Low body fat content prior to declining day length in the autumn significantly increased growth and reduced weight dispersion in farmed Atlantic salmon *Salmo salar* L.

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Running head: Body fat and growth in Atlantic salmon

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ABSTRACT:

Based on the regulatory effects of body fat on appetite and seasonal variations in fat deposition and growth of Atlantic salmon, the present study tested the hypothesis that body fat content prior to declining day length in the autumn can significantly modulate growth rate. The growth rate of salmon (mean initial body weight, BW=2.3 kg) with different muscle fat content prior to autumn, subjected to natural photoperiod and temperature, during a 7-months period (mean final BW=6.6 kg) was studied. In August, three fish groups (HF, LF and 0.5LF group) with significantly different muscle fat content (HF=16.4%, LF=13.2% and 0.5LF=11.3%), individually marked with PIT-tag, were mixed into the four net pens and fed a standard high-energy diet until March the following year. The muscle fat content prior to the autumn had a highly significant ($P < 0.0001$) effect on growth during the seven month main-dietary period, even after identical fat stores among the groups were restored, indicating a more complex explanation than just a lipostatic regulation mechanism. Mean thermal growth coefficients were HF=2.9, LF=3.4 and 0.5 LF=3.9, resulting in increased final weight gain for LF and 0.5LF of 590 g. and 980 g., respectively, compared to the HF group. The LF groups obtained a significantly higher homogeneity in BW and shape than HF fed fish in March, optimizing automatic gutting and filleting at slaughter. The improved growth response among the LF groups by reducing lipid levels can potentially be utilized in closed and semi-closed production units where photoperiod can be manipulated.

INTRODUCTION:

Fish that encounter setbacks induced by nutritional deficit, feed deprivation or sub-optimal conditions often display increased feed consumption (hyperphagia) and compensatory growth (CG) when circumstances are normalized (Ali, Nicieza, & Wootton, 2003; Foss & Imsland, 2002; Metcalfe & Monaghan, 2001). The degree of CG in fish vary and is often categorized based on the growth catch-up ability (Ali et al., 2003). Feed restriction or deprivation induce changes in body energy by depleting lipid stores, and during the course of CG and hyperphagia, body weight and lipid reserves are gradually restored (Ali et al., 2003; Bull & Metcalfe, 1997; Jobling & Miglavs, 1993; Metcalfe & Thorpe, 1992). The lipostatic model is often discussed within the circumstances of CG responses in fish (Jobling & Johansen, 1999; Johansen, Ekli, Stangnes, & Jobling, 2001). The lipostatic regulation hypothesis identifies adipose tissue and stored lipids to have an important role in governing appetite (Jobling & Johansen, 1999; Keesey & Corbett, 1984; Kennedy, 1953). The model implies that the amounts of stored fat has a negative feedback control on feed intake and is important for the regulation of energy homeostasis. Hence, CG is not only a response to recover body weight, but also a strong response to restore lipid levels and thereof CG will cease once this is achieved (Ali et al., 2003; Jobling & Johansen, 1999; Johansen, Ekli, & Jobling, 2002). Johansen et al., (2002) showed that altering body lipids of juvenile salmon by dietary administration of low-fat feeds yield similar growth responses as deprivation or feed restriction *per se*.

In modern high-fat diets for salmonids, lipids of marine and vegetable origin are the main sources of energy and support growth efficiently if essential fatty acids requirements are met (Bell et al., 2001; Thomassen & Røsjø, 1989; Torstensen, Lie, & Frøyland, 2000). Because

74 salmonids have a high ability to utilize large amount of lipids efficiently for growth, high-fat
75 diets with up to 380 g kg^{-1} of fat are commonly used in intensive salmon farming (Torrissen et
76 al., 2011). However, salmonids also have the capacity to store large amounts of excess fat as
77 triacylglycerols mainly in the muscle and visceral cavity (Aursand, Bleivik, Rainuzzo, Leif, &
78 Mohr, 1994). Body lipid content of farmed salmonids correlates with fish size, dietary fat
79 level and feed intake (Aksnes, 1995; Hemre & Sandnes, 1999; Torstensen, Lie, & Hamre,
80 2001). Like other anadromous species, Atlantic salmon display seasonal changes in feed
81 intake, growth and lipid deposition during the seawater phase (Mørkøre & Rørvik, 2001).
82 Farmed Atlantic salmon display elevated deposition of lipids in muscle and increased
83 retention of lipids in whole body during declining day length in autumn, with a concomitant
84 increase in feed intake, somatic growth and condition factor (CF) (Alne, Oehme, Thomassen,
85 Terjesen, & Rørvik, 2011; Dessen, Weihe, Hatlen, Thomassen, & Rørvik, 2017; Mørkøre &
86 Rørvik, 2001; Rørvik et al., 2010). This is particularly pronounced for salmon reared at high
87 latitudes that experience long winters and late spring, which results in reduced lipid levels and
88 CF prior to summer and autumn.
89
90 The recent increase in automation of fish processing at slaughter requires uniform body
91 weight (BW) and shape among the salmon for optimal efficiency and quality of products such
92 as gutted fish and fillets. Increased uniformity of BW and CF reduces the need for manual
93 gutting/filleting of very small or large individuals. Due to this, the homogeneity in body shape
94 and mass of salmonids are important parameters in salmon farming industry and low
95 dispersion in BW and CF are beneficial at time of harvest. The homogeneity of BW may be
96 strongly influenced by events occurring during the production cycle, i.e. disease outbreaks,
97 handling stress, reduced seawater tolerance or competition of feed (McLoughlin, Nelson,
98 McCormick, Rowley, & Bryson, 2002; Ryer & Olla, 1996; Taksdal et al., 2007; Usher,

Talbot, & Eddy, 1991). The dispersion in the distribution of BW, length and CF are often assessed by calculating the coefficient of variation (CV). The CV of BW for farmed salmon grown from 70 until 300 g. and from 60 until 500 g. fed either in excess or restrictively for period followed by unrestricted feeding, are reported to vary from 9 to 13% and 16 to 21%, respectively (Johansen et al., 2001). In the latter study, no significant differences was observed in the CV of BW between fish fed in excess and fish fed restrictively.

The majority of studies regarding growth responses related to lipid content are based on in-house laboratory experiments with small juvenile salmonids under constant conditions. To our knowledge, few have investigated grow out salmon with different lipid content subjected to seasonal environmental changes in photoperiod and temperature. Due to the regulatory effects of body fat on appetite and the observed fat storage in salmon linked to the seasonal cues, the present study tested the hypothesis that lipid status prior to declining day length in the autumn functions as a significant growth regulator. Accordingly, the growth rate for three groups of salmon with different muscle fat content prior to autumn, subjected to natural photoperiod and temperature, was studied throughout a 7-months period. About each second month, weight samplings and analysis of muscle fat content was conducted to investigate any relationship between fat accumulation and periodic growth rate, and to identify the duration of a potential lipostatic regulatory effect. Changes in visceral fat, CF, length, and the dispersion in BW and CF were further assessed.

MATERIAL & METHODS:

This experiment was conducted in accordance with laws and regulations that control experiments and procedures in live animals in Norway, as overseen by the Norwegian Animal

Research Authority. Stunning and sampling of fish were performed in accordance with the Norwegian Animal Welfare act. Fish were treated as production fish up to the point of tissue sampling which was only conducted after the fish were put to death.

