	658 ± 2.3	< 0.001
SFR		1.33 ± 0.02	1.38 ± 0.01	0.054
FI, g^{-1} $fish^{-1}$		452 ± 9	527 ± 2	< 0.001
FCRb		0.81 ± 0.01	0.80 ± 0.00	0.126
FCRg		0.98 ± 0.01	0.93 ± 0.00	0.013

SFR, specific feed intake; FI, feed intake; FCRb, biological feed conversion ratio
FCRg, feed conversion based on gutted weight

Table 5: Biometric parameters at each sampling point (mean ± SEM, n = 4)

Dietary group		LP	HP	Dietary effect (*P*-value)
11 June, Sampling 1, end of 3 mm diet				
Body weight, g		150 ± 3	151 ± 4	0.849
Gutted body weight, g		129 ± 3	131 ± 3	0.646
Body length (fork), cm		23.9 ± 0.1	24.0 ± 0.2	0.554
Condition factor (CF)		1.10 ± 0.01	1.09 ± 0.01	0.571
Condition factor gutted (CFg)		0.94 ± 0.01	0.94 ± 0.01	1.000
Carcass yield, %		86.1 ± 0.2	86.8 ± 0.3	0.102
Visceral score, 1-5		1.3 ± 0.1	1.5 ± 0.1	0.139
23 July, Sampling 2, end of 4 mm diet				
Body weight, g		274 ± 2	277 ± 2	0.393
Gutted body weight, g		233 ± 2	238 ± 2	0.087
Body length (fork), cm		29.0 ± 0.0	29.2 ± 0.2	0.234
Condition factor (CF)		1.12 ± 0.01	1.11 ± 0.02	0.526
Condition factor gutted (CFg)		0.95 ± 0.01	0.95 ± 0.01	1.000
Carcass yield, %		85.0 ± 0.4	86.0 ± 0.3	0.072
Visceral score, 1-5		1.4 ± 0.1	0.9 ± 0.1	0.017
24 September, Sampling 3, end of 6 mm diet				
Body weight, g		815 ± 20	926 ± 6	0.002
Gutted body weight, g		685 ± 16	796 ± 7	0.001
Body length (fork), cm		38.7 ± 0.3	39.9 ± 0.2	0.023
Condition factor (CF)		1.40 ± 0.01	1.46 ± 0.02	0.025
Condition factor gutted (CFg)		1.18 ± 0.01	1.25 ± 0.01	0.008
Carcass yield, %		84.1 ± 0.2	86.0 ± 0.2	< 0.001
Visceral score, 1-5		2.7 ± 0.1	2.4 ± 0.1	0.106

Initial sampling before sea transfer, 29 March: body weight; 92.8 ± 0.3 g, length; 19.1 ± 0.0 cm, condition factor; 1.33 ± 0.01

Table 6: Whole body composition of lipids, protein and energy at each sampling point (mean ± SEM, n = 4)

Dietary group		LP	HP	Dietary effect (*P*-value)
11 June, Sampling 1, end of 3 mm diet				
Lipids (%)		9.6 ± 0.3	9.0 ± 0.0	0.075
Protein (%)		17.9 ± 0.1	18.1 ± 0.2	0.287
Energy (MJ kg^{-1})		8.0 ± 0.1	7.7 ± 0.0	0.098
23 July, Sampling 2, end of 4 mm diet				
Lipids (%)		10.9 ± 0.1	9.2 ± 0.3	0.003
Protein (%)		17.1 ± 0.1	17.2 ± 0.1	0.357
Energy (MJ kg^{-1})		8.4 ± 0.1	7.8 ± 0.2	0.011
24 September, Sampling 3, end of 6 mm diet				
Lipids (%)		16.4 ± 0.1	16.0 ± 0.3	0.301
Protein (%)		16.9 ± 0.1	17.6 ± 0.1	0.004
Energy (MJ kg^{-1})		10.3 ± 0.1	10.3 ± 0.1	0.867

