Ontogenetic effects of diet during early development on growth performance, myosin mRNA expression and metabolic enzyme activity in Atlantic cod juveniles reared at different salinities


1. Introduction

Recent findings have indicated that diet during the early larval period could influence juvenile quality, growth and general development in Atlantic cod (Gadus morhua) (Imsland et al., 2006; Koedijk et al., in press), but the differences in physiological adaptation to environmental changes suggested a more fundamental effect of diet at early life stages on juvenile performance (Imsland et al., 2006). This persisting effect of nutrition during early developmental stages on long-term growth has largely remained unexplored and implies a critical time frame during larval development in which optimal diet is required to ensure a successful adaptation to environmental changes throughout life. That growth differs with different diets is not new, but that diet during early life could to some extent determine growth capacity of juvenile fish and affect later growth or survival performances through physiological, structural or biochemical differences between animals fed different larval diets is worth examining in more detail. Exactly how early-life diet influences growth or physiological adaptation to environmental changes is largely unknown. More knowledge on this process could potentially reveal important nutritional and physiological information that could be applied to the cod aquaculture industry in order to optimize larval diets as well as to increase juvenile quality, the main bottleneck in commercial aquaculture of this species.

Larval food quality or quantity has been shown to affect metabolic enzyme activity in larval fish (Lamarre et al., 2007), but whether this effect persists into the juvenile stage of fish is unknown. In the current study the catabolic capacity was analyzed in juveniles by measuring three key metabolic enzymes in muscle and liver tissue. Lactate dehydrogenase (LDH) was measured as a marker for anaerobic glycolysis, malate dehydrogenase (MDH) as a marker for the aerobic metabolic capacity, and aspartate aminotransferase (AAT) as a marker for amino acid metabolism. Expression of myosin, the most abundant protein of muscle,
was also investigated in the current study, as the myosin transcripts content was previously reported as a potential biochemical marker for muscle growth in rainbow trout, *Oncorhynchus mykiss* (Overturf and Hardy, 2001) and spotted wolfish, *Anarhichas minor* (Imsland et al., 2006). The main aim of this study was to test the hypothesis that juveniles fed with zooplankton as a larval diet showed higher juvenile growth rates with a higher level of metabolic enzyme activities and myosin expression when compared to juveniles that had been fed with enriched rotifers as a larval diet. A secondary objective was to determine whether reduced salinity affects growth positively in cod juveniles.

2. Materials and methods

2.1. Animals

Detailed information on the used rearing practices is previously described in Koedijk et al. (in press). In short: cod larvae from one egg batch hatched on 4th of April 2006 at a commercial cod producer in western Norway. At 1 dph the larvae were transported to the High Technology Centre in Bergen where they were randomly divided over 8 experimental tanks. Larvae in four tanks were fed with natural zooplankton (1–36 dph; Z group) and larvae in the remaining four tanks were fed with enriched rotifers (1–36 dph; R group). A third group (ZRZ group) was fed with zooplankton (1–22 dph), after which diet changed to enriched rotifers (22–36 dph). The rotifer enrichment procedure consisted of short time enrichment (2–3 h) with a mixture of oil emulsion (40–60%), protein (30–50%) and vitamins (2–3%). In all tanks green water technique was used (*Rhodomonas sp.* and *Isochrysis sp.*). Temperature was stable at 8–9 °C, salinity was 32‰, density was 14 larveL−1, and the light regime was kept at 16 h light, 4 h dark and 4 h twilight (LD20:4). Live feed was fed until weaning onto dry feed at 36 dph (Ewos AgloNorse Standard, containing 59% protein, 21% fat, 10% ash and 1% fibre).

After weaning, the fish from the zooplankton group were significantly larger. Before the juvenile experiment started the zooplankton fish were reared at 10 °C, and the rotifer fish at 14 °C to reduce the size difference during a period of two months. After this period the temperature difference was reduced. One month before the juvenile experiment started both fish groups were both reared at 12 °C, fish were individually tagged intraperitoneally (Trovan® Passive Transponder tags), and randomly divided into eight 1 m² square covered grey fiberglass tanks (with a rearing volume of 400 L) with integrated light.