The experiment was conducted in seawater on the Norwegian west coast (Ekkilsøy, Norway 3° 03' N, 7° 35' E) at Nofima research center from August 2011 to March 2012. In July 2010, the fish were transferred to seawater as S1 smolt, at which time the BW was 62 g. From the 10 to 12 of May 2011, the post-smolt were re-stocked into three net-pens (343 m^3) with 650 fish per pen. Prior to this, all individual fish were measured for weight and length, and tagged using passive integrated transmitter tags (PIT-tags) placed in the body cavity just posterior to the gut. The average BW per pen was 1085 g. (SD = 79 g.) and each pen received different dietary treatments: a high-fat diet (HF), a low-fat high-protein diet (LF) or half the ration of the low-fat high-protein diet (0.5LF). The 0.5LF-group were given half the amount of the feed provided to fish administrated the LF-diet the day before. Skretting (Averøy, Norway) produced the feeds and the composition of the HF diet was (wet weight, as is basis): dry matter 93.4%, crude protein 33.5%, crude lipid 34.1%, nitrogen-free extract (NFE) 21.2%, ash 4.6% and gross energy of 25.1 MJ kg^{-1}. The composition of the LF diet was (wet weight, as is basis): dry matter 91.7%, crude protein 49.9%, crude lipid 17.5%, NFE 17.1%, ash 7.2% and gross energy 21.7 MJ kg^{-1}. The three dietary treatments were fed from 12 of May until 9 of August (pre-dietary phase). May 12th, the fish were sampled for analysis of initial muscle fat content and biometric data. The analysis showed the following (mean ± SE, n = 30): BW: 1087 ± 97g, initial muscle fat: 12.2 ± 1.1% and initial CF: 1.10 ± 0.06. After ending the pre-dietary phase, the PIT-tag, BW and length of all individual fish in the three pens were recorded. In addition, fish from each pen were sampled for analysis of muscle and visceral fat content. The pre-dietary feeding phase generated three fish groups with significantly different

149 ($P < 0.05$) muscle fat content, visceral fat and visceral mass (Table 1). During the pre-dietary
150 phase, 2.5%, 0.6% and 0.3% fish died in the HF, LF and 0.5LF group, respectively. The
151 majority of mortality occurred from May until mid-June and was not related to any disease
152 outbreak (non-specific morality).
153
154 **(Table 1).**
155
156 At the 10 to 11 of August, the fish were restocked from the three original pens used in pre-
157 dietary phase into four new pens (125 m^3). Each of the four pens contained 50 fish from each
158 of the three pre-dietary treatments (HF, LF and 0.5LF), 150 fish in total (Fig 1). During the
159 period from 11 of August until termination at 20 of March 2012 (main-dietary phase), the
160 pens were fed isonitrogenous and isoenergetic diets produced by Ewos (Bergneset, Norway)
161 (Table 2). The current experiment was an integrated part of a large study were potential
162 effects of dietary oil source were investigated. Therefore, two pens in the main-dietary phase
163 were fed a diet with a marine oil profile (MO), whereas the two other pens were fed a diet
164 with a rapeseed oil profile (RO). The marine oil diet (MO) had an inclusion of 70 % South
165 American fish oil and 30 % of rapeseed oil. The rapeseed oil diet (RO) had an inclusion of 70
166 % rapeseed oil and 30 % South American fish oil. During the main-dietary phase, the pellet
167 size was changed from 7 to 9 mm in December due to the increase in BW of the fish.
168
169 **(Fig. 1 and Table 2)**
170
171 In both periods, feed was administrated using automatic feeders (Betten Maskinstasjon AS,
172 Vågland, Norway) and uneaten feed was collected as described in Einen, Mørkøre, Røra &
173 Thomassen (1999) and corrected for the recovery of dry matter as described by Helland,

Grisdale-Helland & Nerland (1996). The fish groups (except the 0.5LF group during the pre-dietary phase) were fed to satiation and the feed ration was set at 5-10 % in excess (*ad libitum* feeding). The fish were fed four times a day until October 2011, after this, the fish were fed three times a day until termination in March 2012. Adjustments of the feed ration was done according to the daily amount of uneaten feed collected. Due to the stocking of 50 fish from each of the pre-dietary treatments into each net pen, it was not possible to determine the feed intake or feed utilization of the different pre-dietary groups during the main-dietary phase. The pens were checked for mortalities daily and the dead fish were collected and weighed. The fish were exposed to natural variations in photoperiod and sea temperature during the experiment (Fig. 2).

(Fig. 2)

Three samplings were performed during the main-dietary phase; from 9 to 11 October 2011, from 6 to 9 December 2011 and the final sampling and termination of the experiment was conducted from 20 to 22 March 2012. At each sampling, all fish were anaesthetized (MS-222 metacaine 0.1 g L^{-1}, Alpharma, Animal Health, Hampshire, UK) and the PIT-tag, fork length and weight of each individual fish were recorded. All fish were starved two days prior to the samplings in August and October, and three days prior to the samplings in December and March to avoid feed matter in the gastrointestinal system. At each sampling, 10 fish from each pre-dietary group in all the pens were sampled. The sampled fish at each sampling point were selected so that the mean weight corresponded to the mean weight of all the fish in the respective fish group within each pen (as all possible fish were weighted and PIT-tag read). After anesthetization, a blow to the head was used to kill fish sampled for analysis. Then the gill arches were cut and the fish were bled out in ice seawater. Length and weight of each

individual fish sampled for analysis were recorded after bleeding and the fish visually tagged. The fish were then gutted and filleted by hand during the pre-rigor state. Norwegian Quality Cut, NQC (NS9401, 1994) from the left fillet was photographed and the fat content was predicted by digital image analyses (PhotoFish, AKVAgroup, Bryne, Norway), as described by Folkestad et al. (2008). The visceral mass of the sampled fish were pooled on group level, homogenised and frozen at - 20°C for later analyses of total lipid content as described by Folch, Less & Stanley (1957). The proximate composition of crude protein, lipid (acidic-hydrolysis method), starch and moisture of the diets were analysed according to the methods described by Oehme et al. (2010). To determine the fatty acid (FA) composition of the diets, lipids were first extracted according to Foch et al. (1957), and a sample of 2 ml from the chloroform–methanol phase was dried under N_2 gas, then the residual lipid extract was trans-methylated overnight with 2',2'-dimethoxypropane, methanolic HCl and benzene at room temperature according to Mason & Waller (1964). Finally, the methyl esters were separated by gas chromatography and individual FA were identified as described in Røsjø et al. (1994).

The growth rate of the fish are presented as the thermal growth coefficient (TGC), and were calculated as described by Iwama & Tautz (1981): $TGC = [(M_1^{1/3} - M_0^{1/3}) \times (\Sigma T)^{-1}] \times 1000$, where M_0 and M_1 are the initial and final BW, respectively, and ΣT is the sum of day degrees during the period (feeding days x average temperature, °C). The mean TGC for the total main-dietary phase was calculated as the weighted arithmetic mean of the periodical TGC to balance these values in relation to their relative contribution to the weight gain.

All fish sampled and killed for analysis were starved and bled. The calculation of visceral-somatic index is therefore based on BW with minimal blood content and no feed material in the gastrointestinal system. Visceral-somatic index (VSI), was calculated as: Y (g) x BW (g)$^{-1}$

x 100, where *Y* is the measured visceral mass. The visceral mass was defined as all mass in the abdominal cavity except liver, heart, kidney and swim bladder. The CF was calculated as: 100 x *BW* (g) x fork length (cm) $^{-3}$. The dispersion in the distribution of BW, length and CF were assessed by calculating the CV: (standard deviation x mean value^{-1}) x 100.

The results were analysed by the General Linear Model (GLM) procedure in the SAS 9.4 computer software (SAS Institute Inc., Cary, NC, USA). Mean results per fish group in each pen were initially subjected to a two-way analysis of variance (ANOVA) to evaluate the effects of muscle fat content due to the pre-dietary phase (0.5LF, LF and HF), main-dietary treatment (oil source; MO and RO-diet) and their interaction (pre-diet x main-diet). As the statistical analysis showed that neither oil source nor the interaction term has significant effects on the traits studied, the data was analysed using pre-dietary treatment as the only experimental factor (one-way ANOVA). Significant differences among experimental groups within treatments were indicated by Duncan's multiple range test. Least-square means (lsmeans) comparison were also used to identify differences among variables within treatments. The Pearson product-moment correlation coefficient was used to describe the association between two variables. Linear regression analysis were conducted using Microsoft excel. The proportion of total variance explained by the model was expressed by R^2 and the level of significance was chosen at $P \leq 0.05$. Tendencies was identified at P = 0.05 – 0.1. The results are presented as means ± SEM, if not otherwise stated.

No significant effects of the main-dietary treatment (RO and MO-diet) or interaction term (main x pre-diet) *per se* were detected on the traits examined during the main-dietary phase. Thus, only the effects of body fat content due to the pre-dietary treatment are presented in the

results. No significant differences in mortality among the pre-dietary groups were observed during the main-dietary phase (24 out of 650 fish, 3.6%).

