

Growth and muscle cellularity of diploid and triploid Atlantic cod (*Gadus morhua* Linnaeus, 1758) larvae

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Summary

The aim of this study was to compare somatic growth and muscle fibre development in diploid and triploid siblings of Atlantic cod (*Gadus morhua* Linnaeus, 1758) during the larval stage. Newly hatched larvae were transferred into 200 L tanks, three tanks per ploidy group (70 larvae L⁻¹, continuous light, gradually increasing seawater temperature 7-11 °C and flow rates 50-117 Lh⁻¹). Larvae were fed rotifers from 2-22 days post hatch (dph), *Artemia* 19-31 dph and weaned onto a microparticulate diet from 26 dph until the end of the experiment. Measurements of growth (dry weight, standard length) and muscle cellularity were taken at intervals between 1 and 44 dph. Ploidy groups showed a similar performance throughout the trial, although a marked stagnation in growth was observed for triploids during the weaning from *Artemia* onto dry feed. Overall, diploid and triploid cod larvae showed a similar development in muscle fibre growth pattern during the experimental period. For both groups, the total number of fast muscle fibres showed a 10-fold increase (from 384 to 3462), whereas the diameter of fast fibre increased from 8.9 to 13.3 µm (mean number from all treatments). Thus, a temporary

but significant effect of triploidy on fast muscle fibre growth pattern was observed in 19 dph larvae in terms of fibre size and number, with triploids showing larger mean fast fibre diameter (11.62 ± 0.63 vs. 10.05 ± 0.34) and a lower number of fibres with diameter $< 5 \mu\text{m}$ than their diploid siblings. Thus, this was found to be related to larvae size and to the differences in total fast fibre cross sectional areas rather than to ploidy status. Overall, our results suggest possible deficiencies in nutrients' digestion and absorption of triploid cod larvae particularly during the transitional period from live food to inert diets.

1. Introduction

During early stages, rapid growth is directed to organs associated to food intake and swimming, enabling fish larva to catch prey items and avoid predators (Galloway et al., 1999; Osse and VandenBoogaart, 1997). As for adult fish, the axial musculature of fish larvae represents the largest and fastest growing organ (Alami-Durante et al., 1997) and can constitute up to 40 % of the total body mass during early stages (Galloway et al., 1999).

In fish, muscle development through hyperplasia can be divided into three phases: embryonic, stratified and mosaic hyperplasia. During embryonic myogenesis, the adaxial and posterior cells generate respectively the superficial and deep muscle cells (Devoto et al., 1996; Rescan, 2005 and 2008), components of the primary myotome (Stellabotte and Devoto, 2007). Although during early stages both fibre types have an aerobic metabolism, they will then differentiate into slow (red) aerobic and fast (white) anaerobic fibres around metamorphosis (Johnston, 1999). From that stage onwards,

growth of the primary myotome will occur by stratified hyperplasia. During this phase, fibres from the dermomyotome (external cells) move from the outer to the inner surface of slow fibres to position in discrete germinal zones situated mainly at the dorsal and ventral regions of the myotome (Hollway et al., 2007; Stellabotte and Devoto, 2007). Finally, mosaic hyperplasia which is characterized by the formation of new fast fibres between the existing fibres will give rise to an assortment of fibre sizes. The onset of mosaic hyperplasia varies between species, occurring late in fast-growing species capable of reaching large ultimate size or being greatly reduced (not occurring) in slow growing species with small ultimate size (Johnston, 1999).

Skeletal muscle in fish shows high phenotypic plasticity to environmental clues like temperature, swimming activity and food availability (Johnston, 2006). For some fish species, myotomal muscle growth might also be affected by ploidy status. For instance, it is known that triploidy may be associated with greater cell size in various tissues as reported in zebrafish *Danio rerio* (Hamilton, 1822), various salmonids and marine species, and such effects may extend to muscle fibres (see Benfey, 1999; Feindel et al., 2011; Piferrer et al., 2009). However, comparison of muscle growth between diploid and triploid fish remains scarce and mostly limited to juvenile and adult stages. In Atlantic salmon, Johnston et al. (1999) found that the embryonic phase of myogenesis was relatively little affected by ploidy status in all-female (AF) populations, whereas diploids displayed ~30% more white muscle fibres than triploids in a normal sex-ratio (NSR) population at first feeding. This suggests that differences in muscle fibre growth between these ploidies might be related to the stage of development. The number, diameter

and size distribution of fast fibres are directly related to growth (Johnston, 1999). Thus, improved knowledge of possible effects of triploidization on muscle growth and cellularity may contribute to elucidate the variable and contradictory growth performance of triploids *versus* diploids.

