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Growth and Gut Morphology of Diploid and Triploid Juvenile Atlantic Cod (*Gadus morhua*)

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Abstract

The objective of this paper was to compare the growth and gut morphology of juvenile diploid and triploid Atlantic cod (*Gadus morhua*) reared under similar conditions. Individually tagged 36-week old diploid (mean weight 49.3 ± 13.8 g) and triploid (mean weight 43.6 ± 11.2) juvenile cod were measured at intervals during a 29-weeks growth trial. Data for weight, length, condition factor (K), hepato-somatic index (HSI), gonado-somatic index (GSI), Relative Gut Length (RGL), and pyloric caeca number were collected and results were analyzed in relation to ploidy status, gender and family contribution. At the end of the

experiment, only one family (M2xF3) had many representatives with a relatively even distribution of sexes and ploidies. Diploid females were significantly heavier and had higher K than triploid females in the M2xF3 family (body weight 371.2 ± 120.2 vs. 298.4 ± 100.7g; K 1.1 ± 0.1 *vs.* 0.93 ± 0.1) but no differences were found between diploid and triploid males. In the other families (pooled data), no differences in body weight were found between the ploidy groups. In general, triploids had a shorter intestine (RGL) and fewer pyloric caeca than their diploid siblings regardless of gender suggesting possible impairments in nutrient utilization and growth.

Introduction

Norway is the leading producer of farmed fish in Europe with over a million tonnes being produced each year (FAO 2012), most of which is Atlantic salmon *Salmo salar* L. In an attempt to diversify the aquaculture industry, production of some marine finfish species has been attempted. Atlantic cod *Gadus morhua* L. has received attention because of its economic importance but several biological, technical and market issues have created problems for development of cod culture into a profitable industry. Early sexual maturation that can reduce fish growth, survival and fillet quality, the release of eggs from net pens and the accidental release of farmed fish have raised questions about the sustainability and ecological impact of cod farming (Jensen, Dempster, Thorstad , Uglem & Fredheim 2010).

Triploidy impairs gonad development and creates fish that are usually sterile. Triploid male cod are gametically sterile and there is a significant suppression of gonad development in females (Peruzzi, Rudolfsen, Primicerio, Frantzen & Kauric 2009; Feindel, Benfey & Trippel 2011). The simplicity and reliability of the methods used to induce triploidy, have made this a common way to produce sterile fish (Maxime 2008; Piferrer, Beaumont, Falguiere, Flajshans, Haffray, Colombo 2009).

There may be physiological and morphological differences between diploid and triploid individuals within a species and these differences may influence performance under certain environmental conditions (Benfey 2001; Benfey & Bennett 2009; Piferrer et al., 2009; Leclercq, Taylor, Fison, Fjelldal, Diez-Padrisa, Hansen & Migaud 2011). Diploid and triploid individuals differ in gastrointestinal tract physiology and morphology (Cantas, Fraser, Fjelldal, Mayer & Sorum 2011; Peruzzi, Jobling, Falk-Petersen, Lein & Puvanendran 2013) and such differences could be hypothesized to play a role in determining the digestive efficiency and subsequent growth of fish that differ in ploidy status. In this study, we compare the growth, condition and gut morphology (Relative Gut Length or RGL and pyloric caeca number) of diploid and triploid cod reared under similar conditions during the juvenile stage. The fish originated from a multifactorial crossing design that gave 8 half-sib diploid and triploid families.

Material and Methods

Ethics

All procedures involving fish handling and treatments were conducted in accordance to the guidelines set by the National Animal Research Authority (Forsøksdyrutvalget, Norway). The project (ID number 4158) was approved by the Animal Care Committee at the University of Nordland. The Mørkvedbukta Research station (University of Nordland) is certified for animal experimentation (March 9th, 2010) by the National Animal Research Authority (Forsøksdyrutvalget, Norway). The corresponding author (course attendance 11-07, October 2011) and all people involved in animal experimentation received official training approved by the National Animal Research Authority of Norway (Forsøksdyrutvalget, Norway).

Fish origin and handling

Gametes from 2nd generation (2008 year class, 3 years old; 3-4 kg weight) Atlantic cod (*G. morhua*) reared at the Norwegian National Breeding Program, Tromsø (Northern Norway, 69°N, 19°E) were used to produce diploids and triploids. Eight half-sib families were established by crossing 4 males (M) and 8 females (F): M1xF1, M1xF2, M2xF3, M2xF4, M3xF5, M3xF6, M4xF7 and M4xF8. Shortly after fertilization and rinsing with seawater, the eggs were drained on sieves and eggs from each cross were pooled and divided into two groups. One group of eggs (2/3 of total) received a hydrostatic pressure shock (TRC-HPC™ Pressure machine, TRC Hydraulics Inc. New Brunswick, Canada) of 8500 psi for 5 min applied 50 min post-fertilization at 3.6°C (Trippel, Benfey, Neil, Cross, Blanchard & Powell 2008). The remaining group of eggs (1/3 of total) was not exposed to pressure treatment and served as the control. Eggs