Initial sampling before sea transfer, 29 March: Lipids; 12.0 ± 0.2 %, Protein; 17.3 ± 0.1 %, Energy; 8.8 ± 0.0 MJ kg^{-1}

FIGURE CAPTIONS:

Figure 1 A: Ambient sea temperature (°C) and hours of daylight during the trial. B: The measured oxygen level (mg l^{-1}) during the trial. Diets used (3, 4 and 6 mm) and the duration of the feeding periods (months) are indicated in the top of the figure

Figure 2: Changes in muscle fat content, % w/w (lines) and viscero-somatic index, % (bars) for S1 Atlantic salmon fed isoenergetic diets with high (HP) or low (LP) protein/lipid ratio. Significant differences between dietary groups within each sampling (11 Jun, 13 Jul and 24 Sep) are indicated by * over the lines and different letters on bars. The diets used (3, 4 and 6 mm) before the samplings are shown in the parenthesis. Data are presented as means ± SEM, n = 4

Figure 3: Changes in body weight (lines) and thermal growth coefficient (bars) for S1 Atlantic salmon fed isoenergetic diets with high (HP) or low (LP) protein/lipid ratio. Significant differences between dietary groups within each sampling (11 Jun, 13, Jul and 24 Sep) or feeding period (Apr-Jun; 3 mm, Jun-Jul;4 mm and Jul-Sep; 6 mm) are indicated by * over the lines and different letters on bars. Data are presented as means ± SEM, n = 4

Figure 4: Relative nutrient retention (% of ingested) of protein (A), lipid (C) and energy (E), and the absolute nutrient retention of protein (g 100 g^{-1} feed; B), lipid (g 100 g^{-1} feed; D) and energy (MJ kg^{-1} feed; F) for S1 Atlantic salmon fed isoenergetic diets with either a high (HP; white bars) or a low (LP; gray bars) protein/lipid ratio. Significant differences between dietary groups within each feeding period (Apr-Jun, Jun-Jul and Jul-Sep) are indicated by different letters over the bars. Data are presented as means ± SEM, n = 4

FIGURES:

FIGURE 1:

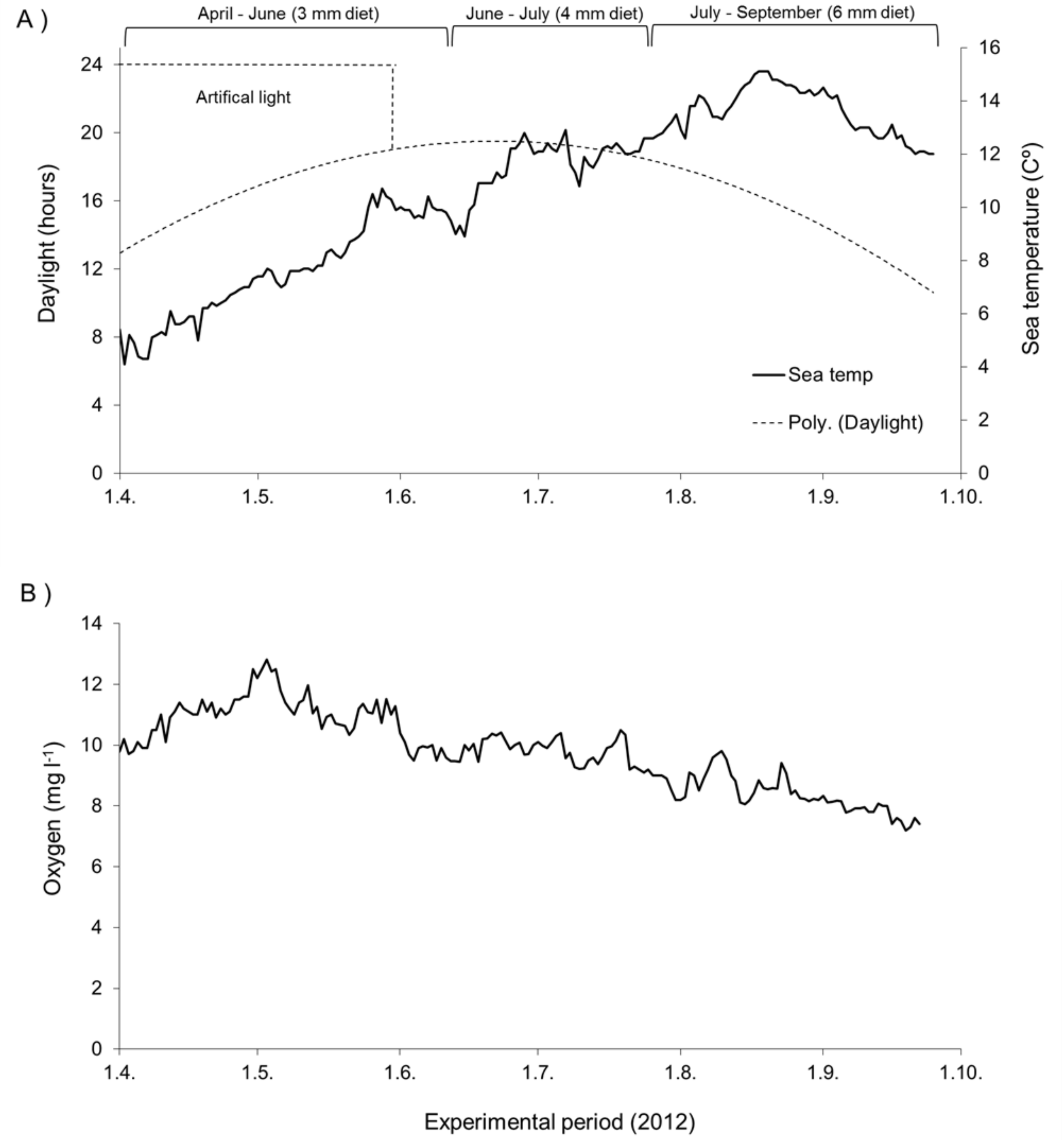


FIGURE 2:

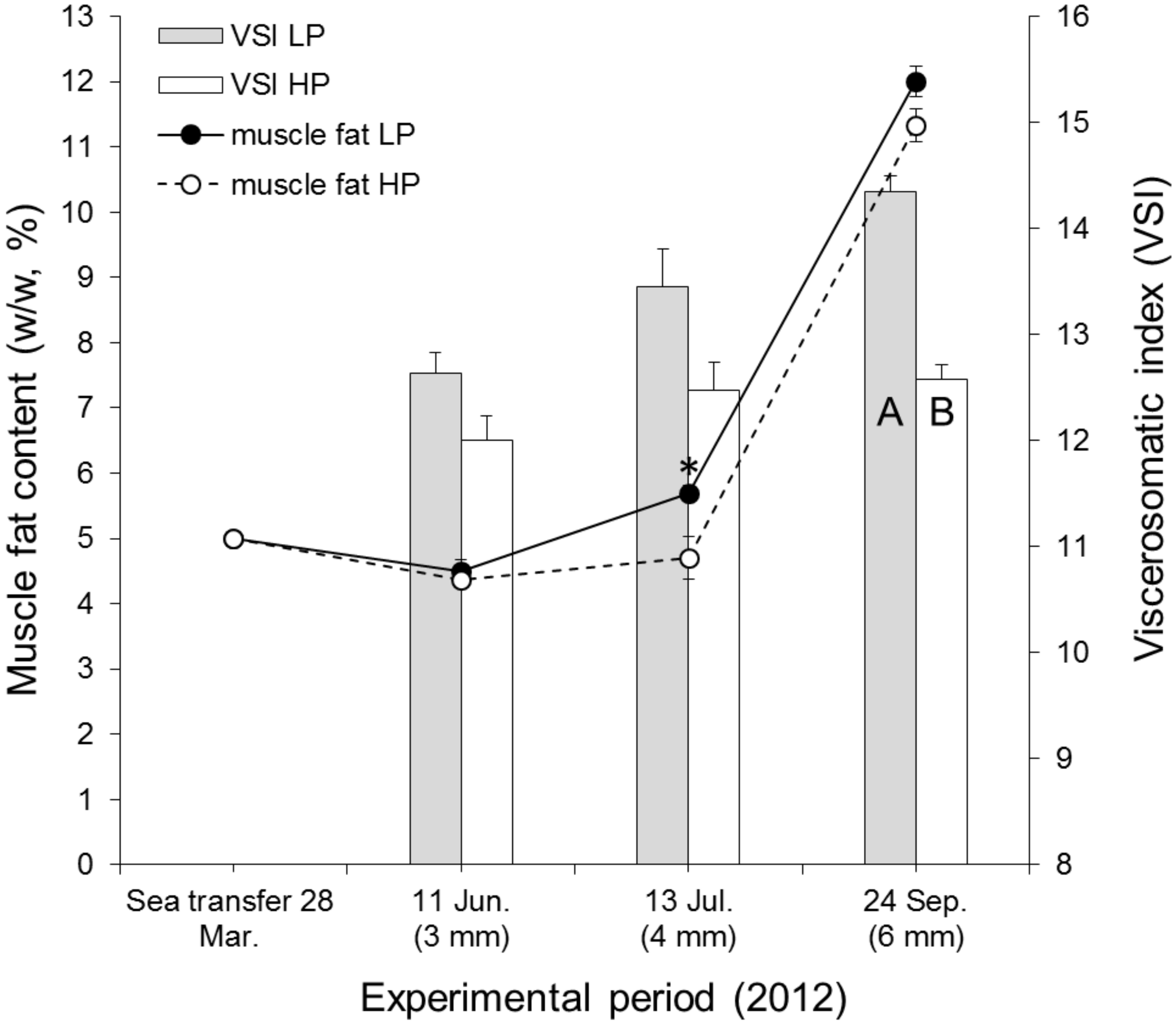
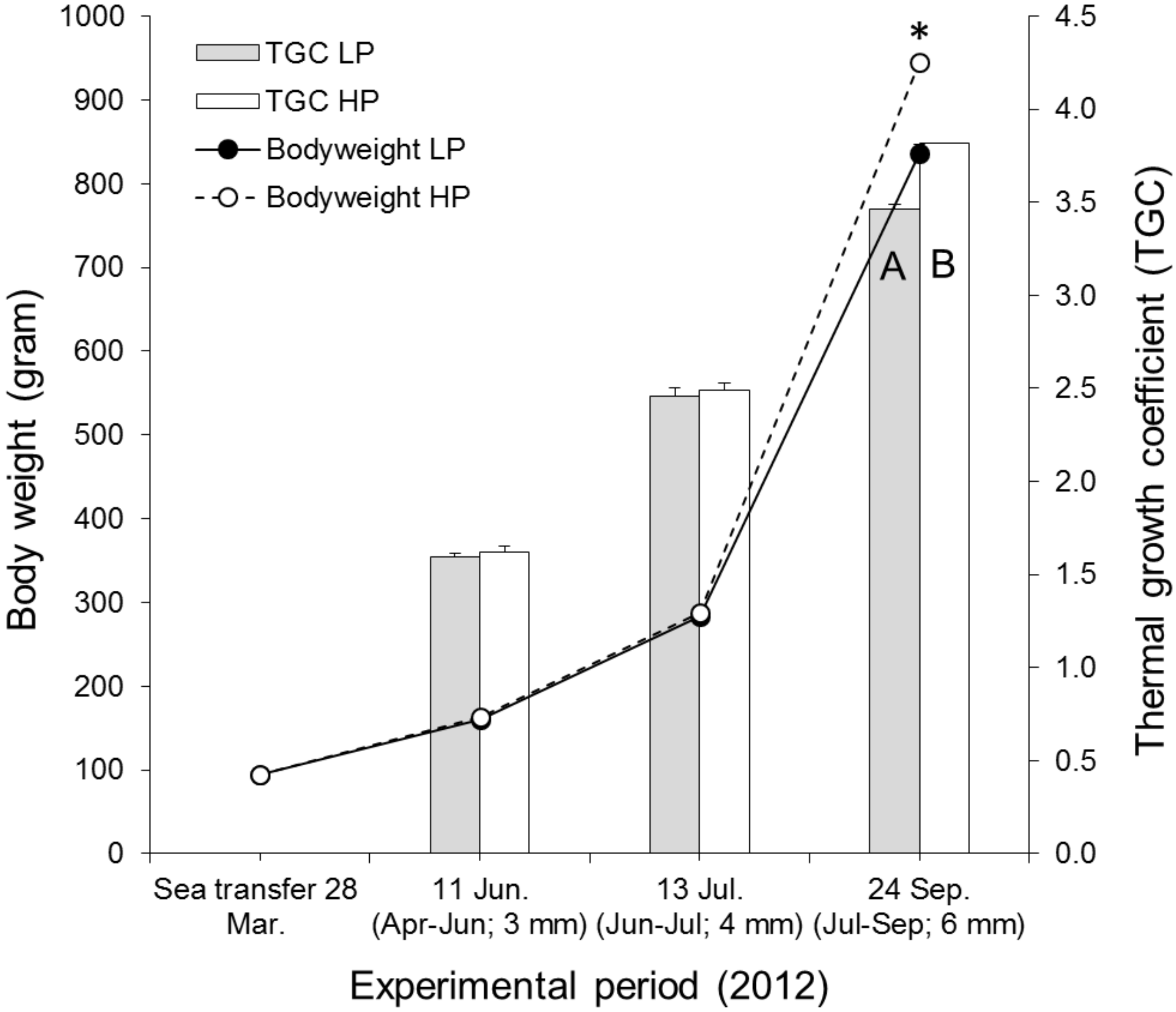