2.2. Experimental design and rearing conditions

The experiment was designed as a 2 × 2 × 4 factorial design with two salinities (15‰ or 32‰) in combination with two temperatures (10 °C or 14 °C), with the three fish groups within each tank (R; Z; ZRZ) per treatment (high and low salinity) in replicate. Each tank consisted of two salinities (15‰ or 14 °C), with the three fish groups within each tank (R; Z; ZRZ) per treatment (high and low salinity) in replicate. Each tank consisted of eight experimental tanks. Larvae in four tanks were fed with natural zooplankton (1–36 dph; Z group) and larvae in the remaining four tanks were fed with enriched rotifers (1–36 dph; R group). A third group (ZRZ group) was fed with zooplankton (1–22 dph), after which diet changed to enriched rotifers (22–36 dph). The rotifer enrichment procedure consisted of short time enrichment (2–3 h) with a mixture of oil emulsion (40–60%), protein (30–50%) and vitamins (2–3%). In all tanks green water technique was used (*Rhodomonas sp.* and *Isochrysis sp.*). Temperature was stable at 8–9 °C, salinity was 32‰, density was 14 larveL−1, and the light regime was kept at 16 h light, 4 h dark and 4 h twilight (LD20:4). Live feed was fed until weaning onto dry feed at 36 dph (Ewos AgloNorse Standard, containing 59% protein, 21% fat, 10% ash and 1% fibre).

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2.3. Growth

Growth sampling was performed at the onset of the experiment and thereafter at three week intervals. All growth (weight and specific growth rates) estimates in the present study were based on individually tagged fish, whereas results on feed conversion efficiency and daily feed consumption were based on the total biomass per tank. Specific growth rate (g-day−1, SGR) was calculated according to:

\[ SGR = \left( e^{g} - 1 \right) 100 \]

where \( g = \left( \ln(W_2) - \ln(W_1) \right) / (t_2 - t_1)^{-1} \) and \( W_2 \) and \( W_1 \) represent weights (g) at days \( t_2 \) and \( t_1 \), respectively.

2.4. Feeding efficiency

Feed conversion efficiency (FCE), total feed intake (C_T) and daily feeding rate (F) were calculated on a tank and treatment level because the three first-feeding groups were reared within the same tanks and division between these groups could therefore not be made during the main growth trial. C_T is the total ingested feed (g) over the experimental period, calculated from the difference between feed and waste feed. Feed was provided in excess for two hours daily (0800–0900 and 1400–1500). Feed that was not consumed by the fish was flushed out of the tank by the normal water flow through a bottom outlet, collected and pellets were counted manually to obtain the amount of unused feed as a fraction of the fed amount. Feed conversion efficiency (FCE) was calculated as: \( FCE = \left( 100 \times C_T / W_1 \right) \), and the feeding rate as: \( F = \left( 100 \times C_T / W_1 \right) \).

2.5. Tissue collection

Tissue samples from muscle and liver tissue were collected at the onset of the experiment (from untagged fish), and thereafter at twice six week intervals (from tagged fish), and consisted of eight anaesthetized fish (Metacain, 0.2 g L−1) from each of the three first-feeding groups from each of the four treatments (n = 96 at each sampling). Fish and individual tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

2.6. Myosin mRNA expression

Myosin expression was quantified as levels of myosin heavy chain mRNA by real-time reverse transcription PCR (qRT-PCR) using SYBR Green Quantitative RT-PCR Kit from Sigma (St. Louis, Missouri) and the MyiQ single-color Real-Time PCR detection system from BioRad (Hercules, California). Levels of Myosin mRNA were normalized to 18S rRNA. Primers specific to myosin heavy chain and 18S were designed using available sequences for *Gadus morhua* (GenBank Accession Numbers AY093703 and U14347 for Myosin and 18S respectively). Primer sequences were as follows (5′–3′): Forward primers; Myosin-F, CAGAAGCTTAAAGGTTGC and 18S-F, GGCTGAAAAGTGAACCT GC; Reverse primers, Myosin-R, GCAGGCTTCTTCTTATCTCTC and 18S-R, GCCCGTCTTTCATGATTA−3′.