(ca. 200 ml group^{-1}) were shipped by air-freight after 60 day degrees (d°, 3.7 ± 0.3°C) to the Research Station of Mørkvedbukta, University of Nordland (67°N, 14°E). On arrival, the eggs were treated with the wide spectrum fungicide Pyceze (Novartis Ltd., Litlington, Near Royston, UK; 0.8 ml L^{-1} water for 6 minutes) and then incubated until hatching. Communal rearing was carried out during the larval and nursery phases following standard rearing protocols. In brief, larvae were reared in twelve 80 L black, cone-bottomed tanks at densities of 100 larvae L^{-1}. The water exchange was gradually increased over time (10 to 53 L hr^{-1}). Continuous light (600 lux) and a temperature regime of 6 to 11 °C ± 0.3°C were applied. Dead larvae were removed daily. The larvae were fed on short term (five hours) enriched (Multigain, Biomar, Norway) rotifers (*Brachionus plicatilis*) until 29 days post hatching (dph) and enriched *Artemia* (Multigain, Biomar, Norway) from 21 dph onwards. The weaning period with microdiets (Skretting AS, France) started at 34 dph and larvae were fed dry feed only from 41 dph to 55 dph. Then, fish were transferred to 1m^3 circular tanks, exposed to continuous light, and reared at a temperature of 7.3-7.6 °C, salinity of 34 ± 0.5 ppt, and oxygen saturation of 75 – 85 %. Fish were fed on commercial diets (Skretting AS, Norway) following the manufacturer's feeding protocols until they were 40-50 g. From 2 to 5 months (week 8 – 20 of age), the fish were size-graded three times. At week 8, fish were graded into three size groups (<4mm, 4-5mm, >5mm) which corresponded to a wet weight of 0.5, 0.9 and 1.4 g respectively. A month later fish were sorted using 6mm sorting grids, where fish under < 6mm (1.3 g) were placed in one tank and fish larger than 6mm (3 g) were placed in two rearing tanks. At the age of 5 months, fish were

graded using 8mm sorting grids and divided into three size groups: 3.9 g (one tank), 5.5 g (two tanks) and 6.1 g (two tanks). Prior to grading, random samples of fish were weighed and average wet weight was estimated in order to use the appropriate grid size. Once most fish had reached a weight of 43-49 g (36 weeks), they were individually PIT-tagged (APR350 Handheld Reader, Agrident GmbH, Steinklippenstrasse 10, D-30890 Barsinghausen) and blood samples were taken to identify their ploidy status. Fish were anesthetized (70 mg L^{-1} MS-222) and tags inserted by making an incision of 1-2mm under the pectoral fin using a scalpel. Blood samples were collected from the caudal vein using heparinized syringes. After recovery in aerated seawater, the fish were placed in temporary holding tanks until ploidy had been determined and then allocated to 6 rearing tanks according to their ploidy status, 3 for each ploidy, with 75 fish in each tank. Fish were fed on commercial diets following feeding protocols provided by the feed company (Skretting AS, Norway) throughout the growth trial which lasted for 29 weeks. Fish were held in $1m^3$ circular units, under environmental conditions similar to those of the early juvenile phase. The initial stocking density was 3.2 - 3.7 kg m^{-3} and had reached 16.6 - 23.9 kg m^{-3} by the end of the experiment.

Measurements

Fish growth, condition and gut morphology

To assess individual growth, body weight and total length were recorded on five occasions during the trial, when the fish were 36, 44, 51, 62 and 65 weeks of

age. Before measurements, fish were anesthetized (70 mg L^{-1} MS-222) and body weight (W, ±0.5 g) and total length (BL, ±1mm) recorded. PIT-tag numbers were also read for fish that had retained their tags until the time of measurement. Condition factor (K) was calculated from the weight and length data using the formula $K = 100WBL^{-3}$.

At the end of the experiment (65 weeks of age), fish were anesthetized (MS222, 70 mg L^{-1}), and killed with a sharp blow to the head. Wherever possible PIT-tag numbers for individual fish were recorded. The fish were then dissected and the liver, gastrointestinal tract and gonads removed. The gastrointestinal tract was excised and flushed with ice-cold saline solution (0.9% NaCl), stretched to a relaxed position and the length of the intestine measured to the nearest mm from the pyloric sphincter to the anus. The Relative Gut Length (RGL) was calculated as: RGL= Intestine Length (cm) / Total Length (cm). Pyloric caeca were cut at their junction with the upper intestine and fixed in 10% neutral buffered formalin. For analysis, the pyloric caeca were rinsed overnight in running tap water and the total numbers of pyloric caeca were counted. The hepato-somatic index (HSI) and gonado-somatic index (GSI) were calculated as the weight of the organ relative to total body weight, expressed as a percentage. A fin clip from the dorsal fin of each fish was collected and preserved in 96% ethanol at 4ºC for genotyping.

Analytical methods

Ploidy validation

Blood samples were diluted (1:1000 v/v) in PBS (pH=7, 0.2M) and stained with Propidium Iodide (PI) (Peruzzi, Chatain, Fauvel & Menu 2005). Dimethyl sulfoxide (DMSO) (10% v/v) was added to the samples after 1 hour of PI-staining for short-term storage (-80 °C) prior to flow cytometry analysis. Ploidy was determined using a FACScan (Becton Dickinson, San Jose, CA, USA) flow cytometer. Approximately 20,000 nuclei were recorded per sample. Ploidy was assessed by calculating the ratio of the mean fluorescence intensity and fish were considered triploid when the ratio was 1.5 ± 0.1. The flow-cytometry data were analyzed using CyFlow v. 1.2.1 software (©Pertthu Thero & CyFlow Ltd).