RESULTS:

The muscle fat content increased by 8.1% for 0.5LF fish, 5.6% for the LF group and 3.6% for HF group from August to October (Fig 4A1). Thus, during an 8-week period of declining day length, the initial significant differences in muscle fat content was equilibrated. TGC was highest for the 0.5LF group, intermediate for the LF group and lowest for the HF group (Fig 5A). The growth rate and the increase in muscle fat content from August to October showed a significant positive linear relationship, and the increase in muscle fat explained 81% of the variation in growth (Fig 3). From August to October, the growth rates were therefore highly affected by the pre-dietary treatment (ANOVA: $R^2 = 0.97$, $P < 0.001$). The muscle fat did not differ significantly between the pre-dietary treatments in October or December (Fig. 4A1), but pre-diet still significantly influenced the growth rates (ANOVA: $P < 0.05$, $R^2 = 0.51$) and the TGCs were similar, relatively, to the period from August to October (0.5LF > LF > HF), although no significant differences was found between LF and HF group. In the period December to March, the TGC for the 0.5LF and LF group were significantly higher ($P < 0.05$) than the HF group (Fig 5A). At the end of the main-dietary phase, the muscle fat content of the LF group was significantly lower ($P < 0.05$) than the 0.5LF group, and tended to be lower ($P < 0.1$) than the HF group (Fig 4A2).

(Fig. 3 and 4)

The BW of the LF group reached a similar BW as the HF fish in October, whereas the 0.5LF group reached a similar BW as the HF group in December (Fig 4B1). At the end of the trial in March, the LF group (6.87 ± 0.07 kg.) had a significantly higher ($P < 0.05$) BW than the HF group (6.40 ± 0.16 kg.) (Fig 4B2). The 0.5LF group (6.62 ± 0.12 kg.) had numerical higher BW than the HF group, however, no statistically significant difference was detected. From August 2011 to March 2012, the 0.5LF group gained 980 g. and the LF group gained 590 g. more relative to the BW of the HF group (Fig 5B). The overall weighted mean TGC during the main-dietary phase were 3.9 for the 0.5LF group, 3.4 for the LF group and 2.9 for the HF group. Hence, the pre-dietary treatment and consequently the fat status in August 2011 had a clear and significant effect on growth, weight gain and the changes in BW throughout the whole main-dietary phase, with a total duration of seven months.

(Fig. 5 and 6)

No significant differences in length between LF and HF group were detected during the trial (Fig 6B1). The strong growth spurt of the 0.5 LF group resulted in no significant differences in length between the 0.5 LF (75.9 ± 0.2 cm.) and HF group (76.4 ± 0.8 cm.) at the trial termination in March. However, the LF (77.9 ± 0.1 cm.) group was significantly longer ($P < 0.05$) than the 0.5LF group (Fig 6B2). The 0.5LF group that had the lowest CF in August, ended up having the significantly highest CF at termination (Fig. 6A1 and A2). The overall development in CF correlated well with the changes in muscle fat during the study ($r = 0.98$, $P < 0.01$). Significant positive overall correlations were also observed between the final CF and mean TGC ($r = 0.88$; $P < 0.001$), and between the final CF and total weight gain ($r = 0.88$; $P < 0.001$).

297 The visceral fat content of the HF group was consistently highest, although only significant in
298 October (Fig 7). The VSI of the LF group (8.5 ± 0.1) was significantly lower (ANOVA: $P <$
299 0.01) than the HF group (9.0 ± 0.1) in October, whereas the VSI of the 0.5LF group (8.7 ±
300 0.1) was not different from the LF or HF group. No significant differences in VSI were
301 detected in December (overall mean; VSI: 8.8 ± 0.1) or March (overall mean; VSI: 9.8 ± 0.2).
302
303 **(Fig. 7)**
304
305 The 0.5LF group had the highest CV_{BW} at the end of the pre-dietary phase (Fig 8A). From
306 August to October, the CV_{BW} of the 0.5LF group decreased and no significant difference in
307 CV_{BW} was observed at the samplings in October and December. However, at termination in
308 March, the HF group had a significantly ($P < 0.05$) higher CV_{BW} compared to both LF and
309 0.5LF group. The CV_{CF} was lowest for the LF group and similar for the HF and 0.5LF group
310 at the end of the pre-dietary phase (Fig 8B). At the sampling in October, after the large
311 increase in fat deposition, growth and CF, the 0.5LF group had the highest CV_{CF}. The
312 variation within the CV of CF for the 0.5LF group was at this time very high and no
313 significant differences between the groups was detected. The CV_{CF} for the HF group
314 increased gradually from October to March. In line with the CV_{BW}, the HF group had a
315 significantly ($P < 0.05$) higher CV_{CF} compared to the 0.5LF and LF group at termination. No
316 significant differences in the CV_{LENGHT} was detected during the experiment (results not
317 shown).
318
319 **(Fig. 8)**
320
321

DISCUSSION:

The coinciding increase in fat and improved growth shown by the 0.5LF and LF group compared to the HF group in the beginning of main-dietary phase (August and September), seem to reflect a growth response similar to CG and lipostatic regulation observed in previous studies in the field and laboratory (Ali et al., 2003; Jobling & Johansen, 1999; Johansen et al., 2002, 2001). The obtained growth rates, fat increase and weight gain from August to October, together with the high feed intake (on pen basis), indicate that the 0.5LF and LF group had increased feed consumption and hyperphagic behaviour. In addition to the high growth rate of the 0.5LF and LF groups, the increase in muscle and visceral fat content during August and September were substantial for these two groups. However, the muscle fat of the HF group also increased during this period (16.4%→20.0%). The TGC of the HF group had an average of 3.0, which is regarded as a normal and sufficient growth rate (Austreng, Storebakken, & Åsgård, 1987; Thorarensen & Farrell, 2011). Thus, improved growth in the LF groups from August to October, compared to the HF group, is not a result of impaired growth due to adiposity in the latter group, but rather a stronger response among the fish in the LF and 0.5LF group. The growth responses from August to October differ from the observations of Johansen, Sveier, & Jobling (2003), where Atlantic salmon fed a high fat diet during both the build-up and main phase, maintained their body fat levels after the build-up phase, at the same time as feed intake was down-regulated and growth impaired. In the present study, the salmon were exposed to natural photoperiod, as opposed to the study by Johansen et al. (2003), where the salmon were held under continuous light (24L:0D). It has been suggested that reduction in day length is an important environmental factor that trigger the salmon to assess its current mass during this time of the year (Maclean & Metcalfe, 2001). It may also apply for energy status and body condition (Kadri, Mitchell, Metcalfe, Huntingford, & Thorpe, 1996). In

addition, high retention of dietary lipid, elevated fat deposition, increased CF and rapid growth are observed during the autumn period (Alne et al., 2011; Dessen et al., 2017; Kadri et al., 1996; Mørkøre & Rørvik, 2001). Hence, the influence of natural seasonal cues might be the main reason for the observed differences in growth between the present study and the one of Johansen et al. (2003).

In October, two months after the start of the main-dietary phase, muscle fat and CF were restored in both the LF and 0.5LF group compared to the HF group. This observation shows that Atlantic salmon is able to rapidly replenish lipid stores and body condition during the autumn following a feeding period of a low-fat diet or restricted ration of this diet. In contrast, the visceral fat content among the groups maintained about the same pattern thought out the study. The level or severity of restricting lipid deposition during pre-dietary phase was highly negatively related with the magnitude of the subsequent growth response from August to October. This was particularly linked to the relative muscle fat content at termination of the pre-dietary phase prior to autumn. The degree of CG response seem also related to the level of deviance in body condition, length and mass in the restricted or deprived fish groups compared to their non-treated counter-specifics (Alvarez & Nicieza, 2005; Johansen et al., 2001; Johnsson & Bohlin, 2005; Johnsson & Bohlin, 2006). Although the deviance in mass and length may have contributed to the growth response in the present study, the small difference between the LF and HF groups in August and the strong correlation between muscle fat and growth, indicate that fat/energy status seem to be the most important growth regulator during August and September. The increased growth and rapidly replenishment of lipid stores suggest a robust mechanism for the regulation of body fat, and are in line with the observation of Silverstein, Shearer, Dickhoff & Plisetskaya (1999).

