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Muscle growth of triploid Atlantic cod juveniles (*Gadus morhua*)

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Running title: Muscle growth of triploid Atlantic cod juveniles

Abstract

This study has investigated the muscle growth of diploid and triploid Atlantic cod (*Gadus morhua*) juveniles raised in replicate tanks over a period of 29 weeks and analysed at three sampling points (February, June and September). Data for weight, length, condition factor (K), muscle fibre growth and myogenic progenitor cells (MPCs) number were collected and results were analysed in relation to body growth and ploidy

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status. Diploids were significantly heavier than triploids throughout the trial (~10-20%) and had K in June and September samplings. Over the whole period, the rate of muscle fibres' recruitment was 318 fibres day^{-1} and 252 fibres day^{-1} for diploid and triploid cod respectively. The larger body weight of diploids resulted in a total number of fast fibre number of 114979 compared to 91086 in triploids. The average diameter of the 2.5 % of the smallest fibres (2.5th percentile) was higher in diploids than triploids at the start of the trial, with a reversed picture for the average of the upper 2.5 % (97.5th percentile) at the end of the trial. The probability density function of the estimated muscle fibre diameters showed similar fibre size distribution between size-matched diploids and triploids at all sample points. The peak fibre diameter was approximately 25 μm in February and increased to approximately 50 μm in June and September, irrespectively of ploidy. *Pax 7* were used as molecular markers for MPCs. A positive correlation between *Pax 7*$^+$ cells and total body length was observed only among triploid fish at the onset of the experiment.

Introduction

The skeletal muscle in most teleost fish accounts for a largest bulk (65 %) of the body mass (Johnston, Strugnell, McCracken & Johnstone 1999). Three different types of fibres can be identified; the fast-white muscle, the intermediate-pink and the slow-red fibres which are organized into discrete layers within the myotome. Unlike mammals in which the number of muscle fibres is fixed late in gestation, muscle fibre recruitment (hyperplasia) in many fish species, continues throughout much of their life cycle. Muscle growth in fish is characterized by two phases; 1) embryonic that includes formation of embryonic muscle fibres and an undifferentiated myogenic progenitor cells

(MPCs) population and 2) post-embryonic phase characterized for hyperplasia (stratified and/or mosaic). Stratified hyperplasia is defined as muscle fibre recruitment restricted to growth zones in the lateral margins of the myotomal muscle (Johnston, 1999). Mosaic hyperplasia (muscle fibre recruitment scattered throughout the myotomal muscle) is predominant in fish that reach a large ultimate body and is initiated around the first feeding stage and continues into the adult stage resulting in a large increase of cell numbers, particularly in fast muscle. The source for hyperplasia and hypertrophy is thought to be a population of MPCs scattered throughout the myotome which are equivalent to mammalian satellite cells (Johnston, Bower & Macqueen 2011). These cells are also responsible for repair and maintenance of the skeletal muscle mass. The MPCs are provided by the external cell layer during the late embryonic phase (Koumans & Akster 1995). The external cell layer is present during the late embryo and larval stages and consist of both primary dermal endothelial cells and proliferative MPCs. The MPCs migrates through the somite and contribute to a second wave of lateral fast muscle fibres (Hollway, Bryson-Richardson, Berger, Cole, Hall & Currie 2007). The specification to a myogenic fate of these cells is controlled by myogenic regulatory factors such as *myf-5* and *myoD* whereas the activation and proliferation into myoblasts are controlled by the myogenin, *MRF-4* and *MEF-2* genes (Johnston *et al.* 2011). Further, the myoblasts either fuse to form myotubes (hyperplasia) on the surface of existing fibres or are absorbed into maturing fibres leading to the expansion of the diameter (hypertrophy). During the larval and early juvenile stages of several species, MPCs are observed interspersed between muscle fibres (Johnston 2001), while in adult fish they are observed between the sarcolemma and basal membrane of muscle fibres. The c-Met receptor (receptor for hepatocyte growth factor) and M-cadherin protein are expressed in quiescent satellite cells.

Another marker is the transcriptor factor paired box protein 7 (*Pax 7*) which plays also important regulatory roles in the development of diverse cell lineages. In studies with adult mice muscle, Seale (2000) found that *Pax 7* expression appears specific to the satellite cell myogenic lineage and suggested that satellite cells would not exist or fail to proliferate in absence of *Pax 7*.

Several studies have shown the myotomal muscle ability to respond to environmental factors like temperature, water flow regime and chemical composition, photoperiod, but also food availability, parasites (Johnston 2001, Johnston 2006; López-Albors, Ayala, Gil, García-Alcázar, Abellán, Latorre, Ramírez-Zarzosa & Vázquez 2003) and genetic factors (i.e. family, strains, ploidy status) (Johnston *et al.* 1999; Johnston, Alderson, Sandham, Mitchell, Selkirk, Dingwall, Nickell, Baker, Robertson, Whyte & Springate 2000; Johnston 2001). With respect to ploidy status, triploid fish and other organisms are expected to have fewer and larger cells in organs and tissues, including muscles. For example, triploid rainbow trout, *Oncorhynchus mykiss* had fewer myoblasts per unit weight of muscle tissue (Johnston 1999), different fibre size distribution in fish of < 30 cm (Suresh & Sheehan 1998) and fewer fast muscle fibres (Poontawee, Werner, Müller-Belecke, Hörstgen-Schwark & Wicke 2007) when compared to their diploid siblings. However, the effect of triploidy in muscle growth dynamic may depend on the stage or age of the fish as reported for Atlantic salmon, *Salmo salar*. Johnston and co-authors (1999) found a slightly more advanced development of myotubes and myofibrils in triploid than diploid embryos. However, during the freshwater and seawater stages (fish < 42 cm fork-length), triploids had fewer muscle fibres per myotome. Furthermore, MPCs seem to be affected by triploidy induction as well. For instance, the c-met expressing cells were more abundant in diploid than in triploid Atlantic salmon smolts (Johnston *et al.* 1999).