Genotyping

Genomic DNA was extracted from fin clips using an E-Z96 Tissue DNA Kit (OMEGA Bio-tek, Norcross, GA, USA) following manufacturer's instructions. Ten microsatellite loci were analyzed: *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35 and *Gmo*37 (Miller, Le & Beacham 2000), *Gmo*2 and *Gmo*132 (Brooker, Cook, Bentzen, Wright & Doyle 1994), *Tch*11 and *Tch*13 (O'Reilly, Canino, Bailey & Bentzen 2000). For all microsatellite primer sets, the protocol for amplification and fragment analysis of Westgaard & Fevolden (2007) was modified to allow 2.5ul reaction volume in the PCR, carried out using a Qiagen Multiplex PCR kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's procedures. The PCR included an initial denaturizing step at 95°C for 15 min, followed by 22 cycles at 95°C for 30 s, 56°C for 3 min and 68°C for 1 min, and a final elongation step at 60°C for 30 min. The amplified alleles were separated using an ABI 3130 XL sequence analyser (Applied Biosystems, Foster City, CA, USA)

and scored with Genemapper® software v3.7 package (Applied Biosystems, Foster City, CA, USA). Parental assignment was performed manually and the genotypes of candidate parents were compared with those of the offspring. Candidate parents were excluded if a mismatch occurred at one or more of the loci. For the analysis of triploid fish, the two maternal alleles were coded as a single allele as detailed in Hernández-Urcera, Vera, Magadán, Pino-Querido, Cal & Martínez (2012).

Data selection criteria and analyses

The numbers of fish analyzed are shown in Table 1. For individual growth (W, BL), data of M2xF3 fish (dataset 1, Table 1) recorded at all five sampling points were analyzed according to gender and ploidy. Data for weight (W), length (BL), condition (K), GSI, HSI, and gut morphology (RGL and pyloric caeca number) recorded at the end of the trial for M2xF3 and remaining families (dataset 2, Table 1) were analyzed by family, gender and ploidy. This dataset includes PIT-tagged fish and fish that lost their PIT tag but could be assigned to individual families. Data for individual initial weights of the fish that lost their PIT-tags during the study are not available, but individual data for family, final weight and morphometrics from these fish were collected for analysis. When necessary, data of body weight and length were logarithmically (log 10) transformed while K data were arcsine transformed to normalize distributions. RGL data were logarithmically transformed, pyloric caeca numbers data were square root transformed and somatic index data (GSI and HSI) were arcsine transformed prior to analysis. All transformed data were tested for normality of distribution (Shapiro Wilk's test) and homogeneity of variance (Levene's test)

before analyses. Normally distributed data were compared using a one-way ANOVA. When differences between means were found, post-hoc analyses were conducted using paired comparisons (Tukey's HSD) for homogeneous data and a 2-t (assuming non equal variances) for non-homogeneous data. Non parametric testing (Kruskal-Wallis, Moods Median Test) was used for non-normal distributed data. ANCOVA was used to analyze data of HSI and GSI with ploidy as factor and sex and body weight as covariates. Correlations between final body weight and RGL or pyloric caeca number were analyzed using linear regression analysis (scatterplot with regression fit) and Pearson's correlation coefficient. To analyze the number of diploid and triploid individuals scored in each half-sib family, a CHISQ test ($n > 5$) and an Exact Binomial Test ($n < 5$) were employed. Data were analyzed using the program Minitab version 16 (Minitab Statistical software Inc., US) and a significance level of $P < 0.05$. Data are presented as means ± SD.

Results

Representation by family

All diploid and triploid fish (n=342) could be assigned to parental pairs. Of the 8 families produced, one (M1xF1) was not represented at the final assessment and three families (M1xF2, M2xF4 and M3xF7) had low numbers of representatives irrespective of ploidy status (Table 2). Two families (M3xF8 and M4xF6) were represented by more diploids than triploids, whereas the opposite was observed for M2xF3 and M4xF6 ($P < 0.01$). Diploids and triploids were

most evenly represented in the M2xF3 family and their growth throughout the trial was analyzed separately.

Growth of M2xF3 family

Growth (W and BL) of this family was analyzed using data from fish that were recorded at all five sampling points (dataset 1, Table 1). For both sexes, body weight and length were similar for the two ploidy groups throughout the experiment (Fig. 1A-B).

Body size, condition and gut morphology

Results from the last sampling (65 weeks of age) were analyzed for the M2xF3 family and for the remaining families (pooled) as two separate groups (dataset 2, Table 1).

M2xF3 family

For the M2xF3 family, differences in body weight and condition factor (K) were found for diploid and triploid females at 65 weeks of age (Fig. 2A, C). Diploids were heavier (371.2 ± 120.2 g vs. 298.4 ± 100.7 g, $P < 0.05$) and had higher K (1.08 ± 0.07 vs. 0.93 ± 0.1, $P < 0.001$) than triploids. Body lengths were similar for diploids and triploids (Fig. 2B). Results of ANCOVA showed an effect of body weight on HSI. Diploid females had higher HSI values than triploid females (9.51 ± 1.24 % vs. 8.09 ± 2.17 %, $P < 0.01$), but no differences were found between diploid and triploid males (Fig. 3A). Both ploidy and gender had a significant effect on GSI. The gonads of female and male triploids were relatively smaller than those of diploids of the same gender (F: 0.21 ± 0.08 %

vs. 0.59 ± 0.19 %, $P < 0.001$; M: 0.11 ± 0.08 % vs. 0.22 ± 0.13 %, $P < 0.001$; Fig. 3B).