The aim of this study was therefore to investigate the effect of triploidization on muscle fibre growth dynamics in Atlantic cod larvae reared under similar conditions.

2. Material and methods

All husbandry procedures and experimental protocols were conducted in accordance to the guidelines set by the National Animal Research Authority (Forsøksdyrutvalget, Norway).

Fish husbandry and treatment

Gametes origin and fertilization

Gametes were obtained by stripping 6 females and 3 males of the 2nd generation (2008 year class, 3 years old; 4-5 kg body weight) selected Atlantic cod broodstock reared at Cod Juveniles AS – hatchery. Egg fertilization and induction of triploidy were conducted in November 2012. Male and female gametes were pooled in two separated containers prior to fertilization. Sperm and eggs were gently mixed (2 ml sperm . 300 ml eggs⁻¹ or ca. 300.000 eggs) followed by addition of 6 °C seawater and gametes left undisturbed for 15 minutes in a temperature-controlled room at 6 °C to allow completion of the fertilization process before being rinsed thoroughly with 6 °C seawater. Two-third of the total volume of eggs was used for triploid

production, while the remaining egg volume served as untreated diploid control.

Triploidy induction

Triploid fish were produced by a hydrostatic pressure treatment applied to 180 minute degrees eggs (30 min post-fertilization at 6 °C). For this purpose, fertilized eggs and 6 °C seawater were poured into the chamber of a pressure device (TRC-HPC™ Pressure machine, TRC Hydraulics Inc. New Brunswick, Canada). The hydrostatic pressure was then quickly elevated manually until it reached 8500 psi and kept constant for 5 min, following the protocol of Trippel et al. (2008). Post treatment the eggs were gently poured into a 280 L incubator. Diploid and triploid eggs were incubated in separate incubators (two per ploidy group) at 6.0 °C with a flow rate of 3 – 5 L min⁻¹ until hatching and the eggs were treated with a surface disinfectant (Pyceze, Novartis Ltd., Litlington, Near Royston, UK; 0.8 ml L⁻¹ seawater for 6 min). Seawater at the incubation room was taken from an inlet at a depth of 50 m, filtered through a Bernoulli filter to remove particles over 20 µm, skimmed, aerated and cooled with seawater controlled by a thermostat.

Larval rearing

Newly hatched larvae were transferred into 200 L water volume, green, slightly cone-bottomed tanks (triplicates per ploidy group) with a density of 70 larvae L⁻¹. To estimate the number of larvae to be transferred into the tanks, samples of 2-liters volume were taken from each incubator (2 per ploidy group) after lowering the water level to concentrate the larvae and counting

the larvae contained in a 100 ml graduated cylinder. Water flow rate was kept constant at 0.6 L min⁻¹ until 29 (dph). During the co-feeding (*Artemia*-dry feed) and dry feed periods water flow rate was increased to 1.1 and 2 L min⁻¹, respectively. Seawater temperature was gradually increased from 6 to 10 °C ± 0.3 °C (1 - 10 dph) and kept constant thereafter until the end of the experiment (44 dph) by a heating system with a thermostatic device. Continuous light conditions (600 lux, measured at the water surface) was applied to each tank. Larvae were fed on rotifers (*Brachionus plicatilis*, Cayman strain), short-term enriched with OriGreen (Skretting AS, France) from 2 to 22 dph, added to the tanks in 4 meals of 8000 individuals L⁻¹ every six hours. A solution of Neptune (60 ppm tank⁻¹; Skretting AS, France) was added to the tanks as green water technique simultaneously with the rotifers. *Artemia* enriched with OriGold (Skretting AS, France) was offered to the larvae at a rate of 3000 individuals L⁻¹, 3 meals during the co-feeding period with rotifers (19-22 dph) then at 6-h intervals from 23 to 31 dph. The weaning onto Gemma Micro 150-300 (Skretting AS, France) was from 26 dph until the end of the experiment. Dead larvae were removed as a part of the daily maintenance.