Numerous results suggest that the performance of triploids is species-specific (Piferrer, Beaumont, Falguière, Flajšhans, Haffray & Colombo L 2009), but also depends on the induction method, husbandry practices and the stage of the life cycle being compared. Since somatic growth is closely related to that of the muscle tissue, studies of the possible effects of triploidization on muscle growth could elucidate the variable results in growth performance observed in triploid fish (Blanc, Poisson & Valleé 2001; Taylor, Sambraus, Mota-Velasco, Guy, Hamilton, Hunter, Corrigan & Migaud 2013). Atlantic cod is a promising species for the diversification of the aquaculture sector in northern countries but the development of the industry is hampered by early maturation of fish. The use of sterile triploids may help improving the culture performance and welfare of fish under commercial farming operations. The adoption of the triploidy technology would also address environmental concerns regarding the release of gametes from sea cages or potential interbreeding between farm escapees and wild stocks. The aim of the present study was to investigate and compare the muscle fibre growth dynamics of triploid and diploid Atlantic cod (*G. morhua*) juveniles for a better characterization of these fish for culture purposes and in view of the establishment of a more profitable and sustainable cod farming industry.

Material and methods

Animal ethics

Fish handling and treatment procedures were conducted according to the Norwegian "Animal Protection Law" and all people involved in the experiment received official

training approved by the National Animal Research Authority of Norway (Forsøksdyrutvalget, Norway).

Fish husbandry and origin

Fertilization, triploid induction and incubation were conducted at the Norwegian National Breeding Program, Tromsø (Northern Norway, 69°N, 19°E). In brief, Atlantic cod (*G. morhua*) eggs and milt were obtained by stripping eight females and four males from 2nd generation selected fish (2008 year class, 3-years old; 3-4 kg weight) producing eight half-sib families. Following fertilization, the eggs were pooled and treated according to Campos Vargas, Hagen, Solberg, Jobling & (2014). Briefly, a proportion of the fertilized eggs (2/3 of total) was exposed to hydrostatic pressure shocks (TRC-HPC™ Pressure machine, TRC Hydraulics Inc. New Brunswick, Canada) of 8500 psi (58.600 kPa) for 5 min applied 50 min post-fertilization (3.6 °C) following Trippel, Benfey, Neil, Cross, Blanchard & Powell (2008). Untreated eggs (1/3 of total) served as diploid controls. The eggs were then shipped on 60-day degrees (d°, 3.7 ± 0.3 °C) to the Research station of Mørkvedbukta, University of Nordland (67°N, 14°E). The resulting larvae and juveniles were communally reared until the onset of the experiment (average weight of ~40g). For details regarding larval and juvenile rearing conditions, see Campos Vargas *et al.* (2014).

Experimental growth trial

The growth trial was initiated when fish reached an average weight of 40 g (February 2012, 36 weeks of age), and the juveniles from the two largest size grading were individually PIT-tagged and placed in two mixed (both size classes) temporal holding tanks awaiting ploidy verification (two weeks). Fish were then allocated to six rearing

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tanks according to their ploidy status, three for each ploidy, with 75 fish in each tank. For details on fish handling and rearing conditions see Campos Vargas *et al.* (2014).

Analytical methods

Ploidy analysis

Ploidy level of individual fish was determined by flow cytometry as described in Campos Vargas *et al.* (2014). Briefly, blood samples were stained with Propidium Iodide and analyzed using a FACScan (Becton Dickinson, Franklin Lakes, NJ, US). Ploidy was assessed by calculating the ratio of the mean fluorescence intensity of triploid (3n) to diploid (2n) and fish were considered triploid when the ratio was 1.5 ± 0.2. The flow-cytometry data were analyzed using the software CyFlow v. 2.1.2. All fish were confirmed to be of the correct ploidy before the experimental groups were established.

Sampling procedures

Samples for muscle cellullarity studies were taken in February (n=14 (2n), n= 14 (3n)), June (n= 24 (2n), n= 24 (3n)) and September (n= 34 (2n), n= 34 (3n)) corresponding to the start, middle and end of the growth trial. The juveniles were sacrificed with an overdose of MS-222 prior to being scaled (W, ± 0.5 g) and measured (BL, ± 1 mm). Condition factor (K) was calculated from body weight and length using the formula: K = 100 WBL^{-3}.

Histological sections were taken following adapted methods from established protocols (Hagen, Solberg & Johnston 2006; Johnston *et al.* 2000). In summary, whole body

steak (0.5 cm thick) of each individual was cross-sectioned at the level of the anterior to the third dorsal fin ray and digitally photographed. Then, three blocks from the dorsal right side of each steak were mounted using Cryomatrix (Thermo Fisher Scientific) and snap frozen in isopentane (60 sec) cooled to near its freezing point (-159 °C) using liquid nitrogen. Samples were wrapped in aluminum foil and stored in a liquid nitrogen container until sampling was completed and transferred to a -80 °C freezer. Blocks were cut at -20 °C in a cryostat (Microm HM 550, MICROM International GmbH) to obtain 8 µm thick histological sections, which were dehydrated before being stored at -80 °C for further processing.