A difference was found in gut morphology between diploids and triploids. Both female and male triploid cod from the M2xF3 family had significantly shorter intestines (RGL) than their diploid siblings (F: 0.92 ± 0.11 vs. 1.11 ± 0.1, $P < 0.001$, M: 0.98 ± 0.14 vs. 1.13 ± 0.14, $P < 0.01$; Fig. 3C) and also had fewer pyloric caeca (F: 217 ± 38 vs. 300 ± 59, $P < 0.001$, M: 226 ± 35 vs. 283 ± 58, $P < 0.001$; Fig. 3D).

Remaining families (pooled data)

Body weights and K of diploids and triploids were similar (Fig. 4A, C). On the other hand, triploid females were longer (36.46 ± 2.12 cm vs. 32.69 ± 3.75 cm, $P < 0.01$; Fig. 4B) than diploid females. No significant differences were recorded for males. The HSI was similar for diploids and triploids of the same gender (Fig.5A). With respect to GSI, both sex and ploidy status affected GSI. The GSIs of female and male triploids were lower than those of female and male diploids (F: 0.14 ± 0.04 % vs. 0.55 ± 0.1 %, $P < 0.001$; M: 0.15 ± 0.17 % vs. 0.3 ± 0.29 %, $P < 0.05$; Fig. 5B).

Female triploids had shorter intestines (RGL) than diploids: (1.08 ± 0.11 vs. 1.23 ± 0.19, $P < 0.05$; Fig. 5C) but no differences were found between male diploids and triploids. Triploid males and females had fewer pyloric caeca than their diploid siblings (F: 235 ± 16 vs. 267 ± 59, $P <0.05$, M: 219 ± 43 vs. 276 ± 65, $P < 0.01$; Fig. 5D).

A significant correlation between body weight and RGL was observed in both ploidy and gender groups (2n F: r= 0.344, $P < 0.05$; 3n F: r= 0.557, $P < 0.001$; 2n M: r= 0.542, $P < 0.001$; 3n M: r= 0.454, $P < 0.01$) whereas body weight and pyloric caeca number were significantly correlated only in diploid males (r= 0.368, $P < 0.05$).

Discussion

The eggs of eight females were fertilized with the sperm of 4 males to create 8 half-sib families but, at the end of the trial, the contribution of each half-sib family was significantly different. One family was not present and other families showed unequal contributions of diploid and triploid fish. Only one family (M2xF3) was evenly represented in both ploidy groups and with relatively large numbers of individuals. Differences in family contribution have previously been reported for Atlantic cod. Garber, Tosh, Fordham, Hubert, Simpson, Symonds, Robinson, Bowman & Trippel (2010) studied family contribution when progeny were mixed as eggs, newly hatched larvae or juveniles. When families were mixed as eggs, progeny from only 37% of families were present at harvest. By contrast, progeny from every family were present at harvest when mixing took place at the larval or juvenile stage. The authors attributed this differential survival among families mixed as fertilized eggs to several factors including egg quality, additive genetic effects (specific parental crosses) and variability in larval growth leading to competition and cannibalism. All these factors could

have contributed to the differential survival among families observed in our study.

In our study, the two half-sib families sired by male 4 resulted in opposite contributions of diploid and triploid offspring (Table 2). This was not observed in Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum) (Shrimpton, Heath, Devlin & Heath 2012), where survival of diploid and triploid half-sib families during egg incubation was significantly affected by ploidy but without any female, male or parental interaction effect. To our best knowledge, there are no other studies reporting family and ploidy effects on fish survival including information on parental interaction. Comparison of a large number of paternal and maternal half-sib families would be needed to investigate this in detail.

Diploids and triploids of the M2xF3 family showed similar growth throughout the trial but there was a trend towards a higher body weight in favor of diploid females at final sampling (Fig.1A, dataset 1) and this became significant when all fish of this family were included in the analyses (Fig. 2A, dataset 2). As such, our results suggest that during the juvenile stage, a poorer performance of triploids compared to diploids may be linked to the growth of females rather than males. This is opposite to observations made during the adult stage, where positive effects of triploidization for growth and carcass yield, especially in females, have been reported by Feindel et al. (2011). Derayat, Magnússon, Steinarsson & Björnsson (2013) reported no differences in growth between large diploid and triploid cod, but that might have been due to the fact that fish were still immature (22-months old fish). In a recent study focusing on the effect of triploidization on the growth, survival and development of deformities from the