Analytical methods

Larvae were randomly sampled from each tank and anaesthetized with Tricaine methanesulfonate - Finquel vet. (Western Chemical Inc., WA, USA) directly after sampling. Thereafter, larvae were treated as described for the respective analytical methods.

Triploidy assessment

2-day old larvae from control and treated groups were anaesthetized, rinsed with distilled water, individually placed into 1.5ml Eppendorf tubes, and deep frozen (-80 °C) until analysis. Larvae were then prepared for propidium iodide (PI) flow cytometric analysis as described by Peruzzi et al. (2007). The DNA content was measured using a FACScan (Becton Dickinson, San Jose, CA, USA) flow cytometer, based on 20.000 nuclei counts per sample. Ploidy was assessed by calculating the ratio of the mean fluorescence intensity of triploid to diploid and fish were considered triploid when the ratio was 1.5 ± 0.1 . The flow-cytometry data were analyzed using the software CyFlow v. 1.2.1 (©Perthu Thero and CyFlow Ltd).

Growth

For dry weight (DW), standard length (SL) and myotome height (MH) measurements, n= 90 larvae from each ploidy group were randomly sampled from the incubators (1 dph) immediately prior to being placed into their respective tanks. Thereafter n= 30 larvae per tank were sampled at 8, 18, 29, 36 and 44 dph. Larvae were rinsed in distilled water, photographed with a Stereo light microscope equipped with a Olympus Colour - View Illu camera (Olympus, Soft Imaging Solutions, GmbH, Oslo, Norway) before being transferred to tin pre-weighed capsules (SÄNTIS Analytical AG, Landhausstrasse 1, CH-9053 Teufen, Switzerland), and dried at 60 °C for a minimum of 24 hours (depending on larval size). Dry weight (DW) was measured to the nearest 0.1 µg on a microbalance (Mettler Toledo UMX2, Columbus, OH, USA) and the standard length (SL) was measured from the tip of the snout to the end of the notochord by image analysis (Cell P

software Olympus Soft Imaging Solutions, GmbH, Oslo, Norway). The daily weight increase (mg, % day⁻¹) of cod larvae was calculated according to Ricker (1958) as: %DWI = (exp^g – 1)100; where g is the growth coefficient = (ln W₁ – ln W₀) (t₁ – t₀)⁻¹ and W=weight and t=time at sampling.

Muscle cellularity

For muscle cellularity analysis n= 5 larvae per tank were sampled at 1, 8, 19, 29, 36 and 44 dph, rinsed in distilled water and the total length (TL) was recorded. Larvae were sectioned transversely to the body axis at post-anal level, and the anterior part were mounted using cryomatrix (Anatomical pathology, Bergmann AS, Oslo) in aluminum capsules (SÄNTIS Analytical AG, Landhausstrasse 1, CH–9053 Teufen, Switzerland) and snap frozen (60 sec) in isopentane cooled to its near freezing point (-159 °C) in liquid nitrogen. Histological sections were cut at -20 °C in a cryostat (Microm HM 550, MICROM International GmbH) to obtain 2 µm thick histological sections, stained in Harris haematoxylin (Merck KGaA, Darmstadt, Germany) after dehydration and mounted using glycerol gelatin (Sigma-Aldrich, USA) on poly-L-lysine treated slides. Sections were analyzed with a light microscope (Axioscop 2 mot plus; Carl Zeiss INC., Germany) equipped with a camera. The area and diameter of 200-600 fibres (dependent on size) from the left epaxial (dorsal) and hypaxial (ventral) side of the steak of white muscle sections were calculated for each fish using the software Axio Vision (Rel. 4.2, Carl Zeiss INC., Germany). The total fibre number was calculated as [10⁶ x total cross-sectional area of fast muscle (mm²) x number of analyzed fibres] [total area of analyzed fibres (µm)]⁻¹. The fibre density (number of

fibres per unit area (mm^2) was calculated as $[10^6 \times \text{the number of fibres measured}] [\text{total area of analyzed fibres } (\mu\text{m})]^{-1}$.