- *Morphometrics*

Histological sections were stained in Harris haemotoxylin (Sigma-Aldrich) and mounted using glycerol gelatin (Sigma-Aldrich) on poly-L-lysine treated slides. Sections were analyzed with a light microscope (Axioscop 2 mot plus, Carl Zeiss) equipped with a camera. The area and diameter of 1000 fibres from random locations within the fast muscle sections were calculated for each fish using the Axio Vision software (v. 4.2, Carl Zeiss). The total fibre number was calculated as: $[10^6$ x total cross-sectional area of fast muscle (mm^2) x number of analyzed fibres] / [total area of analyzed fibres (µm)]. The total fibre density (number of fibres per unit area (mm^2)) was calculated as: $[10^6$ x the number of fibres measured] / [total area of analyzed fibres (µm)]. The total cross-sectional area (TCA) of the steak was calculated using the Sigma Scan pro software (v.5.0, Systat, Inc.).

- *Fibre size distribution*

Fish of similar body length, 7 fish in February and 10 fish in June and September were used per ploidy group for the investigation of the muscle fibre size distribution. The 2.5th and 97.5th percentiles of fast fibre diameter were used as estimates of the minimum and maximum fibre diameter, respectively.

- *Immunohistochemistry*

Histological sections from February (2n, n=14, 3n, n=14, 2 sections per fish) and September (n= 12 per ploidy group, 3 sections per fish) sampling were further analyzed after applying an immunohistochemical protocol. Myogenic progenitor cells (MPCs) were identified using a primary antibody to *Pax 7* (Johnston, Abercromby, Vieira, Sigursteindóttir, Kristjánsson, Sibthorpe & Skúlason 2004) with a dilution rate of 1:1000. As second antibody the IgG anti-rabbit (Biotin conjugated, Cat. No. B 8895, Sigma) with a dilution rate of 1:400 was used. The antibodies were diluted in 1% (v/v) TritonX100, 1.5% (m/v) BSA (Bovine serum albumin) in PBS (Phosphate Buffered Saline) at their respective dilution rates. Before staining, sections were fixed in acetone for 10 min and then placed in a solution of 5% (v/v) normal goat serum, 1% (v/v) TritonX100, 1.5% (m/v) BSA in PBS for 60 min to rehydrate and block endogenous material. Sections were then washed in PBS for 5 min and incubated overnight in a humidity chamber at 4°C after applying to each section the primary antibody solution (100 µl). Post incubation, sections were rinsed for 3 x 3 min in PBS and placed for 10 min in Peroxidase blocking reagent, followed by a 3 x 3 washing steps in PBS and incubated with the second antibody (100 µl) for 1 hour. After 3 x 3 min washes in PBS, sections were incubated for 30 min in a 1:50 dilution of ExtrAVidin-Peroxidase 1% (v/v) TritonX100, 1.5% (m/v) BSA in PBS followed by 3 x 3 min wash in PBS. The slides

where then being developed using 3-amino-9ethylcarbazole (AEC) solution for 5 min, giving a strong red color to the antibody complex. Post rinsing with distilled water, sections were mounted using glycerol gelatin. The quantification of cells stained with *Pax 7* was determined at a magnification of 10X in six randomly selected fields per section. The total number of myogenic *Pax 7*$^+$ cells was calculated as: [the number of *Pax 7*$^+$ cells counted in the analyzed fields x total cross-sectional area of fast muscle (mm^2)] / [total area of analyzed fields (mm^2)].

Statistical analyses

To compare body weight, total length and condition factor mean values of sampled diploids and triploids, the data was analyzed using one-way ANOVA followed by a t-test for comparisons when significance was established between treatments.

A general lineal model - GLM (ANCOVA) was used to analyze the effect of treatments (ploidy status) and condition factor on diameter, number and density of fibres and total cross section area (TCA). Ploidy status was the fixed factor whereas condition factor (K) was used as covariate. All data were tested for normality distribution using (Shapiro Wilk's W test) after transformation of the data and homogeneity of variance (Levene's test) prior to analysis. When the assumptions for normality and equal variance were not satisfied by transformation of data, the groups were compared by a non-parametric analysis of variance (Mann-Whitney U test). The total number of *Pax 7*$^+$ cells at the different sampling times were compared by a one-way analysis of variance (ANOVA), followed by a t-test for comparisons when a significant difference was found between the treatments. Regression analysis was used to derive relationship between TL and *Pax 7*$^+$ cell number and density. Nonparametric statistical techniques were used to fit smoothed probability density functions (pdfs) to the 1000 fast fibres diameter

measured per individual using a kernel function (Johnston *et al.* 1999). Values for the average smoothing parameter *h* ranged from 0.118 to 0.139. A nonparametric Kolmogorov-Smirnov test was used to check for differences in the pdfs between groups.

All analyses were performed using Minitab version 16 (Minitab Statistical software Inc., US). Data are presented as mean ± SD (n = number of samples) and significance level in all tests was set to P < 0.05.

Results

Muscle fibre growth patterns

Body weight (BW), total length (TL) and condition factor (K) data of the randomly selected fish for muscle cellularity analyses are presented in Table 1.

Table 1. Biometry data (BW = body weight, TL = total length and K = condition factor) of the fish analyzed for muscle cellularity.