For both genes, efficiency and specificity of the reactions were verified before running the samples. Efficiencies (%) of the reactions were estimated using eight points serial dilutions and were 98.5% (r², 0.996) and 106.5% (r², 0.998) for Myosin and 18S respectively. Specificity of each reaction was verified by running a melt curve analysis and by running reaction products on a 6% acrylamide gel. Both methods showed a single specific product for Myosin and 18S reactions.
For all samples, total RNA was extracted from white muscle using TRIzol (Gibco – Life Technologies, Rockville, Maryland) and quantified spectrophotometrically. Integrity of the extracted RNA was verified on a 1% agarose gel. All samples were treated with DNase to remove any possible trace of DNA contamination. Samples were run in triplicate in separate reactions for Myosin and 18 S. qRT-PCR reactions were performed in a single 20 µL reaction by adding 1 µM of each primer and 10 ng total RNA to the ready mix furnished with the kit. Conditions used for real-time RT-PCR were: 2 min at 94 °C, 30 cycles consisting of 15 s at 94 °C followed by 60 s at 60 °C. The fluorescence threshold cycle (CT) was determined using the Optical software from BioRad (Version 1.0). The relative quantity of Myosin mRNA was determined using the Pfaff method (Pfaffl, 2001). This mathematical model allows the evaluation of the respective efficiencies of the genes considered.

2.7. Metabolic enzyme assays

Enzyme activities were measured in triplicate for three enzymes: LDH (EC 1.1.1.27), MDH (EC 1.1.1.37) and AAT (EC 1.4.1.21) on tissue samples (liver and muscle) in 208 individually tagged fish. Frozen tissue samples (−80 °C) were submerged and homogenized (Heidolph Diax 900, Heidolph Instruments GmbH, Schwabach, Germany) in nine volumes of Tris–HCl buffer (100 mmol L−1, pH 7.5) and kept on ice. Homogenates were centrifuged at 10,000 RCF at 4 °C for 3 min. Enzyme activities were measured in supernatant using a spectrophotometer (Ultrospec 3100 pro UV/visible, Biochrom Ltd., Cambridge, UK) with a thermostatic (14 °C) cuvette holder (Perkin Elmer, Wellesley, Massachusetts) for 3 min. Lactate dehydrogenase activity was measured according to (Thibeault et al., 1997), AAT according to (Pelletier et al., 1995), and malate dehydrogenase as modified from LDH activity, using the same buffer solution and a working solution consisting of 0.2 mM NaOH and 5 mM oxaloacetae (pH 7.1) (Bergmeyer, 1974). All enzyme activities were expressed as units·g−1 tissue where one activity unit was equals the formation of 1 µmol of product per min. Total protein content of muscle and liver tissue were measured using bicineonique acid (Smith et al., 1985).

2.8. Statistical analysis

Statistical analyses were performed using SPSS 14.0. Data were tested for normality with a Kolmogorov Smirnov test (Zar, 1996). Homogeneity of variance was tested with Levene’s F-test (Brown, 1974). Data were transformed if necessary. A four-way Model III nested MANOVA (Johnson and Wichern, 1992) where replicate tanks were nested within salinity, temperature and first-feeding group was applied to test for overall differences in specific growth rates, with Wilks’ Lambda (Λ) multivariate test to observe overall trends over time. The equation of the full nested model had the form: $Y = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l(ij) + \epsilon$, where $\alpha_i$ is the factor for salinity, $\beta_j$ is the factor for temperature, $\gamma_k$ is the factor for fish group, $\delta_l(ij)$ is the replicate factor (tank) which is nested in salinity, temperature and first-feeding group. Differences in mean weight at date between groups were tested with a four-way nested ANOVA (Scheffé, 1959; Zar, 1996). If no interactions or tank effect were found the models were reduced and main effects were tested. A Student Newman–Keuls’ post hoc test was applied to identify differences between experimental groups. Metabolic enzyme activities and myosin mRNA expression data were tested with covariate analyses (ANCOVA), with weight as covariate, to test group differences or treatment effects. All tests were performed with a significance level of $\alpha = 0.05$.