larval to the juvenile stage in Atlantic cod, Opstad, Fjelldal, Karlsen, Thorsen, Hansen & Taranger (2013) did not observe any significant differences in weight between diploid and triploid fish up to the age of 87 days. During the juvenile (immature) stage, triploid fish generally grow similar to or less well than diploids depending on the species and rearing conditions (Piferrer et al., 2009). In adult fish, the performance of triploids compared to diploids tends to vary between and within species. For example, in the European seabass, *Dicentrarchus labrax* (Linnaeus), both similar and inferior performance of triploids over diploids has been reported by Felip, Zanuy, Carrillo & Piferrer (1999) and Peruzzi, Chatain, Saillant, Haffray, Menu & Falguiere (2004), respectively. In contrast to results in terms of growth observed between ploidies within the M2xF3 family (Fig. 2A), diploid and triploid fish of the same gender performed similarly in the pooled group of other families (Fig. 4A). In our trial, the study of family and ploidy*family interactions was not possible because of the limited number of individuals and families involved. However, identifying families where their triploid progeny can perform equally or better than diploids is important for a successful production on a commercial scale. Studies on salmonids suggest that individual families may respond differently to ploidy manipulation in terms of survival and growth. For example, the freshwater growth of Chinook salmon was found to be significantly affected not only by ploidy status but also by family (Johnson, Shrimpton Heath & Heath 2004) and male origin (Shrimpton et al., 2012). Furthermore, in studies using multi-generation selected fish, a consistent growth performance ranking was found among some of the families regardless of ploidy. This complies with results on growth and other production traits

reported for diploid and triploid families from different year classes of Atlantic salmon (Taylor, Sambraus, Mota-Velasco, Guy, Hamilton, Hunter, Corrigan & Migaud 2013), suggesting that a selection program based on diploid performance might be applicable to triploid production (but see Friars, McMillan, Quinton, O'Flynn, McGeachy & Benfey 2001). In Atlantic cod, further research should be conducted to examine family*ploidy interactions and level of variance for important production traits within and between families during the hatchery and grow-out phases.

In our study, the differences in HSI observed between diploid and triploid females of the M2xF3 family could be ascribed to differential body mass between the two groups. The fish were young and immature so the differences in HSI were not likely associated to with differential vitellogenic activity and energy allocation for reproduction. Derayat et al. (2013) found higher HSI values in 22-months old diploid cod when compared to their triploid siblings. Similar results have been reported for 30-months old immature diploid and triploid Coho salmon, *Oncorhynchus kisutch* (Walbaum) (Johnson, Dickhoff & Utter 1986). Peruzzi et al. (2004) found significantly lower HSI in both sexes of triploid European seabass compared to their diploid counterparts.

The results on GSI of diploid fish obtained in our study are in accordance with those obtained in diploid cod of similar age (GSI < 1 %, 15 – 18-months old fish) reported by Karlsen, Norberg, Kjesbu & Taranger (2006). In our study, the triploid condition significantly affected gonad development in both sexes and similar results have been reported previously (Derayat et al., 2013). This contrasts with findings for adults, where differences in GSI between ploidies

were only reported for females because of the significant gonadal development of triploid males. As reported by the same authors, suppressed oogenesis resulted in increased carcass yield of triploid over diploid females at two successive spawning seasons. Significantly higher growth of triploids is expected to appear only when diploids become sexually mature, due to the impairment of gonadal development in triploids, particularly in female triploids (Maxime 2008; Piferrer et al., 2009). In Atlantic cod, loss of growth-potential through early sexual maturation under culture conditions represents a major bottleneck in commercial production and the use of triploid fish has generated particular interest (Peruzzi, Kettunen, Primicerio & Kaurić 2007; Trippel et al., 2008; Peruzzi et al., 2009; Feindel et al., 2011).

The presence of a significantly shorter intestine (RGL) and fewer pyloric caeca in triploids compared to diploids (Fig. 3, 5), support the results reported previously for adult Atlantic cod (Peruzzi et al., 2013). These authors found that triploid offspring originating from wild and selected broodstock had significantly fewer pyloric caeca than their diploid siblings. Triploid offspring from wild cod also had a significantly shorter intestine (RGL) than their diploid counterparts. Overall, our results confirm the above findings and may imply that differences in gut morphology between the two ploidies are attributable to the triploid condition *per se* and not to differential survival of diploids and triploids with potentially dissimilar morphological characteristics. There was a positive correlation between body weight and RGL which could indicate that the performance of triploid fish possessing shorter guts was affected. Phenotypic plasticity of gut morphology in response to factors such as habitat and trophic niches (Knudsen,

Amundsen, Jobling & Klemetsen 2008), food deprivation (Bélanger, Blier & Dutil 2002; Blier, Dutil, Lemieux, Bélanger & Bitetera 2007), and genetics (Stevens, Wagner & Sutterlin 1999; Stevens & Devlin 2000, 2005) has been reported. Nevertheless, studies relating growth and gut morphology, particularly with respect to differences between diploid and triploid fish, have not been reported. With regards to the pyloric caeca, because of their involvement in enzymatic digestion and nutrient absorption (Rust 2003), any change in the morphology of these may affect the digestive capacity of fish, and research should be directed towards investigating this.

In conclusion, at the juvenile stage, triploid female cod showed reduced growth and condition in comparison with their diploid counterparts. In addition, the differences observed in gut length and pyloric caeca number between triploids and diploids confirm the presence of a significant ploidy effect on gut morphology in this species. Additional research should compare the digestive capacity of diploid and triploid cod when fed standard and specially-formulated diets in relation to the above findings to extend information about family*ploidy interactions and their potential effects on fish performance.

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Figure Legends

Figure 1. Mean ± SD of individual body growth (A) and total body length (B) of the M2xF3 family, registered at five sampling points during the 29-week trial (dataset 1).