Sampling period	Ploidy	Fish (n)	BW (g)	TL (cm)	K
February	2n	14	49.9 ± 6.7*	18.8 ± 0.9	0.75 ± 0.05
	3n	14	39.8 ± 9.8	17.9 ± 1.7	0.69 ± 0.11
June	2n	24	137 ± 23.3*	24.9 ± 1.8	0.9 ± 0.22*
	3n	24	124.2 ± 18.6	25 ± 1.3	0.79 ± 0.06
September	2n	34	405.2 ± 130.1*	33.3 ± 3.4	1.07 ± 0.14**
	3n	34	328.3 ± 135.8	32 ± 3.5	0.96 ± 0.13

The sampling dates correspond to the start, middle and end of the experiment (fish age of 36, 51 and 65 weeks). Results are means ± SD and significance (ANOVA) are indicated by asterisks (*) *P* < 0.05 and (**) *P* < 0.01.

The diploid group had significant higher average body weight than the triploid group at all sampling points ($P < 0.05$). However, diploids had higher K only in the June (0.9 ± 0.22 vs. 0.79 ± 0.06, $P < 0.05$) and September (1.07 ± 0.14 vs. 0.96 ± 0.13, $P < 0.01$) sampling point. The average total body length was similar between the two ploidy groups. Changes in muscle cellularity with growth are illustrated in Fig. 1A-C. During the 29 weeks trial, the fibre diameter increased from 50 ± 5 µm (February) to 73 ± 6 and 74 ± 10 (September) in diploids and triploids, respectively (Fig. 1A). In the same period, the number of fast fibres increased in diploids from 54905 ± 14125 to 114979 ± 22760 respectively, corresponding to an increase of 318 fibres per day (Fig. 1B). Within triploids, such increase was from 43528 ± 11957 to 91086 ± 19882, corresponding to a gain of 252 fibres per day (Fig. 1B). In contrast, the muscle fibre density decreased in both groups from 389 ± 75 to 183 ± 32 and from 384 ± 68 in to 172 ± 32, respectively (Fig. 1C). At the initial sampling, a significant effect of condition factor (K) on fast fibre number ($P < 0.05$) and TCA ($P < 0.01$, ANCOVA) was found. At this stage, the diploid group had significant higher ($P < 0.05$) number of fast fibres and larger ($P < 0.01$) TCA than the triploid group (Fig. 1B, D). Differences in fast fibre diameter between diploid (54.3 ± 6.2 um) and triploid fish (50.4 ± 5.6 um) were found in the June sample point ($P < 0.05$, Fig. 1A). The results of the ANCOVA analysis indicated a close to significant effect of ploidy on fiber diameter ($P = 0.08$) at this sample point. At the end of the experiment (September), significant differences between ploidies were found in fast fibre number ($P < 0.001$) and TCA ($P < 0.05$). The diploid group had significant higher fast fibre number and a larger TCA compared to the triploid group (Fig. 1B, D). Results from the ANCOVA analysis showed that ploidy status ($P < 0.01$) and K ($P < 0.05$) had a significant effect on fast fibre number, while

only K significantly affected TCA ($P < 0.001$). No differences in fiber diameter or fiber density were observed between the two ploidy groups at this stage.

At the beginning of the experiment (February), the 2.5th percentile was affected by ploidy status ($P < 0.05$) with a higher mean value in diploids (13.4 ± 1.8µm) compared to triploids (11.5 ± 1.1µm). Thus, the maximum diameter (97.5th percentile) did not differ between the two groups (Fig. 2A-B). In the June sample point neither the 2.5th nor the 97.5th percentiles differed between ploidies. However, at the end of the trial, both ploidy ($P< 0.001$) and K ($P< 0.001$) had an effect on the 97.5th percentile with higher values in the triploid group compared to the diploid group (225.2 ± 24.8 um vs. 210.1 ± 24.4 um, $P < 0.05$). Thus, the 2.5th percentile was similar between diploid and triploid groups. A decrease in the 2.5 percentile values was observed in the second sample point compared to the start of the experiment for both ploidy groups (Fig. 2A), while the 97.5th percentile increased during the growth trial for both diploids and triploids (Fig. 2B).

The diploid and triploid group displayed similar fast muscle fibre size distributions at all the three sample points (Fig. 3 A-C). A predominance of muscle fibres < 25 µm was observed at the first sample point (Fig. 3A) and < 50 µm during the last two sample points (Fig. 3B-C). An increase of fibres larger than 50 µm was observed at the end of the trial (Fig. 3C). The diploid group displayed a tendency for fibre diameter < 25 µm at the beginning of the experiment (Fig. 3A) reflecting the higher fibre numbers of diploid group compared to triploid group, although not being significant.

Myogenic cells

In this study, the primary antibody *Pax 7* was used to identify myogenic progenitor cells (MPCs). The immunostained sections showed that regardless of ploidy status, the *Pax 7*⁺ cells were scattered through the myotome (Fig. 4) while more concentrated in the peripheral growth zones and close to the myoseptum.

At the start of the experiment, the total number of *Pax 7*⁺ cells and TL were significantly correlated only in triploids ($P < 0.05$, Fig. 5A) although a trend towards a positive correlation between these two traits could also be observed within triploids ($P = 0.053$). In the same way, the diploid group had nearly significantly ($P = 0.053$) higher total number of *Pax 7*⁺ cells (11913 ± 4969) compared to the triploid group (8878 ± 4619). However, at the end of the experiment neither groups showed a significant relationship between the total number of *Pax 7*⁺ cells and TL (Fig. 5B). At this stage, both ploidy groups had also similar *Pax 7*⁺ cell numbers (50783 ± 16603 vs. 50602 ± 24362).