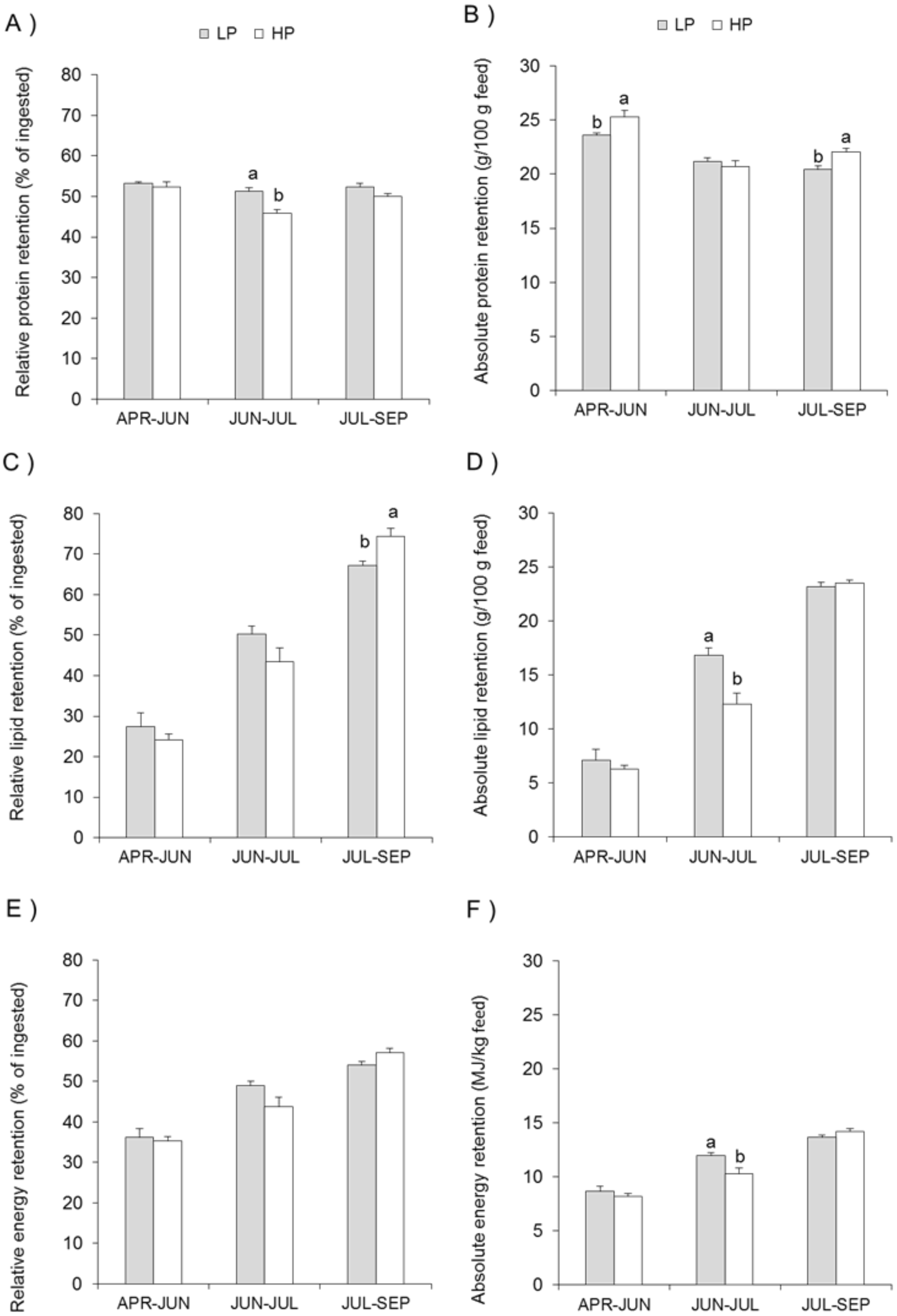