Several studies have indicated that animals displaying CG prioritise the restoration of body condition and fat stores before more resources are allocated to support structural and skeletal growth (Broekhuizen, Gurney, Jones, & Bryant, 1994; Johnsson & Bohlin, 2006). In part, the results of the present study support these observations, as both the relative muscle fat content and CF were quickly restored in the 0.5LF group, but not that quickly restored for BW and length. Some studies have also suggested that structural restoration can be delayed due to the effects of food deprivation or restriction on the endocrine system, involved in the regulation of growth (Björnsson, 1997; Johnsson, Jönsson, & Björnsson, 1996). There is evidence that skeletal and muscle growth are independent processes and that the relationship between length and weight is approximately cubic (Einen, Waagan, & Thomassen, 1998; Jobling, 2002; Mørkøre & Rørvik, 2001). Thus, changes in weight are relatively greater than in length, and the rapid increase in BW and fat content observed among the 0.5LF group in the autumn, may be a factor explaining why length are restored later than body shape and fat content.

The stabilisation of the muscle fat in October coincides with the study of Mørkøre & Rørvik (2001). This may suggest that the capacity of muscle fat deposition has reached an upper limit at this time point. There is documentation that CG responses will cease as lipid stores and body condition are restored to similar levels as the non-affected conspecifics (Johansen *et al.*, 2001; Ali *et al.*, 2003; Alvarez & Nicieza, 2005; Johnsson & Bohlin, 2005). In the present study, the LF and 0.5LF groups continued to grow faster than the HF group both during the periods October to December and December to March. The improved growth of the LF groups from December to March was evident although the relative muscle fat content, CF and BW were restored prior to this period and not significantly different from the HF group. Hence, the observed growth response in this period is not directly related to restoration of fat or BW. The sexual maturation process in Atlantic salmon requires, in addition to photoperiod,

sufficient fat and energy reserves (Kadri et al., 1996; Rowe & Thorpe, 1990; Taranger et al., 2010). The production of gonads are energetically expensive and acquire high-energy investment (Fleming, 1996; Jonsson, Jonsson, & Hansen, 1997). Appropriate and available energy and fat reserves during the spring period seem to be a major factor controlling initiation and proceeding of the maturation process (Thorpe, 1994; Thorpe, Mangel, Metcalfe, & Huntingford, 1998; Wright, 2007). Too low energy and fat levels may arrest the maturation process and postpone reproduction (Duston & Saunders, 1999; Rowe & Thorpe, 1990; Rowe, Thorpe, & Shanks, 1991; Thorpe, 1994; Thorpe, Talbot, Miles, & Keay, 1990). Hence, well growing salmon with a high and stable fat content are more likely to adopt the development pathway of becoming sexual mature (Thorpe, 1994). Following this line of arguments, the stronger growth response observed in both LF groups compared to the HF group prior to the spring period in the present study, may have been triggered by the salmon reproductive life strategy. However, to verify this, the groups of salmon must be studied for a longer period (during late spring, summer and autumn) and measurements of relevant plasma hormones, gonad-somatic index and gene expression of e.g. myosin should be conducted. Unfortunately, this was not possible in the present study. Anyhow, observation of a long-term improved growth response is important for a further development of a dynamic seasonal feeding concept in salmon farming. Not only for traditional sea cage farming, but also in closed and semi-closed production units where photoperiod may be manipulated. Taken into consideration that the initial BW of the 0.5LF group was 738 g. less than the HF group, a relative increase in weight gain of 950 g. more than the HF group is impressive.

When feed availability is restricted, competition for the feed often increase and dominant individuals may try to monopolize the feeding area to obtain larger amounts of feed that is supplied (Maclean & Metcalfe, 2001; Ryer & Olla, 1996). High competition for feed may

422 therefore lead to increased disparities in feed intake and growth that consequently will give
423 higher variation in BW. To minimize such effects, the 0.5LF group was administrated all
424 daily feed in only one ration during the pre-dietary phase. The high dispersion in BW and CF
425 among the HF group at termination of the main-dietary phase indicates that the 0.5LF and LF
426 group had an increase in weight and CF that was more homogeneous than the HF group. This
427 was probably due to the increased growth of LF groups in latter stages of the trial. The
428 possibility that fish among the LF groups displayed aggressive behaviour and tried to
429 monopolize food in this period seem unlikely due to three main factors: i) the HF group
430 showed a normal and satisfying growth with mean TGC of 3.2, ii) feed was administered in
431 excess during the main-dietary phase to ensure *ad libitum* feeding and iii) no or little fin
432 damage were observed at termination.
433
434 In summary, salmon with low body fat levels (LF groups) prior to declining day lengths in the
435 autumn displayed significantly higher growth rate and weight gain compared to the control
436 fish (HF group). The initial differences in muscle fat and CF were restored after only two
437 months, displaying rapid replenishment of lipid stores and body condition. Differences in
438 body fat content prior to autumn had significant effect on growth throughout the whole seven-
439 month main-dietary phase, even after similar body fat stores among the groups were obtained,
440 indicating a more complex explanation than just a lipostatic regulation mechanism. The LF
441 and 0.5LF fed fish obtained a significantly lower variation in BW and CF than the HF fed fish
442 at trial termination. This increased uniformity of BW and CF may reduce the amount of
443 manual gutting and filleting of large and small individuals, which optimizes the efficiency of
444 automatic gutting and filleting of salmon at the time of slaughter.
445

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667 **TABLES:**

668

669 **Table 1.** Biometrics and fat content of Atlantic salmon in August 2011 fed a diet high-fat diet
670 (HF), low-fat high-protein diet (LF) or half ration of the low fat diet high-protein diet (0.5LF)
671 from May until August 2011, referred to as pre-dietary feeding phase. Biometric parameters
672 for all fish are presented as means ± SD, whereas biometric parameters and fat content for
673 sampled fish are presented as means ± SEM together with indications of significant
674 differences.

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Dietary treatment	HF	LF	0.5LF
Biometric parameters, all fish			
Number of fish, n	584	584	602
Bodyweight, g	2651 ± 335	2506 ± 287	1865 ± 253
Fork length, cm	59.1 ± 2.3	59.1 ± 2.1	55.8 ± 2.3
CF	1.28 ± 0.09	1.21 ± 0.07	1.07 ± 0.08
Biometric parameters, sampled fish, n = 20			
Bodyweight, g	2619 ± 70^{a}	2515 ± 63^{a}	1881 ± 47^{b}
Fork length, cm	59.0 ± 0.5^{a}	59.0 ± 0.4^{a}	55.7 ± 0.5^{b}
CF	1.22 ± 0.02^{a}	1.18 ± 0.02^{a}	1.03 ± 0.01^{b}
VSI	11.3 ± 0.4^{a}	9.6 ± 0.2^{b}	8.5 ± 0.1^{c}
Fat content, sampled fish, n = 20			
Muscle fat, %	16.4 ± 0.3^{a}	13.1 ± 0.2^{b}	11.3 ± 0.3^{c}
Visceral fat[†], %	39.0	29.0	26.6

676 CF; condition factor, VSI; Visceral somatic index
677 [†]The analysis of visceral fat content was conducted on pooled samples in August 2011 (n=1)
678 Values in the same row with different letters are significantly different ($P \leq 0.05$) determined by one-way
679 ANOVA followed by Duncan's multiple range test.

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684 **Table 2**. Chemical compositions (wet weight, as is basis) and fatty acid composition (% of
685 total fatty acids) of the diets used in the main-dietary phase.