Discussion

The present study showed little difference between ploidy groups with respect to body growth (overall 8.1 fold for diploids vs. 8.2 fold for triploids) and the investigated fast muscle fibre data (Fig. 1 & 3), apart from a temporal difference in 2.5th and 97.5th percentile at the onset and end of the trial, respectively (Fig. 2A). These results are mirrored in the body mass growth where diploids were significantly heavier than triploids at all three sample points (Table 1), supporting previous findings reporting that effects of triploidy in cod muscle cellularity appear at late juvenile stages (Peruzzi, Falk Petersen, Lein, Puvanendran, Hagen & Jobling 2011). Peruzzi *et al.* (2014) reported

increased hypertrophy of fast muscle fibres in 1.5 – 2 kg triploid Atlantic cod, where triploid fish had ~20% larger fast muscle fibres than diploids. Overall, his would suggest that triploid cod (*G. morhua*) start to express differences in muscle growth pattern in the size range between 0.4 to 1.5 kg. Studies of muscle growth in triploid fish are scarce and show variable results depending on the species and developmental stage. As in Atlantic cod, alterations in hyperplasia and hypertrophy of triploid fish have been observed for salmonids too. For example, Suresh & Sheenan (1998) reported that triploid rainbow trout (*O. mykiss*) had larger but fewer fibres than the diploid control at a body length of < 30 cm. Similarly, triploid Atlantic salmon (*S. salar*) showed fewer fast muscle fibres than diploids during freshwater and seawater phases with diploids having a higher body mass than triploids for a given fork length (Johnston *et al.* 1999). Evidences that triploids possess larger but fewer cells compared to diploids have been provided for other organs and tissues including brain, kidney, liver and blood (Benfey 1999).

Post-embryonic growth in fish depends upon the proliferation and differentiation of a source of undifferentiated dermomyotome cells termed MPCs (Hollway *et al.* 2007; Stellabotte & Devoto 2007). Their density may depend on age and species (Johnston *et al.* 1999) but also be modulated by environmental factors such as thermal regime (Steinbacher, Marschallinger, Obermayer, Neuhofer, Sanger & Stoiber 2011). The use of immunostaining for *Pax 7* has recently been applied as a molecular marker of dermomyotome-derived muscle precursors. In our study, no differences in the number of *Pax 7*^{+} cells were found between diploids and triploids at the start or end of the experiment. However, at the beginning of the trial, both ploidy groups displayed a positive correlation (only being significant for triploids) between the TL of the fish and the number of *Pax 7*^{+} cells. Such positive relationship was no longer present at the end

of the trial. *Pax 7*+ cells are believed to support further growth and this observation might be related to their contribution to the higher hypertrophic growth of muscles fibres in triploids (Fig. 2B) rather than the recruitment of new muscle fibres. There is no previously reported work in Atlantic cod that relates muscle growth and myogenic markers at any developmental stage and in relation to ploidy status. However, Johnston *et al.* (1999) used c-met as muscle precursor marker, and reported that diploid Atlantic salmon smolts had 24 % more abundant c-met positive cells than triploids which in turn contributed to the higher numbers of fibres displayed by diploids at the smolt stage.

In our study, *Pax 7*+ cells of diploids and triploids were found scattered throughout the myotome (Fig. 4) but also more abundant in the peripheral growth zones as well as at the myoseptum. Similar observations have been reported for other species such as pearlfish *Rutilus meidingeri* (Steinbacher, Haslett, Six, Gollmann, Sänger & Stoiber 2006). In pearlfish, *Pax 7*+ cells were found only extremely rarely at the sites of fast muscle mosaic hyperplasia deeper inside the myotome at the larval / juvenile transition (Steinbacher *et al.* 2006). The authors suggested two possible theories for their findings. First, that the transition from cell proliferation to differentiation and becoming myoblast cells is much shorter during mosaic hyperplasia than stratified hyperplasia making *Pax 7*+ cells hardly to detect. Second, that the mosaic precursor cells migrate on a longer distance, probably using myoseptal gaps to reach their final position between the pre-established fibres and only then, these cells begin to differentiate. In contrast to stratified hyperplasia, where the origin of MPCs supporting growth is shown to come from the dermomyotome (Hollway *et al.* 2007; Stellabotte & Devoto 2007; Marschallinger, Obermayer, Sanger, Stoiber & Steinbacher 2009) and more

specifically from the dermomyotome posterior lip (Steinbacher, Stadlmayr, Marschallinger, Sänger & Stoiber 2008), the source of MPC that support mosaic hyperplasia is not well known. However, it is hypothesized that MPCs derived from the dermomyotome are also the source for mosaic hyperplasia in addition to the so-called mosaic cells (precursor cells) located deep and scattered through the myotome after hatching (Hollway *et al.* 2007). The marked increase in muscle growth during mosaic hyperplasia, a growth phase that lasts until approximately 45-50 % of the ultimate fish size, may put this theory in doubt.

In conclusion, diploid and triploid Atlantic cod juveniles (~40-50 g) grew in a comparable manner displaying limited differences in muscle fibres structure and growth over a period of approximately 6 months. Beyond some dissimilarities in muscle hyperplasia in diploids and muscle hypertrophy in triploids, our results suggest that the reported difference in muscle cellularity in late juvenile/adult cod (Peruzzi *et al.,* 2011) takes place between 0.4 and 1.5 kg. Further investigations should be directed to understand the mechanisms underlying recruitment and hypertrophy and other factors that could potentially affect muscle growth in triploids such as feed composition and feeding regimes.