Statistics

When necessary, data were logarithmically (\log_{10}) transformed while data in percentage were arcsine transformed to normalize distributions. All transformed data were tested for normality of distribution (Shapiro Wilk's test) and homogeneity of variance (Levene's test). Normally distributed data were compared using a one-way ANOVA. When differences between means were found ($P < 0.05$), 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-normally distributed data. ANCOVA was used to analyze data of fibre muscle diameter, number and density with ploidy as factor and total cross-sectional area (TCA) and TL as covariates. Non-parametric statistical techniques were used to fit smoothed probability density functions (pdfs) to the measured fast fibre diameters using a kernel function (Johnston et al., 1999). The average value of the smoothing coefficient h varied between 0.117 and 0.141. A pdfs of the fast fibre diameter of one average sized larva of each ploidy group was plotted to show the development during the experiment (1-44 dph). Minitab version 16 (Minitab Statistical software Inc., US) was used for testing of data. Data are presented as mean \pm SEM (N = number of samples).

3. Results

Growth characteristics

DW (mean \pm SEM) at 1 dph were 0.12 ± 0.01 mg larva⁻¹ for diploids (2n) and 0.13 ± 0.0 mg larva⁻¹ for triploids (3n), and increased to 1.28 ± 0.09 and 1.3 ± 0.09 respectively at the end of the experiment (44 dph). No significant differences in DW between the ploidy groups were observed throughout the experiment (Fig. 1A). The DWI (%) did not differ between the two groups during the trial. The highest and lowest growth rate were observed during the rotifer - *Artemia* co-feeding period (2n: 9.9 ± 0.7 %, 3n: 10.6 ± 1.3 %) and during the *Artemia*-dry feed co-feeding period (2n: 2.9 ± 2.1 % vs. 3n: 0.3 ± 2.4 %) respectively.

At the beginning of the experiment, SL and MH increased from 4.47 ± 0.01 mm and 0.25 ± 0.00 mm at 1 dph to 10.33 ± 0.12 mm and 0.98 ± 0.03 mm at 44 dph for diploids respectively. In comparison, the triploid group showed an increase from 4.54 ± 0.02 mm and 0.25 ± 0.00 mm to 10.56 ± 0.13 mm and 1.03 ± 0.03 mm in SL and MH respectively. No significant differences in SL and MH between the two ploidy groups were observed throughout the experiment (Fig. 1B-C). However, triploids showed stagnation in SL and MH during the rotifers - *Artemia* co-feeding period with 8.97 ± 0.41 mm and 0.69 ± 0.0 mm at 29 dph) and 9.27 ± 0.22 mm and 0.71 ± 0.03 mm at 36 dph respectively (Fig. 1B-C).

Muscle growth patterns

The total cross sectional area of fast muscle fibre increased from 0.023 mm² and 0.024 mm² at 1dph to 0.68 ± 0.08 mm² and 0.68 ± 0.05 mm² at 44 dph

for diploids and triploids respectively (Fig. 2). This corresponds to a 24 fold-increase for the 2n group and a 22 fold-increase for the 3n group, respectively. Although no significant differences were found between diploids and triploids at any sample date, the latter experienced a decrease in TCA during the *Artemia* – dry feed co-feeding period (29 dph: $0.29 \pm 0.05 \text{ mm}^2$ and 36 dph: $0.21 \pm 0.02 \text{ mm}^2$) (Fig. 2).

The mean fast fibre diameter at 1 dph increased from $8.87 \pm 0.43 \text{ }\mu\text{m}$ and $9.01 \pm 0.26 \text{ }\mu\text{m}$ for diploids and triploids respectively, to $13.24 \pm 0.58 \text{ }\mu\text{m}$ and $13.38 \pm 0.48 \text{ }\mu\text{m}$ at the end of the trial (44 dph) (Fig. 3A). Significant differences in fast fibre diameter between the two ploidy groups were found at 19 dph only. At this stage, triploids had larger fast fibres than diploids ($11.62 \pm 0.63 \text{ }\mu\text{m}$ vs. $10.05 \pm 0.34 \text{ }\mu\text{m}$) and fewer fast fibres of a diameter $< 5 \text{ }\mu\text{m}$ (Fig. 3B). However, the differences in fast fibre diameter were related to TCA ($P < 0.05$), rather than larval length ($P > 0.05$), while ploidy showed a close to significant effect on fibre diameter ($P = 0.06$), (ANCOVA analysis). Fast muscle fibre measurements from the same location (ventral “hypaxia” left side) of one diploid and one triploid 19 dph larva (a representative larva from each group, 6.1 mm) were color coded and divided into size classes to illustrate that the triploid larvae had fewer small fibres compared to diploid larvae (Fig. 4A-B).