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	7 mm pellet		9 mm pellet	
Diet code	MO	RO	MO	RO
Chemical composition (wet weight, as is basis)				
Dry matter, %	93.2	94.0	93.8	93.9
Crude protein (*N* x 6.25), %	41.2	41.7	34.4	34.6
Crude Lipid, %	31.2	31.4	37.0	35.7
Starch, %	6.2	6.1	6.7	6.8
Ash, %	4.8	4.8	5.1	5.1
NFE[†], %	16.0	16.1	17.3	18.5
Crude protein/lipid ratio	1.32	1.33	0.93	0.97
***Calculated values*[‡]**				
Gross energy, MJ kg^{-1}	24.8	25.1	25.7	25.5
DP, g kg^{-1}	354	359	296	298
DE, MJ kg^{-1}	21.4	21.5	22.2	21.9
DP/DE ratio, g MJ kg^{-1}	16.6	16.6	13.3	13.6
Fatty acid composition (% of total fatty acids)				
C 16:0	12.7	8.5	14.3	9.3
C 18:0	3.2	2.7	3.7	2.9
∑SFA[§]	22.6	15.1	24.0	15.9
C 18:1 n-9	26.8	42.1	23.3	42.5
∑MUFA[¶]	38.1	49.8	36.2	52.8
C 18:2 n-6	8.1	13.9	7.4	13.9
C 18:3 n-3	3.4	6.5	2.9	6.0
C 20:5 n-3	10.1	4.6	11.1	4.0
C 22:5 n-3	1.3	0.6	1.4	0.5
C 22:6 n-3	7.2	3.5	7.5	3.6
∑PUFA[¥]	34.3	30.4	32.7	29.0
SUM EPA+DHA	17.4	8.1	18.6	7.5
n-6/n-3 ratio	0.4	0.9	0.4	1.0

687 MO; Marine oil profile, RO: Rapeseed oil profile, N; Nitrogen, NFE; Nitrogen-free extracts, DP; digestible
688 protein, DE; digestible energy, MJ; Mega joule, SFA; Saturated fatty acids, MUFA; monounsaturated fatty acids,
689 PUFA; polyunsaturated fatty acids.
690 [†]NFE was calculated as = 100 – (protein+lipids+ash+water)
691 [‡]Gross energy, DP and DE were estimated assuming 23.7, 39.5 and 17.2 MJ kg^{-1} as the gross energy content of
692 protein, lipids and carbohydrates, respectively. The apparent digestibility coefficients (ADCs) for protein and
693 lipids used were 0.86 and 0.94, respectively (Einen & Roem 1997), whereas 0.50 was used for NFE (Arnesen &
694 Krogdahl 1993).
695 [§]SFA; C14:0. C15:0, C16:0, C18:0 and 22:0.

696 ¶MUFA; C16:1n-9, C16:1n-7, C17:1n-7,C18:1n-7, C:18:1n-9, C20:1n-7, C20:1n-9,C20:1n-11, C22:1n-
697 9,C22:1n-11,C24:1n-9
698 ¥PUFA; C16:2n-3, C16:3n-4, C18:2n-6,C18:3n-6, C18:3n-3, C18:4n-3, C20:4n-3, C20:2n-6, C20:3n-6, C20:4n-
699 6,C20:5n-3, C22:5-n-3, C22:6n-3.

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718 **FIGURE CAPTIONS:**

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720 **Fig. 1** Schematic overview of the experimental design during the pre- and the main-dietary
721 phase. The squares during the pre-dietary phase represent net-pens fed different diets; HF =
722 high fat diet (black filled square), LF = low fat diet (grey filled square), 0.5LF = half ration of
723 the low-fat diet (white filled square). The large squares in the main-dietary phase represents
724 the net-pens and the squares within the net-pens are the pre-dietary groups.

725

726 **Fig. 2** Ambient daily sea temperature (°C, y-axis) and hours of daylight (hours, z-axis) during
727 the pre-dietary phase (May to August 2011) and the main-dietary phase (August 2011 to
728 March 2012). The length of the different periods are indicate by the different grey colours
729 (light grey = pre-dietary phase, dark grey = main-dietary phase).

730

731 **Fig. 3** Regression line between thermal growth coefficients (TGC) and the increase in muscle
732 fat (%) from August to October in Atlantic salmon fed three different pre-dietary treatment
733 from May to August 2011; high fat diet (black filled squares) = HF, low fat diet (grey filled
734 triangles) = LF, half ration of the low-fat diet (white filled circles) = 0.5LF. Each point
735 represents average per fish group/experimental unit (n = 12).

736

737 **Fig. 4** Muscle fat content (**A1**) and body weight (**B1**) development of Atlantic salmon fed
738 three different pre-dietary treatment from May to August 2011. Values are means ± SEM, n =
739 4 (n = 1 at termination of the pre-dietary phase). Values not sharing common superscript
740 letters within each sampling period are significantly different ($P \leq 0.05$). **A2** and **B2**, present
741 the final muscle fat and BW of the groups, respectively. The values 11.3%, 13.2% and 16.4%

represent the obtained fat content at the beginning of the main-dietary phase (August 2011) for the 0.5LF, LF and HF group, respectively. ns; not significant, *; trend (P < 0.1).

Fig. 5 Thermal growth coefficients (TGC) (**A**) and weight gain (kg) (**B**) of Atlantic salmon fed three different pre-dietary treatment from May to August 2011. Values are means ± SEM, n = 4. Values not sharing common superscript letters within each sampling period are significantly different ($P \leq 0.05$). The values 11.3%, 13.2% and 16.4% represent the obtained fat content at the beginning of the main-dietary phase (August 2011) for the 0.5LF, LF and HF group, respectively.

Fig. 6 Condition factor (CF) (**A1**) and fork length (cm) (**B1**) development of Atlantic salmon. fed three different pre-dietary treatment from May to August 2011. Values are means ± SEM, n = 4 (n = 1 at termination of the pre-dietary phase). Values not sharing common superscript letters within each sampling period are significantly different ($P \leq 0.05$). **A2** and **B2**, present the final CF and fork length of the groups, respectively. The values 11.3%, 13.2% and 16.4% represent the obtained fat content at the beginning of the main-dietary phase (August 2011) for the 0.5LF, LF and HF group, respectively.

Fig. 7 Visceral fat development of Atlantic salmon fed three different pre-dietary treatment from May to August 2011. Values are means ± SEM, n = 4 (n = 1 at termination of the pre-dietary phase). Values not sharing common superscript letters within each sampling period are significantly different ($P \leq 0.05$). The values 11.3%, 13.2% and 16.4% represent the obtained fat content at the beginning of the main-dietary phase (August 2011) for the 0.5LF, LF and HF group, respectively.

767

768 **Fig. 8** Variation in body weight (gram) (**A**) and condition factor (CF) (**B**) assessed using
769 coefficient of variation (CV; mean x SD.$^{-1}$) among Atlantic salmon fed three different pre-
770 dietary treatment from May to August 2011. Values are means ± SEM, n = 4 (n = 1 at
771 termination of the pre-dietary phase). Values not sharing common superscript letters within
772 each sampling period are significantly different ($P \leq 0.05$). ns; not significant

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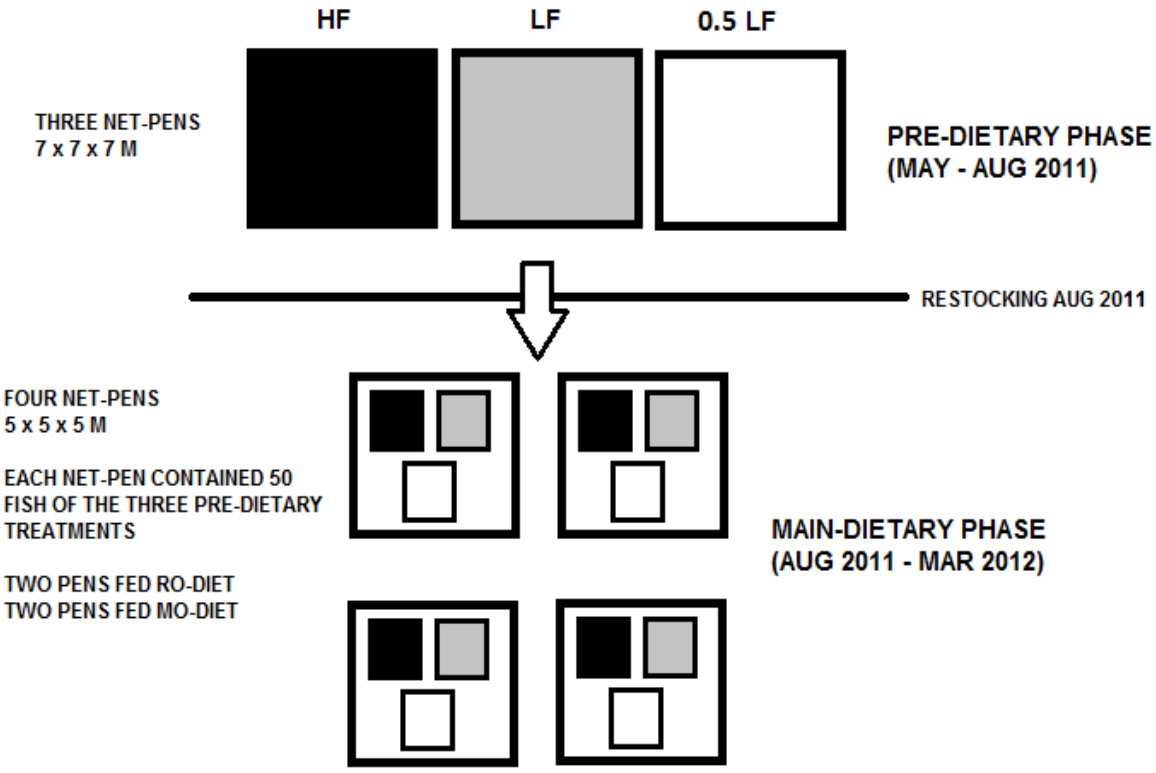
787 FIGURE 1:



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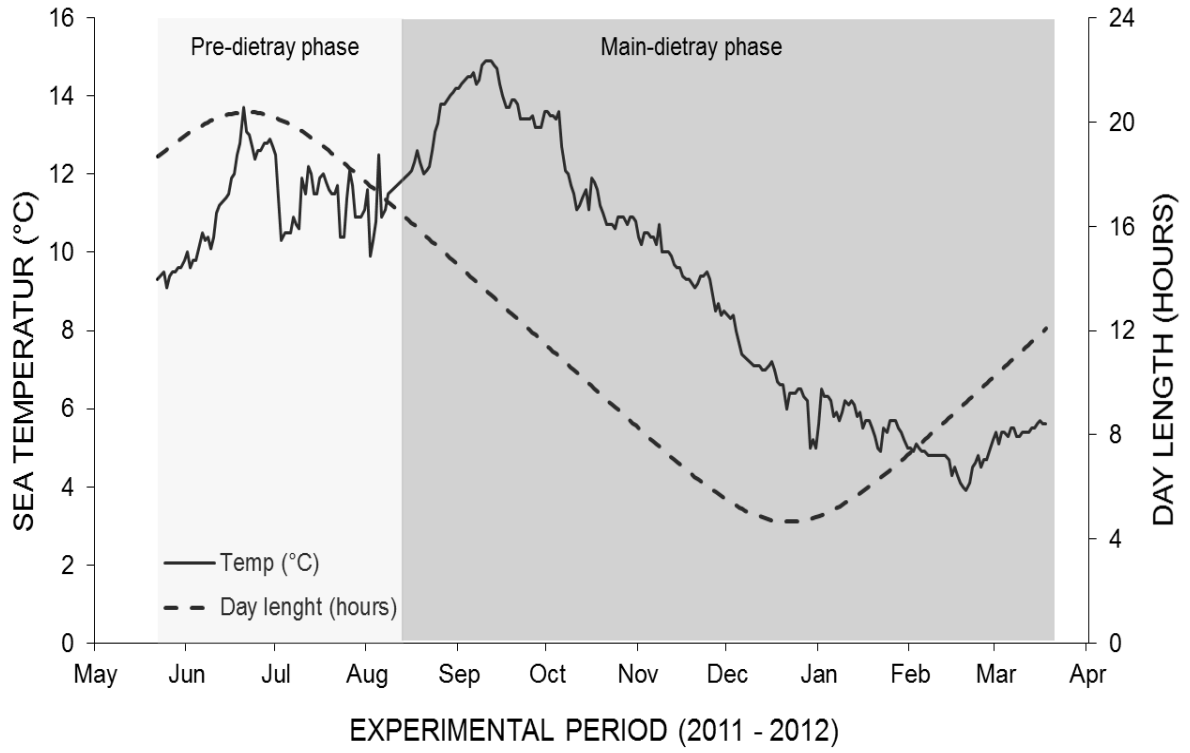
797 FIGURE 2:



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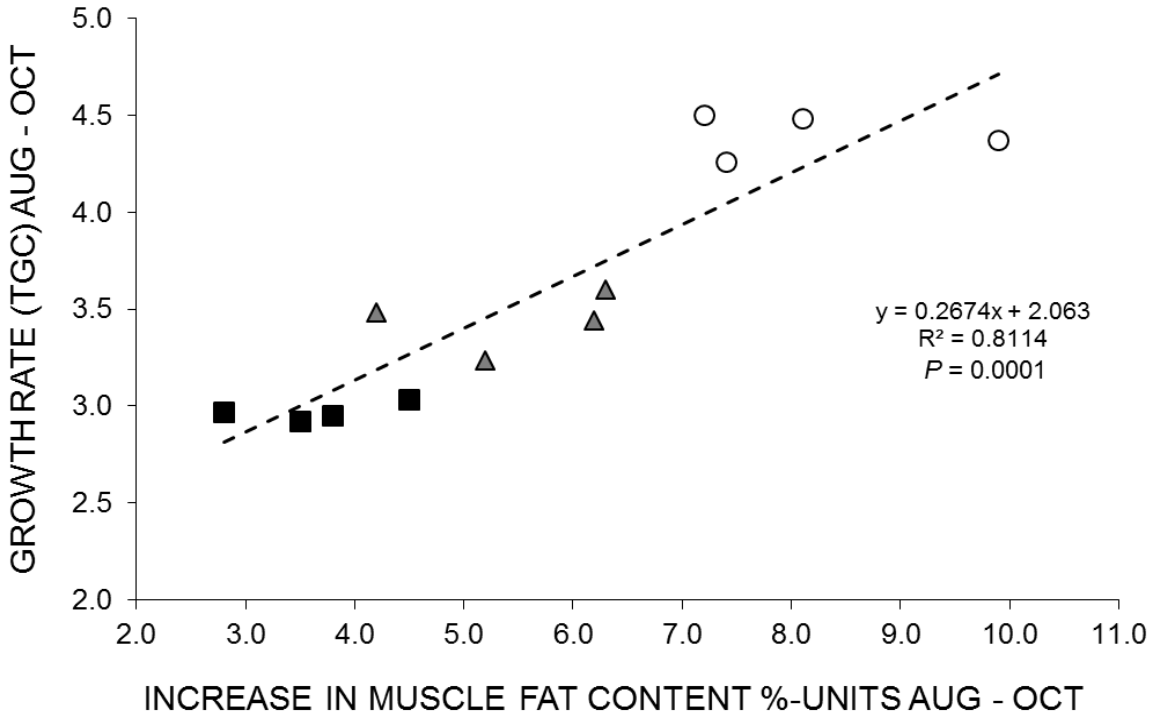
808 FIGURE 3:



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819 FIGURE 4:

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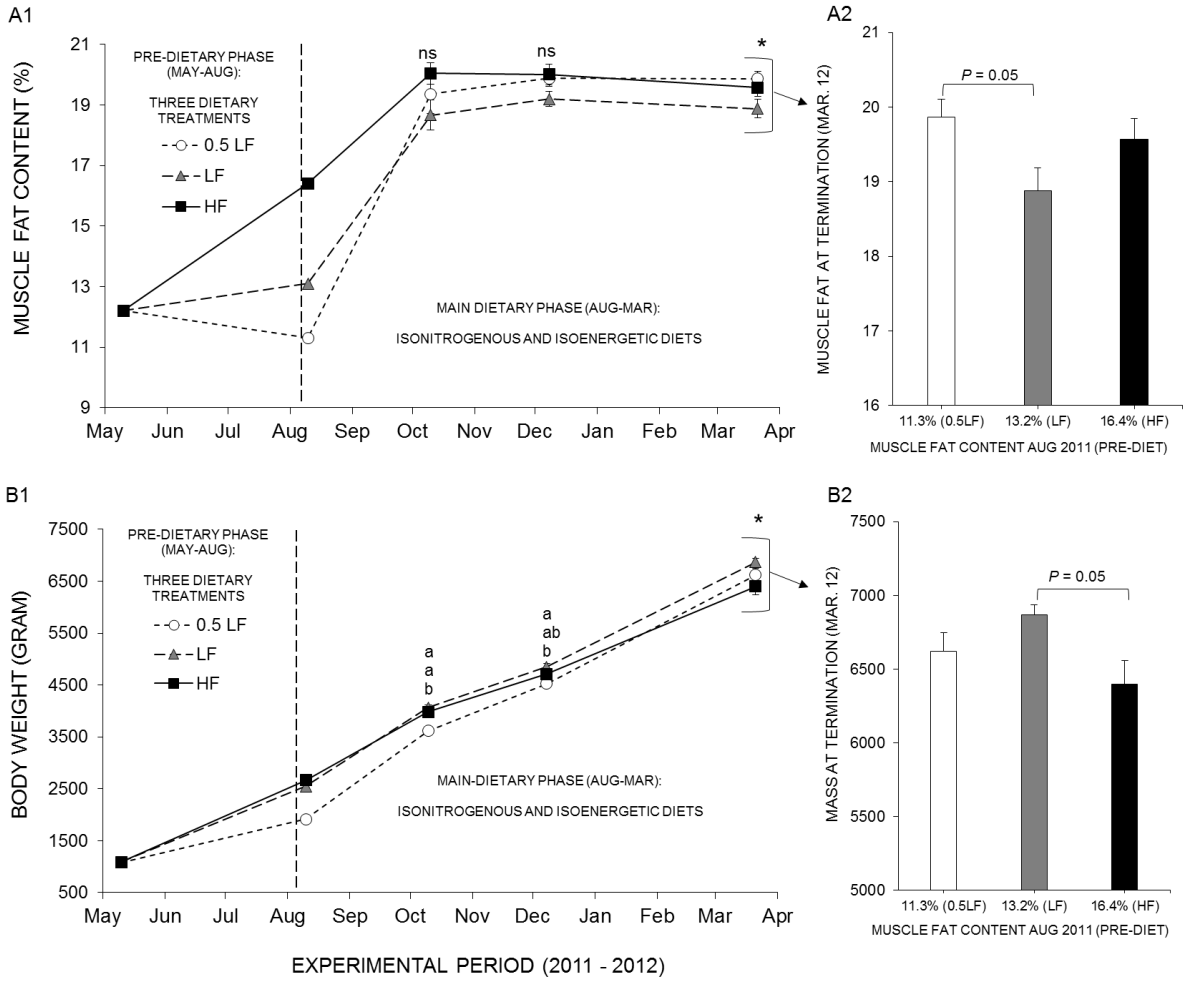

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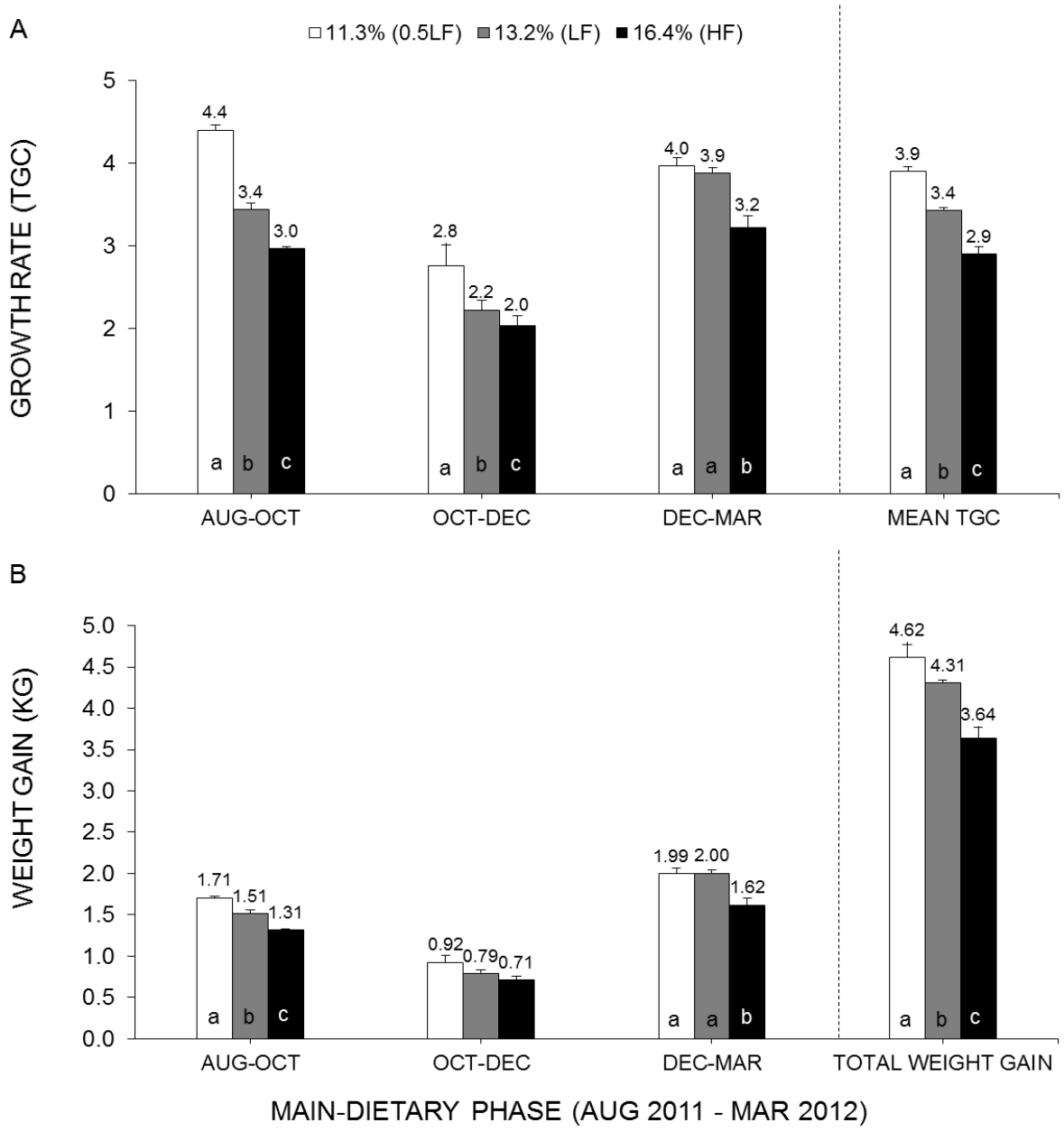
828 FIGURE 5:



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835 FIGURE 6:

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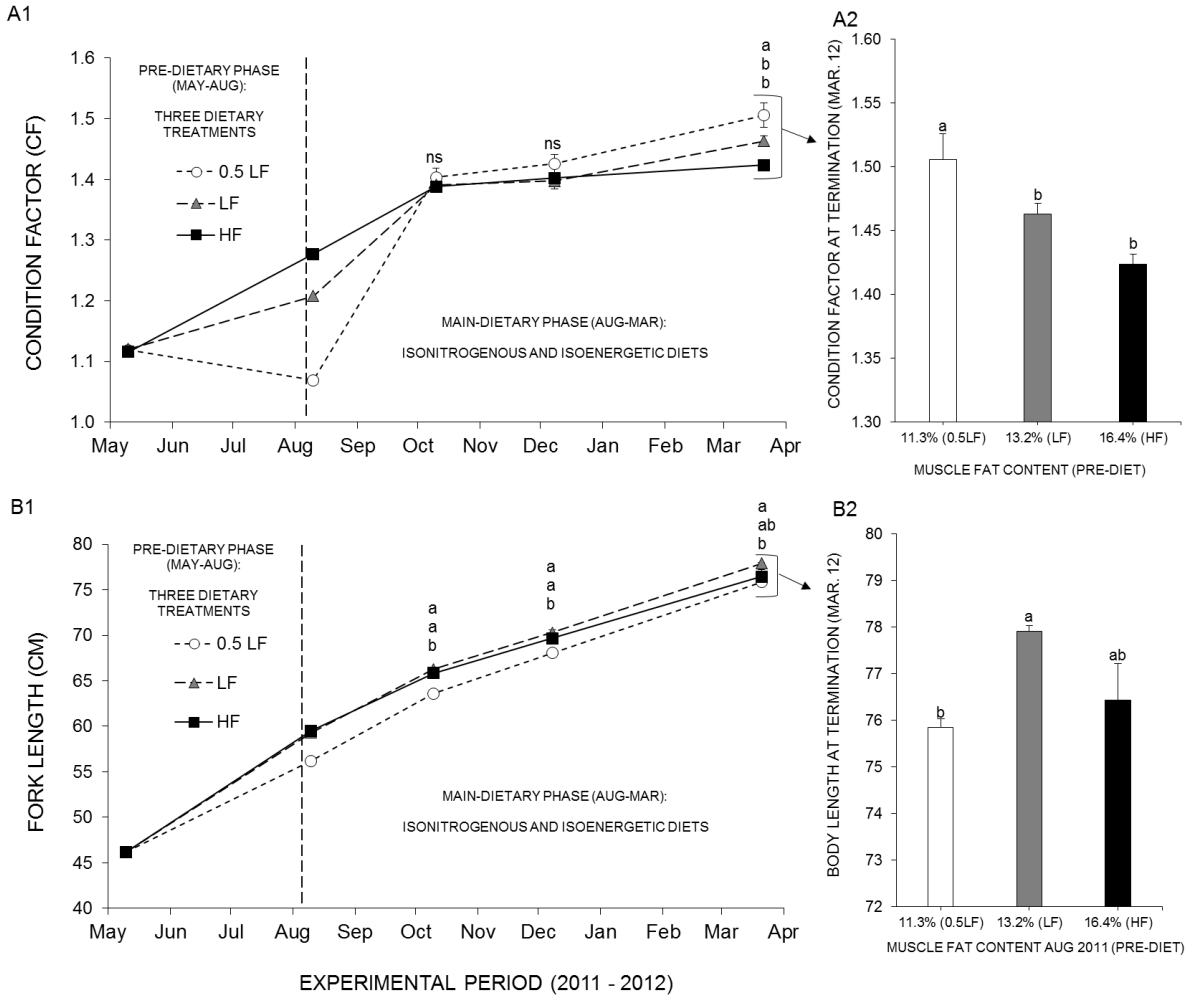