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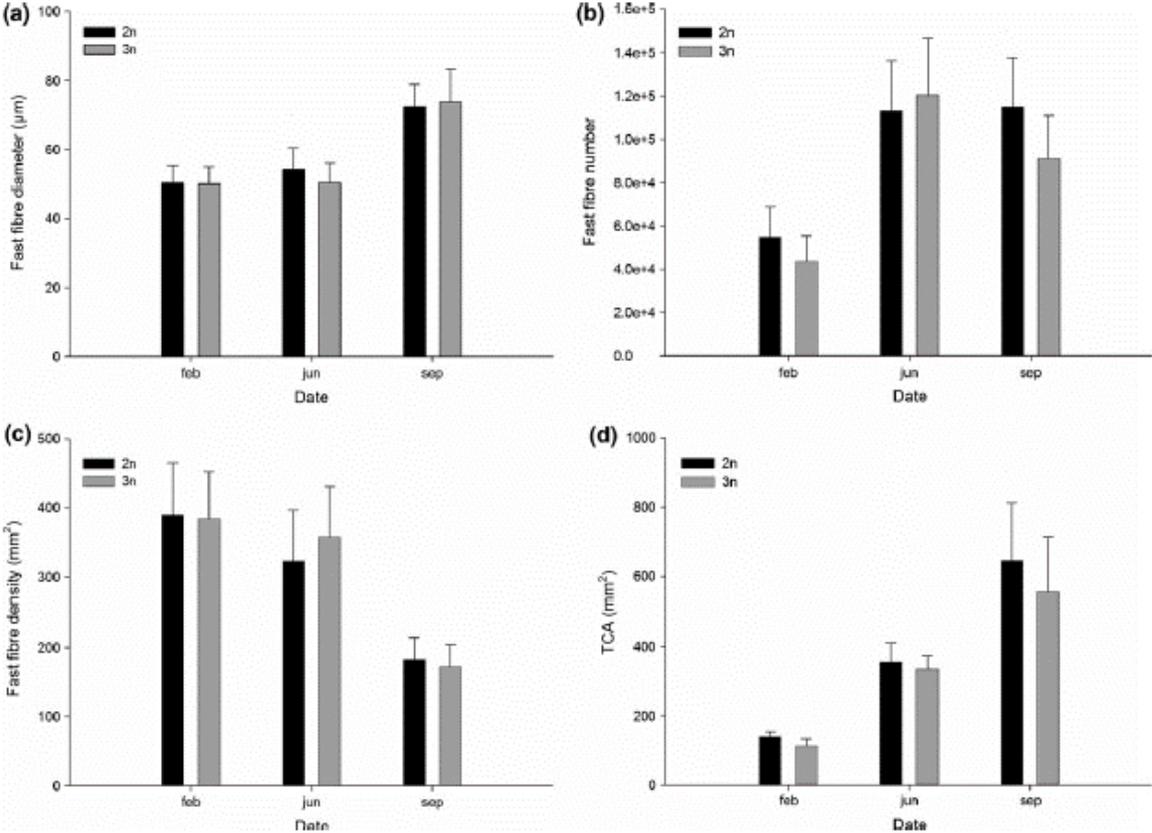
Figures