Figure 2. Body weight (A), total body length (B) and condition factor K (C) of diploid (2n) males (n=13) and females (n=11) versus triploid (3n) males (n=15) and females (n=25) of the M2xF3 family (dataset 2) recorded at the last sampling (65 weeks of age). Significant differences between ploidy groups of a same gender are indicated by asterisks; (*) < *P* 0.05, (**) *P* < 0.01 and (***) *P* < 0.001.

Figure 3. Hepato somatic index HSI (A), gonado somatic index GSI (B), relative gut length (RGL) (C) and pyloric caeca number (D) of diploid (2n) males (n=13) and females (n=11) versus triploid (3n) males (n=15) and females (n=25) of the

M2xF3 family (dataset 2) recorded at the last sampling (65 weeks of age). Significant differences between ploidy groups of a same gender are indicated by asterisks; (*) < *P* 0.05, (**) *P* < 0.01 and (***) *P* < 0.001.

Figure 4. Body weight (A), total body length (B) and fish condition K (C) of diploid (2n) males (n=29) and females (n=26) versus triploid (3n) males (n=13) and females (n=11) of the group remaining families (dataset 2) recorded at the last sampling (65 weeks of age). Significant differences between ploidy groups of a same gender are indicated by asterisks; (*) < *P* 0.05, (**) *P* < 0.01 and (***) *P* < 0.001.

Figure 5. Hepato somatic index HSI (A) and gonado somatic index GSI (B), relative gut length (RGL) (C) and pyloric caeca number (D) of diploid (2n) males (n=29) and females (n=26) versus triploid (3n) males (n=13) and females (n=11) of the group remaining families (dataset 2) recorded at the last sampling (65 weeks of age). Significant differences between ploidy groups of a same gender are indicated by asterisks; (*) < *P* 0.05, (**) *P* < 0.01 and (***) *P* < 0.001.

Tables

Table 1. Number of fish analyzed for growth of the M2xF3 family throughout the 29-week trial (dataset 1) and for growth and gut morphology based on the last sampling (dataset 2). Dataset 1 includes fish for which body weight and length data were obtained for all 5 sampling points throughout the growth trial. Dataset 2 includes fish for which data were collected at the end of the trial, and could be identified to family.

		Females		Males		Total	
		2n	3n	2n	3n	2n	3n
Dataset 1	M2xF3	11	25	13	15	24	40
Dataset 2	M2xF3	16	35	17	24	33	59
	Other families	26	11	29	13	55	24

Table 2. Number of diploid (2n) and triploid (3n) fish assigned to the different half-sib families at the end of the trial (age 65 weeks). Significant differences (Chi-square or Exact Binomial test) between ploidy groups within each family are indicated by asterisks; (*) P<0.05, (**) P < 0.01 or (***) P< 0.001.

Family	Ploidy			ChiSQ	Binomial
	2n	3n	Total	*P*	*P*
M1xF1	0	0	0		
M1xF2	3	0	3		*ns*
M2xF3	67	107	174	**	
M2xF4	1	2	3		*ns*
M3xF7	5	0	5		*
M3xF8	17	1	18	***	
M4xF5	85	4	89	***	
M4xF6	7	38	45	***	

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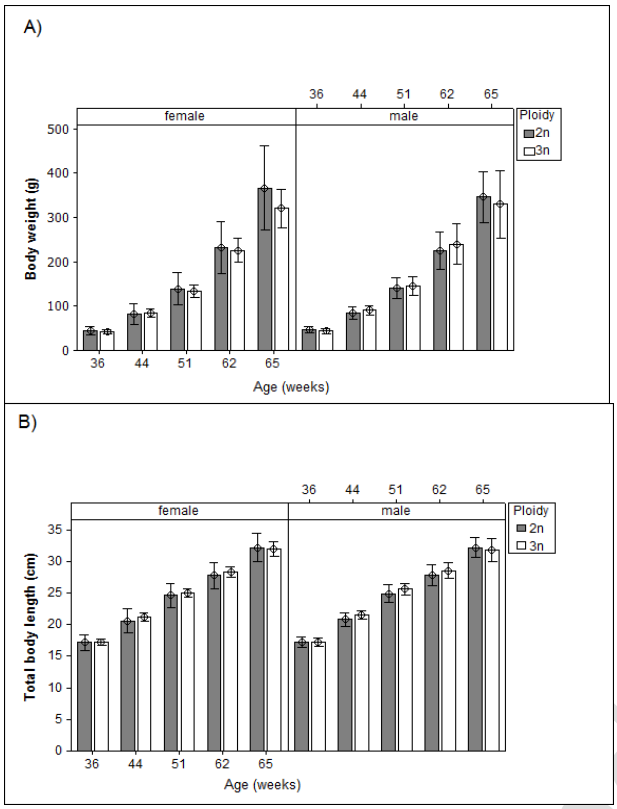