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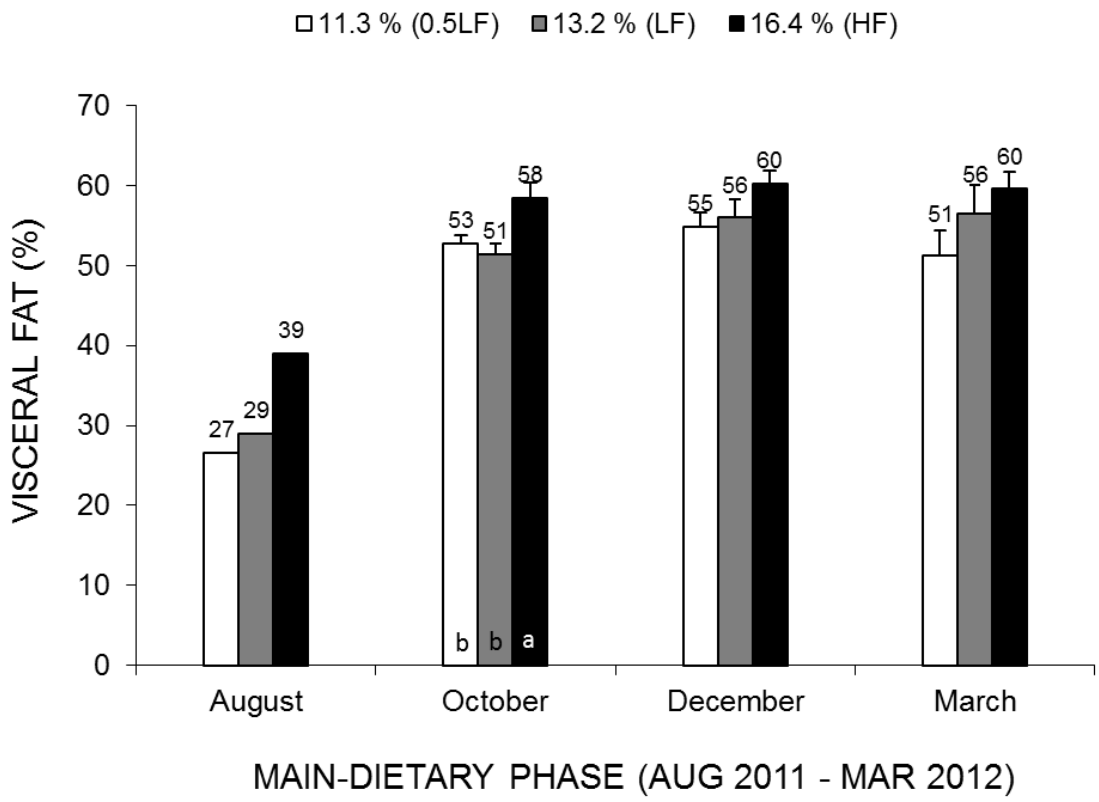
844 FIGURE 7:



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855 FIGURE 8:

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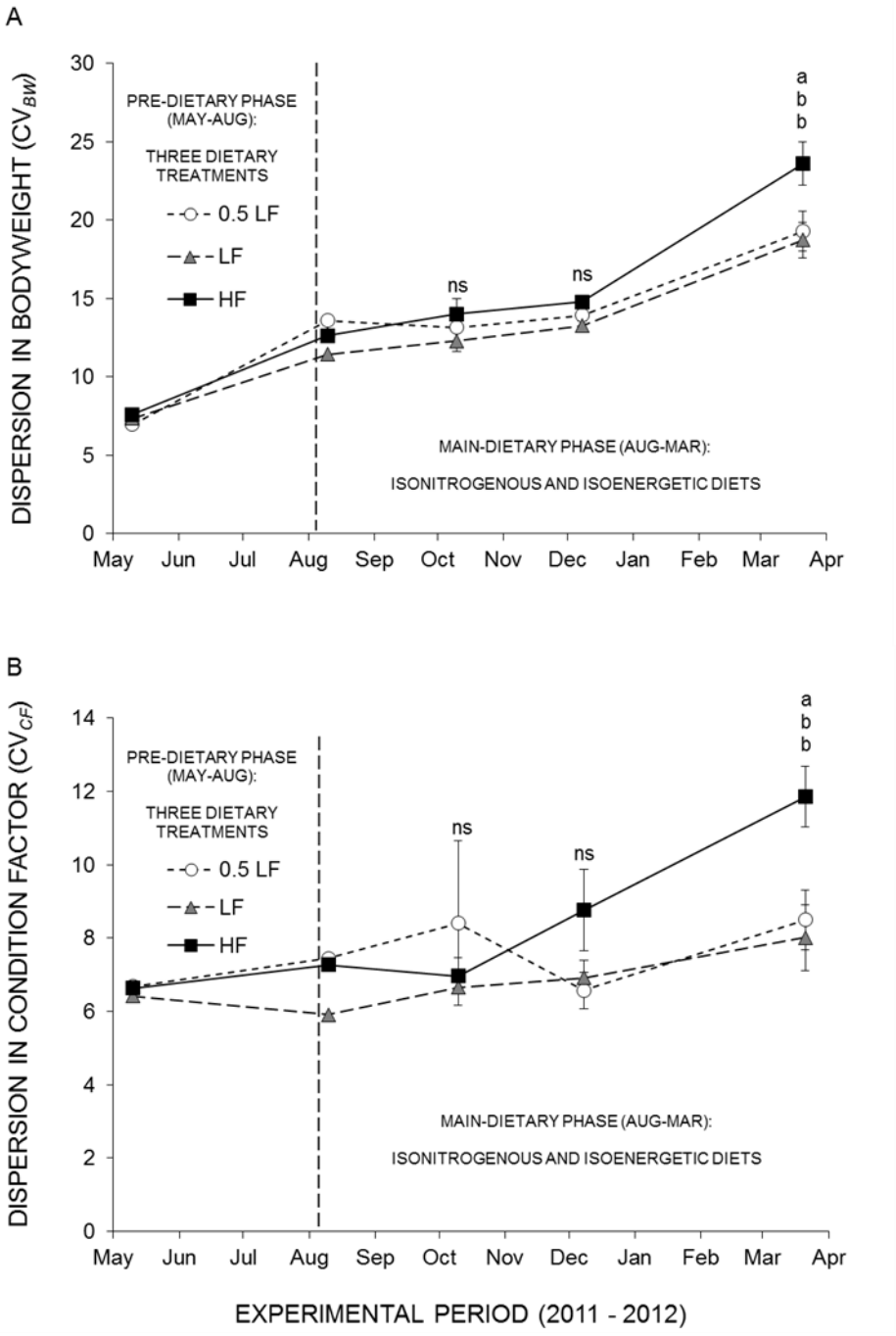

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