Figure 1. Muscle fast fibre diameter (A), number (B) and density (C) and total cross section area (TCA) (D) in diploid and triploid cod (means ± SD). P > 0.05 (ANCOVA).
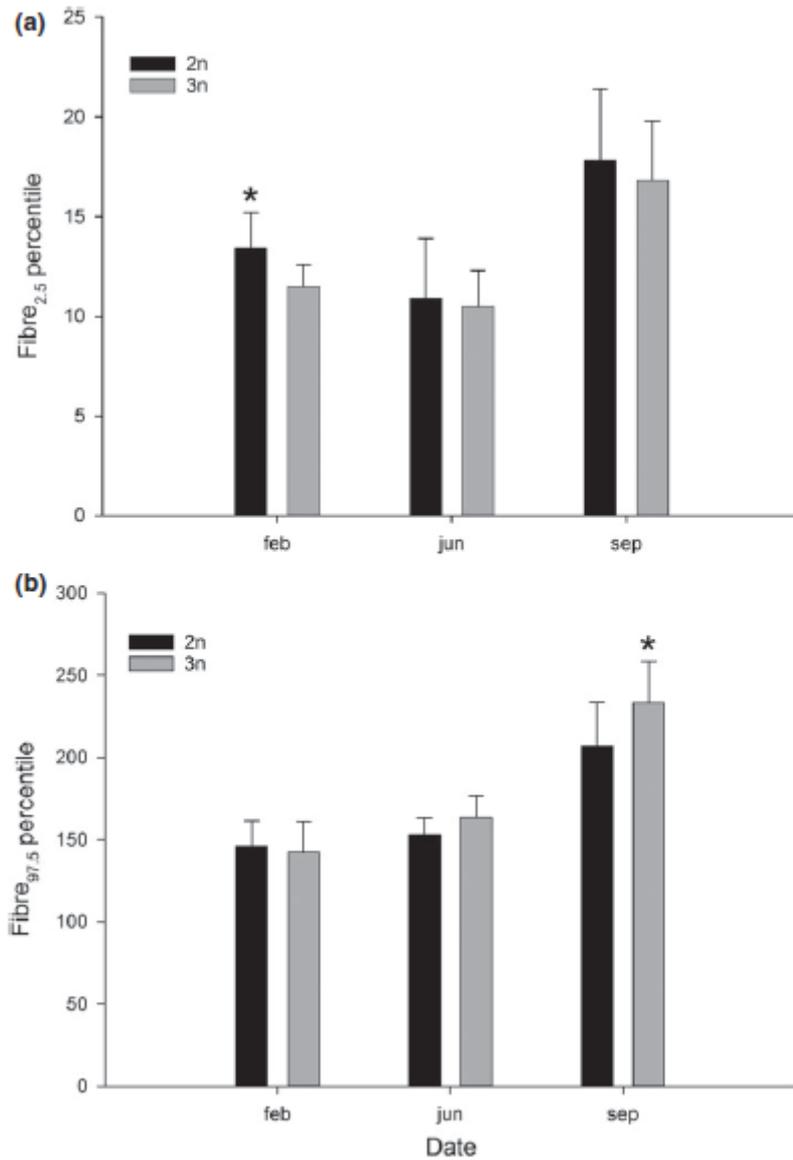
Figure 2. The 2.5th (A) and 97.5th (B) percentile of fast muscle fibres' size distribution in diploid and triploid cod juveniles (means ± SD). Significant differences between the treatments are indicated with asterisks; (*) = P < 0.05, based on ANCOVA and Tukey's comparison tests.
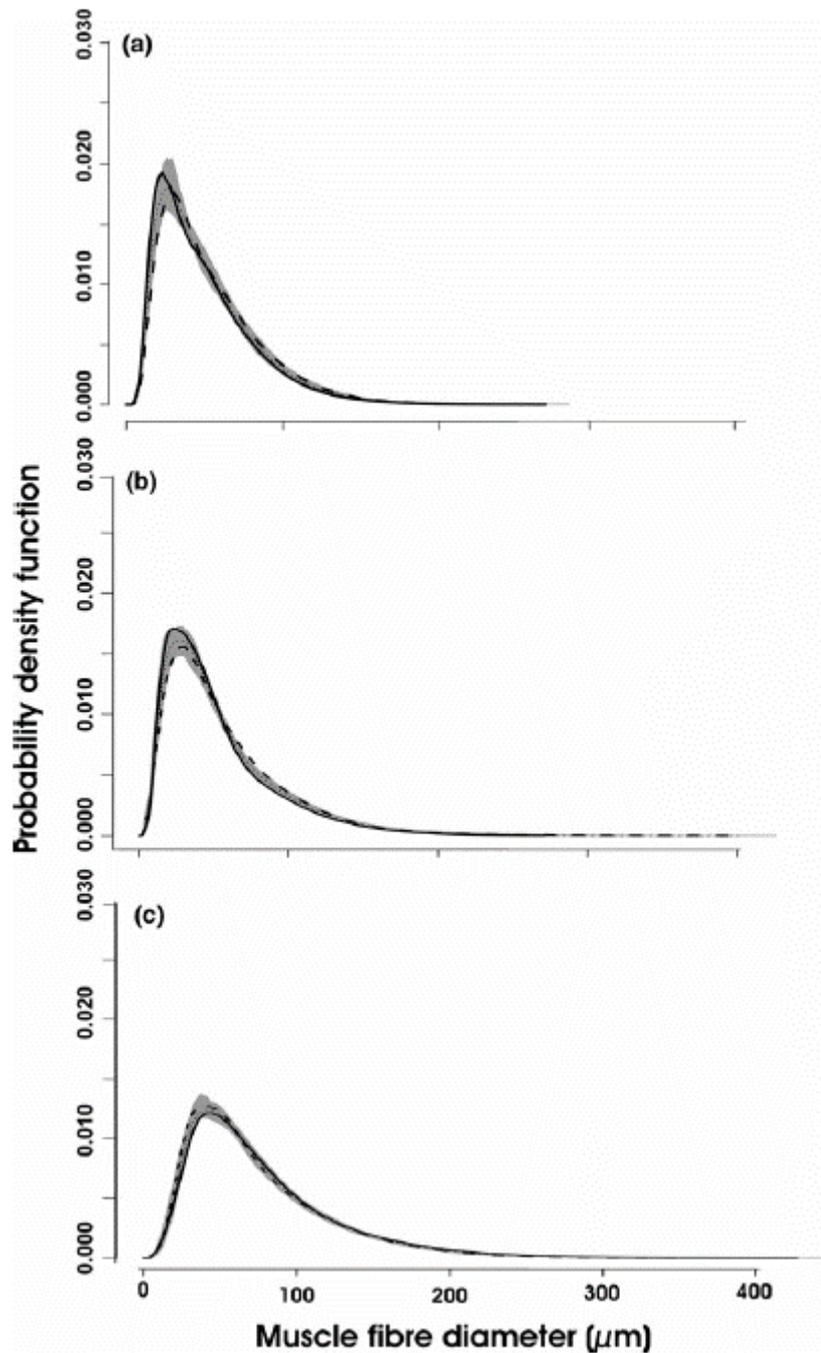
Figure 3. The probability density function (pdf) of fast fibre diameter in Atlantic cod sampled in February (A), June (B) and September (C). The solid (—) and dashed (- -) lines represent the diploid and triploid groups respectively. The dotted line (·····) represents the average probability density function of the combined treatments. The gray shaded area represents a reference band created based on 100 bootstrap