VSI LP
VSI HP
muscle fat LP
muscle fat HP
Muscle fat content (w/w, %)
Viscerosomatic index (VSI)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
8
9
10
11
12
13
14
15
16
*
A
B
Sea transfer 28 Mar.
11 Jun. (3 mm)
13 Jul. (4 mm)
24 Sep. (6 mm)
Experimental period (2012)

FIGURE 3:



TGC LP
TGC HP
Bodyweight LP
Bodyweight HP
Body weight (gram)
0
100
200
300
400
500
600
700
800
900
1000
Thermal growth coefficient (TGC)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
*
A
B
Sea transfer 28 Mar.
11 Jun. (Apr-Jun; 3 mm)
13 Jul. (Jun-Jul; 4 mm)
24 Sep. (Jul-Sep; 6 mm)
Experimental period (2012)

FIGURE 4:


A)
LP
HP
Relative protein retention (% of ingested)
0
10
20
30
40
50
60
70
80
a
b
APR-JUN
JUN-JUL
JUL-SEP
B)
LP
HP
Absolute protein retention (g/100 g feed)
0
5
10
15
20
25
30
b
a
b
a
APR-JUN
JUN-JUL
JUL-SEP
C)
Relative lipid retention (% of ingested)
0
10
20
30
40
50
60
70
80
b
a
APR-JUN
JUN-JUL
JUL-SEP
D)
Absolute lipid retention (g/100 g feed)
0
5
10
15
20
25
30
a
b
APR-JUN
JUN-JUL
JUL-SEP
E)
Relative energy retention (% of ingested)
0
10
20
30
40
50
60
70
80
APR-JUN
JUN-JUL
JUL-SEP
F)
Absolute energy retention (MJ/kg feed)
0
5
10
15
20
25
30
a
b
APR-JUN
JUN-JUL
JUL-SEP