![Fig. 1. Mean specific growth rates (%·day$^{-1}$) for three groups of Atlantic cod juveniles differing in larval history, when reared at two salinities (15‰, 22‰) in combination with two temperatures (10 °C, 14 °C) during four consecutive periods, each lasting three weeks. Data from replicate tanks within each treatment are pooled (SE). Stars within bars indicate a significant difference for that group within each growth period (Student Newman–Keuls post hoc test, P < 0.05).](image-url)
3. Results

3.1. Juvenile growth performance

No initial weight difference was found between fish of different groups (ANOVA, $F=0.38, P=0.69$), or between treatments (ANOVA, $F=1.92, P=0.13$). An overall effect of first-feeding history was found throughout the experiment (three-way, type III, MANOVA (group), Wilks’ lambda ($\Lambda_{0.05} = 0.83$, $P<0.01$), with significantly lower growth rates in juveniles that had a larval feeding history based on enriched rotifers (Fig. 1), leading to the highest final weights in the Z group. No difference occurred in growth rates between fish groups Z and ZRZ at any given treatment (Fig. 1). Within temperatures fish groups showed higher overall growth rates at reduced salinity compared to full salinity (Fig. 2). An interactive effect between salinity and temperature was found on specific growth rates (three-way, type III, MANOVA (salinity x temperature)). Wilks’ lambda ($\Lambda_{0.05} = 3.274$, $P<0.05$). This effect was related to increased growth at low salinity (15‰) and high temperature (14°C) compared to full salinity at 14°C, while the growth difference between the salinity levels did not occur as pronounced at 10°C.

3.2. Mortality, feed intake and feed conversion efficiency

Mortality differed between the four treatments (Student Newman–Keuls multiple comparisons, $P<0.05$, Table 1), and was significantly higher at full salinity, compared to reduced salinity. Neither a temperature effect nor first-feeding effect was found. Feed consumption (Table 1) was lower at reduced salinity at 10°C, but this salinity effect was not observed at high temperature. Feed conversion efficiency was 17% higher in the low salinity groups, compared to the full salinity groups.

3.3. Relative myosin mRNA expression

The three fish groups within each treatment showed no difference in expression level at either sampling date (ANCOVA, $F=0.303$, $P>0.1$, Fig. 3), but a reduction of myosin relative expression over time was observed in all groups, at each environmental treatment (Fig. 3). This reduction was more pronounced at 14°C compared to 10°C within each salinity treatment. A negative correlation between myosin expression and weight were found, while no correlation was observed with growth rate ($P>0.05$).

3.4. Metabolic enzyme activity

An interactive effect (Table 2) between salinity and temperature was found for two of the three measured metabolic enzymes (AAT and MDH), measured in liver tissue (ANCOVA with fish weight as covariate value).
(AAT, $F = 4.02, P < 0.05$), (MDH, $F = 8.6, P < 0.05$) which was related to a weaker negative correlation (AAT), or absence of correlation (MDH) between enzyme activity and weight at $10^\circ C$ and 32‰. No interactive effects occurred in enzyme activities measured in muscle tissue (Table 2). Overall, relations were more pronounced between weight and enzyme activity, than between growth and enzyme activity. Salinity had a significant effect on MDH (ANCOVA, $F = 7.5, P < 0.05$) and AAT (ANCOVA, $F = 13.3, P < 0.05$) in muscle tissue but did not affect any enzymes in liver tissue. Temperature affected LDH significantly in the liver tissue (ANCOVA, $F = 8.7, P < 0.05$) which was expressed by lower enzyme activity at high temperature ($14^\circ C$).

In general, no difference or trend occurred between first-feeding groups in AAT (Fig. 4), LDH (Fig. 5), or MDH (Fig. 6) activity.

![Fig. 4. Aspartate aminotransferase activity in muscle (top) and liver tissue (bottom) of three groups of Atlantic cod juveniles at six week intervals when reared at two salinities and two temperatures. Blue bars = zooplankton; green = zooplankton from 1–22 dph and rotifers from 22–36 dph: brown = enriched rotifers, as determined by diet during the larval period.](image)

![Fig. 5. Lactate dehydrogenase activity in muscle (top) and liver tissue (bottom) of three groups of Atlantic cod juveniles at six week intervals when reared at two salinities and two temperatures. Blue bars = zooplankton; green = zooplankton from 1 to 22 dph and rotifers from 22 to 36 dph: brown = enriched rotifers, as determined by diet during the larval period.](image)