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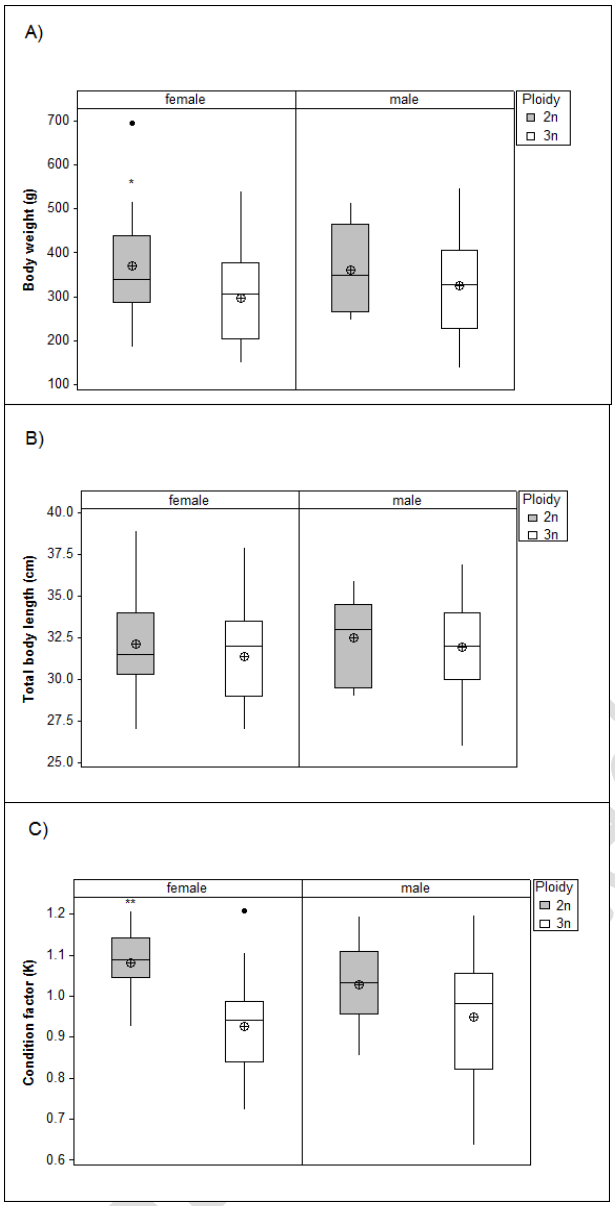

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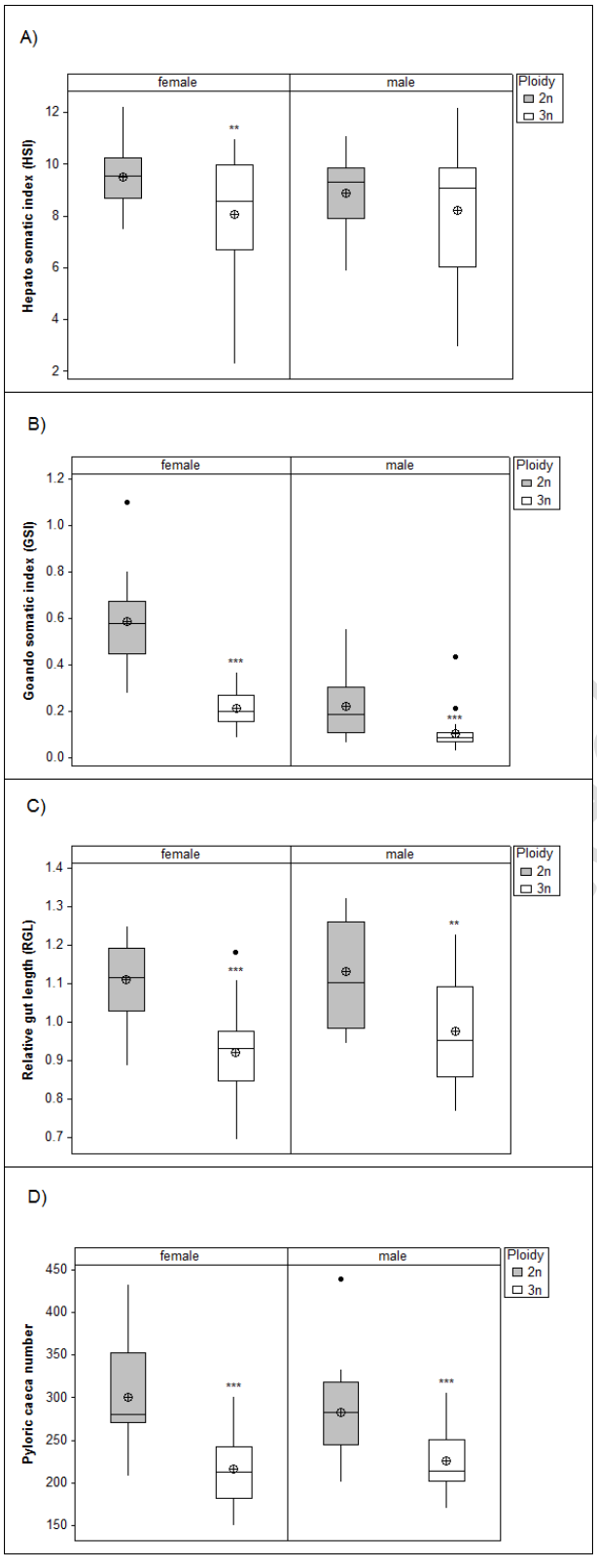