samples of the pdf. The solid and dotted lines do not fall outside the reference band and this indicates that there are no statistical differences between groups (P>0.05).
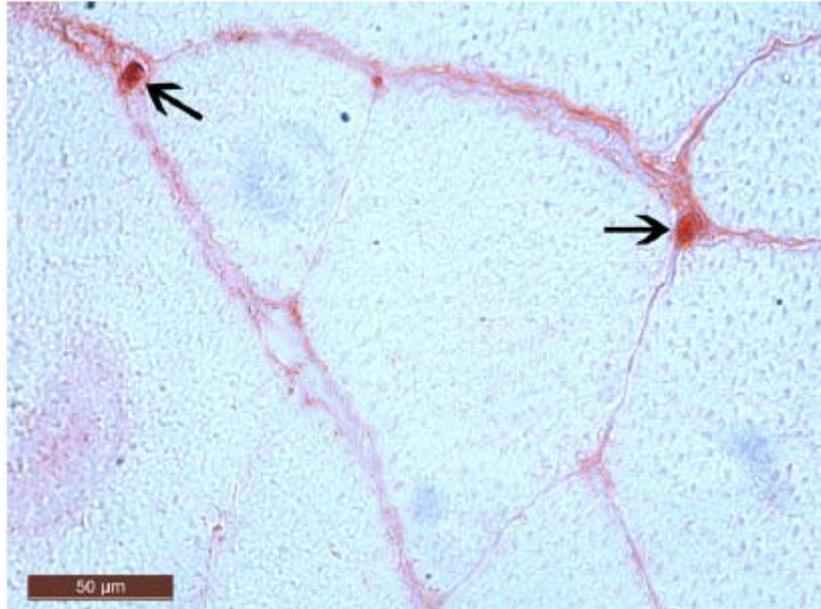

Figure 4. Transverse sections from the central zone of the fast myotomal muscle of Atlantic cod juveniles stained with the molecular marker of myogenic progenitor cells (MPCs), *Pax 7*. Arrowheads indicate *Pax 7*^{+} cells.
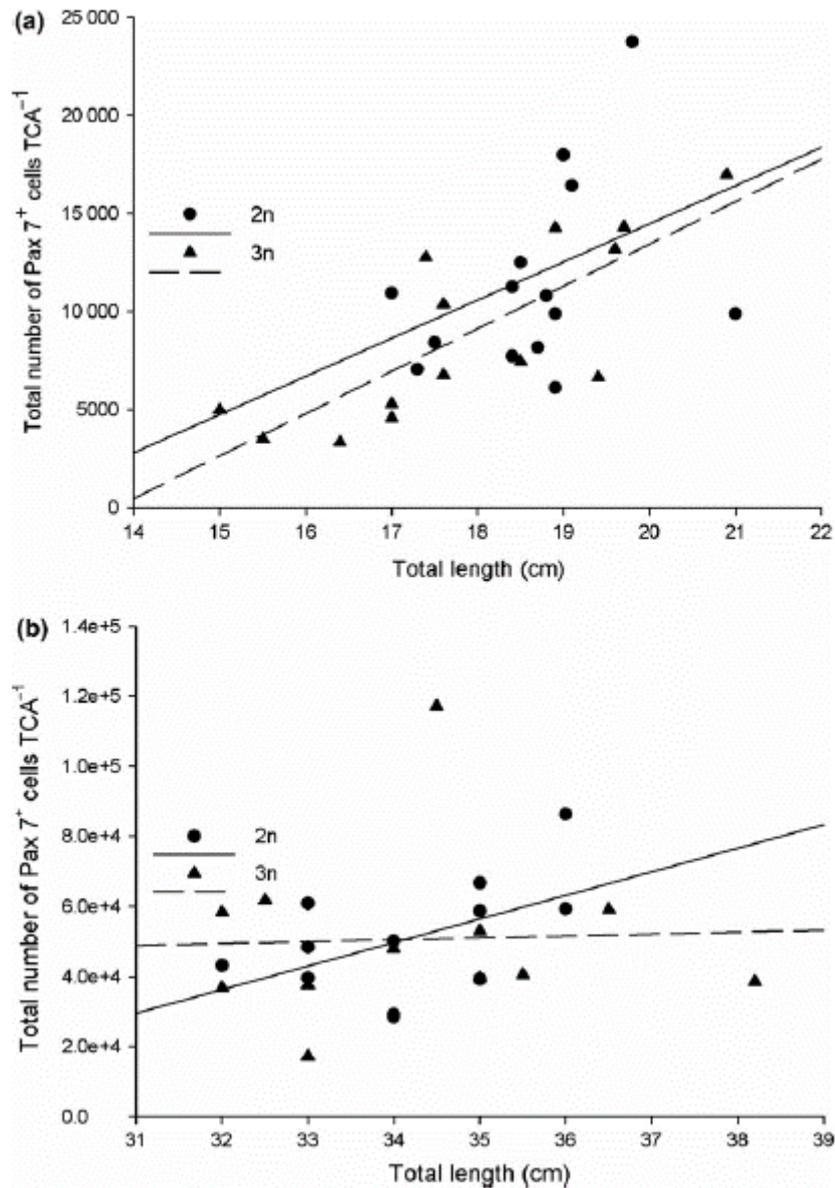
Figure 5. a) The relationship between the number of *Pax 7*^{+} cells and total length (TL) for diploids (filled circles) and triploids (filled triangles) samples taken in February. Linear regressions were fitted to the data with the following equations: total number of *Pax 7*^{+} cells (2n fish) = 24398 -1943*TL* (r^2= 14.7%; P> 0.05) and (3n fish) = 14310 – 2160TL (r^2= 62.6%; P< 0.05).

b) The relationship between the number of *Pax 7*^{+} cells in relation to the total length (TL) of diploids (filled circles) and triploids (filled triangles) samples taken in September. Linear regressions were fitted to the data with the following equations: total number of *Pax 7*^{+} cells (2n fish) = 179147 + 6730TL (r^2= 1.59%; P> 0.05) and total (3n fish) = -13682 + 1834 (r^2= 3.1%; P> 0.05).