4. Discussion

4.1. Ontogenetic effects of larval diet on juvenile growth performance

Our findings show that ontogenetic effects of larval diet clearly influenced juvenile growth rates. Fish with a larval history based on enriched rotifers as a first-feeding diet showed significantly lower growth rates compared to fish that had been fed with natural zooplankton as a larval diet. This is similar to previously reported studies on Atlantic cod in which overall juvenile quality was lower when
fed rotifers as larval diet (Imsland et al., 2006) and that juvenile growth performance was negatively affected when enriched rotifers were fed, compared to a zooplankton diet (Koedijk et al., in press). However, in the current study no growth difference was found between the Z and ZRZ group, or only for 14 days (22–36 dph; ZRZ group) (Koedijk et al., in press). Nutritional contents and limitations of live prey (Hamre and Harboe, 2008), as well as nutritional differences between enriched rotifers and zooplankton have been well documented (van der Meeren et al., 2008). Even though nutritional contents can vary widely across prey species, enrichment procedures, geographical location or season, the main trend is that zooplankton contains higher levels of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) compared to enriched forms of rotifers or Artemia. Two different enriched Artemia diets with similar DHA contents fed to Atlantic cod larvae (37–59 dph) resulted in significantly different mean specific growth rates (10.4 or 6.9%day$^{-1}$) (Garcia et al., 2008a). A similar result was reported for different rotifer enrichment methods where a diet containing the highest DHA content did not result in the highest specific growth rates in Atlantic cod larvae (Garcia et al., 2008b). However, enriched rotifers differ from natural zooplankton not only nutritionally (van der Meeren et al., 2008), but also in size, and size variability, energetic content, swimming speed, behavior (Beck and Turingan, 2007) and general appearance. There are also indications that structural differences related to the absorption of peptides could occur in the digestive tract as induced by diet during early development (Amberg et al., 2008), potentially resulting in higher protein absorption efficiency when fed zooplankton as larval feed. It is likely that all these different aspects, individually as well as interactively, have contributed to differences in growth performance.

4.2. The effect of salinity on juvenile growth

Cold water fish have shown to be able to adapt to reduced salinities relatively easily as studies on wolfishes, A. minor (Foss et al., 2001), A. lupus (Le Francois et al., 2004) and Atlantic halibut, Hippoglossus hippoglossus have indicated (Imsland et al., 2008). Atlantic cod has shown to be able to benefit from intermediate salinity (14‰) as this increased growth rates (Lambert et al., 1994), and is capable to withstand salinity levels as low as 7 g/L without mortality (Provencher et al., 1993). However, our contribution provides the first evidence that an interactive effect occurs between salinity and temperature on growth in cod juveniles. This interaction has been previously observed in juvenile turbot, Scophthalmus maximus (Imsland et al., 2001) and has recently also been described in spotted wolfish (Magnussen et al., 2008). In both previously described studies the interaction resulted in higher optimal temperature at reduced salinities. We found the same effect in cod juveniles as overall growth trajectories increased with decreasing salinity at 14 °C, while this effect did not occur as pronounced at 10 °C while reduced salinity still increased SGR at both temperatures. It is therefore likely that different species could react similarly to reduced salinity in combination with varying temperatures.

Lambert et al. (1994) reported increasing feed conversion efficiencies in a spring group of cod reared at reduced salinity (7‰), but this effect was not found in an autumn group reared at reduced salinity. In our experiment mean feed conversion efficiencies were 17% higher at reduced salinity although these differences were not found to be significant. A study on Atlantic halibut showed besides an increased FCE at reduced salinity also a significant increase in feed consumption with decreasing salinity (Imsland et al., 2008). Our results show the opposite for cod juveniles as feed consumption was found to be lower at reduced salinity at 10 °C, while no effect on feed consumption was observed at 14 °C. It therefore indicates that reduced salinity can increase feeding efficiency in cod, potentially reducing feed costs in aquaculture.

4.3. Metabolic enzyme activity and myosin expression

4.3.1. Metabolic enzyme activity

A negative correlation between growth rates and metabolic enzyme activity level (LDH and MDH) was found within this study, while AAT was positively correlated with growth. This is in line with a reported study by Pelletier et al. (1994) who found correlations between glycolytic enzymes and growth rates in Atlantic cod muscle.
Also, the levels of glycolytic enzymes in spotted wolfish muscle tissue were directly correlated to growth rates (Imsland et al., 2006). However, stronger correlations were consistently found between weight and enzyme activity, rather than growth rate and enzyme activity. These positive relationships between MDH and LDH and weight, together with the negative relationship with these enzymes with growth rates are likely the result of size dependent growth, which generally results in decreasing growth rates with increasing size in Atlantic cod (Folkvord, 2005; Foss et al., 2006). This could explain the relatively low enzyme activity at a relatively small body size, while at the same time growth rates would be relatively high, resulting in negative correlation.