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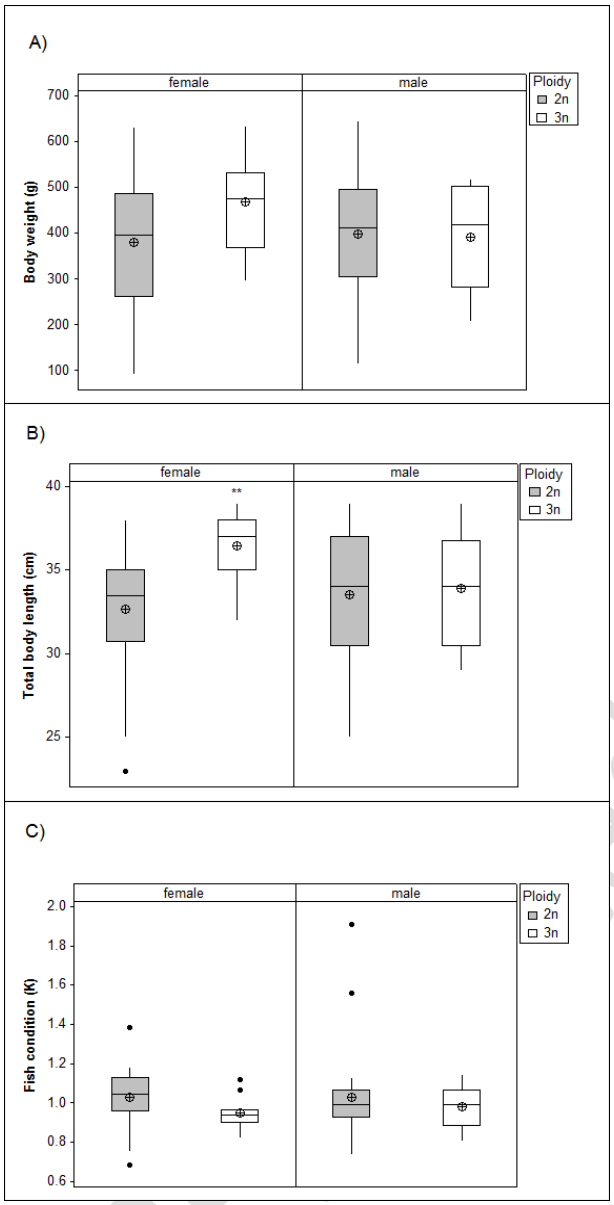

Figure 4

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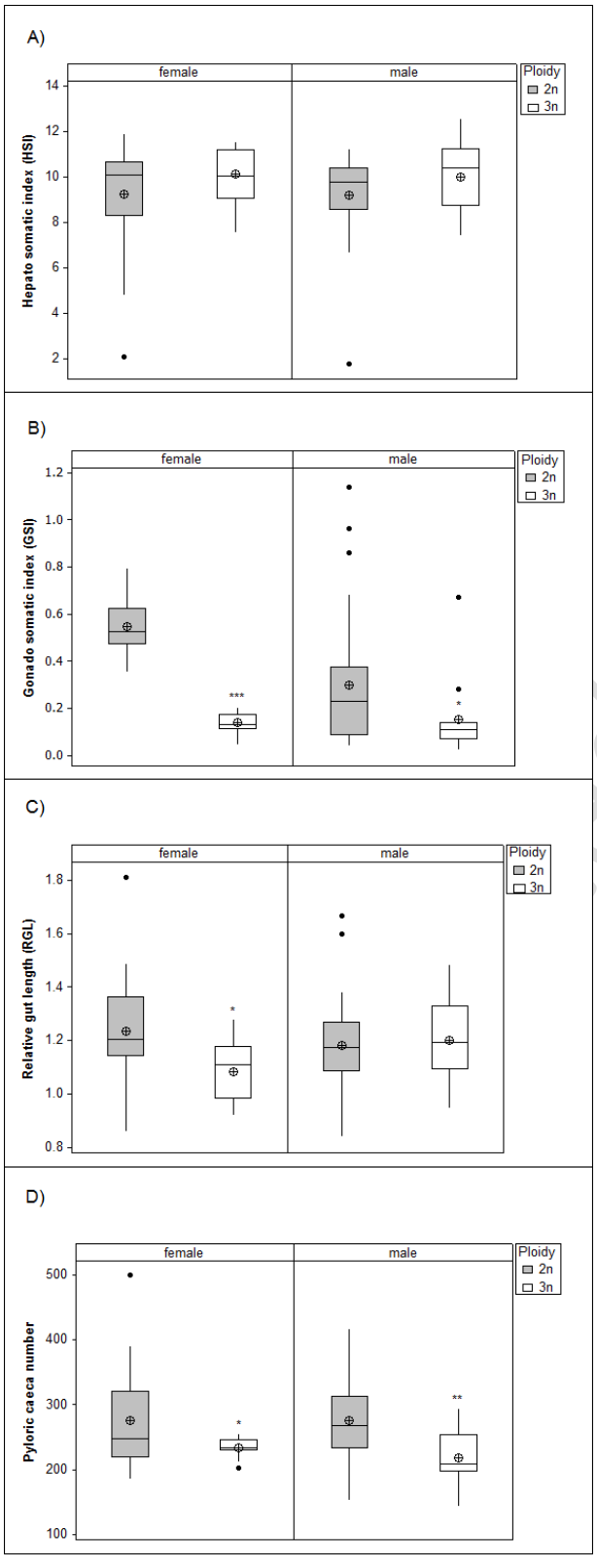

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610 Figure 5

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