Reduced salinity had a significant effect on the catabolic capacity of AAT and MDH in muscle tissue. Liver tissue was not affected by reduced salinity alone, but an interactive effect was observed on the activity level of AAT and MDH. Muscle LDH was the only metabolic enzyme which was not affected by salinity, temperature or interactions and was therefore the most stable across treatments. Pelletier et al. (1994) reported a correlation between growth rate and muscle LDH activity at different growth rates, as was also found in wolfish juveniles (Imsland et al. 2006) and newly-hatched fish (Lamarre et al., 2007). Even though growth rates differed among treatments, there was no difference found in LDH activity level across different growth rates. Instead, a significant correlation with body size (wet weight) at all three metabolic enzymes measured in muscle tissue indicated that the adaptation to reduced salinity in combination with the used temperatures might be size dependent in Atlantic cod. LDH and MDH activities increased with increasing size, and AAT activity decreased with increasing size. This would make the use of these specific metabolic enzymes less evident as a marker for growth rates as was suggested by Imsland et al. (2006).

No ontogenetic effect of diet during early development on metabolic enzyme activity in the juvenile stage was found, even though juvenile growth rates were overall significantly influenced by larval history. Functional development of the digestive tract and rapid somatic growth coincide during the early larval stage of fish (Rannestad et al., 2000). If diet is optimal during larval development growth rates are generally high (Garcia et al., 2008b) and development- mental errors such as malpigmentation (Devresse et al., 1994; McEvoy et al., 1998; Hamre and Harboe, 2008) or deformities (Cahu et al., 2003; Imsland et al., 2006) are reduced. Therefore is diet during early development an influential factor in determining juvenile quality. Less is known about the varying physiological processes of digestion and metabolic activity in juvenile fish related to larval diet. The lack of difference in enzyme activity between the three first-feeding groups and a similar enzymatic response to varying salinities or temperatures may suggest that the growth benefit in the fish groups that received natural zooplankton natural zooplankton for a minimum of 14 days from first-feeding, is higher without having a more efficient metabolic capacity, even though larval diet could affect metabolic activity at the time of the larval feeding period (Segner and Verreth, 1995; Lamarre et al., 2007). Metabolic Together, this assumes that there is no long term effect of first-feeding diet on metabolic capacity and that no physiological limitation to the capacity of the measured glycolytic enzymes and amino acid metabolism is set by diet during early life of cod. This indicates no difference in metabolic efficiency-, or capacity as a result of larval feeding history, and that the measured growth difference has no relationship with the metabolic capacity.

4.3.2. Myosin expression

No correlation was detected between growth rate and myosin mRNA content suggesting that the content of the transcript considered is not a valuable indicator of growth rate in Atlantic cod at this stage of development. Data concerning myosin expression in relation to muscle development are not available for cod of this size but the reduction of the myosin mRNA content observed with time might be a consequence of muscle development at this stage as it is observed in all tanks whatever the treatment or the larval feeding history of fish. Interestingly, this reduction is not as important at 10 °C compared to 14 °C in all groups considered suggesting that temperature modulates the expression of this particular transcript. In vertebrates, myosin heavy chains are encoded in a family of different genes (Weiss and Leinwand, 1996). In carp, Cyprinus carpio L., evidence for at least 28 myosin heavy chain genes have been found (Gerlach et al., 1990) and different isoforms are expressed depending on acclimation temperature (Imai et al., 1996) and developmental stage (Ennion et al., 1999; Fukushima et al., 2009). The reduction of the transcript content observed with time in our study may reflect the switch to the expression of a different isoform (undetected due to the specificity of the primers) or could also reflect an actual reduction of expression of myosin heavy chain as fish grow. Relation between muscle development and myosin expression has never been investigated in juvenile Atlantic cod, but myosin being the most abundant protein of fish white muscle, effect of the modulation of its expression on growth should be further investigated.

5. Conclusion

Larval diet influenced juvenile growth significantly at all treatments but did not affect metabolic enzyme activity, or myosin expression in Atlantic cod. These effects indicate that juvenile growth capacity is determined by larval feed, without influencing enzyme activities or myosin expression on the long term. Further, increased temperature and reduced salinity both increased growth rates significantly.

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