The total number of fast fibre in 1 dph larvae was 387 ± 36 for diploids and 380 ± 22 for triploids, increasing to 3262 ± 296 (diploids) and 3661 ± 232 (triploids) at 44 dph (Fig. 5). This represented an 8.4 fold increase for the diploid and a 9 fold increase for the triploid group. No differences were found

in the mean total number of fast fibre between the two ploidy groups at any sampling date (Fig. 5).

Smooth distributions were fitted to number of measurements (depending on the larval stage) of fast fibre diameter per fish using a kernel function and the corresponding probability density functions (pdfs) plotted. Comparisons of the distribution of fast fibre diameter showed that for 1 dph larvae, the pdf peak corresponded to a muscle fibre diameter of approx. 8 μm in both ploidy groups (Fig. 6A-B). In older larvae, there was a tendency towards an increase in fibre diameter and the peak pdfs shifted towards the right (Fig. 6A-B). However, an exception was observed in 19 dph larvae with diploids showing a peak for 5 μm (Fig. 6A) and triploids for 8 μm fibres (Fig. 6B).

4. Discussion

Growth

During the live food period, triploids grew better than diploids whereas the opposite trend was observed during weaning onto micro particulate diets, although no significant. Similar growth patterns have recently been reported by Opstad et al. (2013), showing that triploid cod are more affected by the shift from live food to dry feed. The authors related such low performance to possible behavioral differences as reported for other species. In Atlantic salmon, triploid fry named “non-feeding fry” were shown to disperse slowly through the water column with more difficulties accepting food compared to their diploid siblings (Cotter et al., 2002). This assumption is supported by recent findings showing that triploid salmon have smaller olfactory bulbs and

larger cerebella and telencephalon than diploids (Fraser et al., 2012). These organs are implicated in functions such as foraging ability, aggression and spatial cognition. If such morphological brain alterations caused by triploidization are confirmed in triploid Atlantic cod too this might explain the stagnant growth observed in our study during the transition between *Artemia* and dry feed.

Contradictory and variable performance results have been published for triploids of several species such as Atlantic salmon (McGeachy et al., 1995; Cotter et al., 2002; Taylor et al., 2011; Taylor et al., 2013), rainbow trout, (Cotter et al., 2002; Taylor et al., 2011; Taylor et al., 2013), Atlantic cod (Opstad et al., 2013), turbot (Cal et al., 2006) and European sea bass (Radaelli et al., 2010) when compared to their diploid siblings. The differences have been related to factors such as unfavorable culture conditions (Maxime, 2008; Piferrer et al., 2009; Tiwary et al., 2004), gamete quality (Taylor et al., 2011), stage of the life cycle (Wagner et al., 2006) and family and/or strain effects (e.g. Blanc et al., 2001; Johnson et al., 2004; Taylor et al., 2013). However, in our study, the marked growth stagnation displayed by triploids during the weaning period, may also indicate deficiencies in nutrients' digestion and/or absorption. For instance, morphological differences in the guts of diploid and triploid siblings have recently been reported for juvenile Atlantic cod (Vargas et al., 2014) and Atlantic salmon (Peruzzi et al., 2014). This has been hypothesized to play a role in determining the digestive efficiency and growth of fish that differ in ploidy status. Overall, this highlights the need for further research on the

nutritional requirements of triploids and the optimization of culture protocols specific for these fish.

Muscle cellularity

In the present study, the somatic growth of diploids and triploids mirrored the two groups' muscle growth dynamics. As larvae grew, an increase in diameter and number of fast fibre was observed in the two ploidies. Differences in muscle cellularity between diploids and triploids were found only at 19 dph (end of the rotifer period, Fig. 3A-B), with larger mean fast fibre diameter and lower numbers of fibres with diameter < 5 μm in triploids than in diploids. This temporal difference might indicate that the recruitment of new fibres was higher in the diploid group, whereas the triploid group showed greater hypertrophy at this developmental stage. However, these differences were attributed to the TCA of the larvae (ANCOVA, $P < 0.05$) rather than a ploidy effect. A marked decrease in the number of fibres < 5 μm following the switch from Artemia to dry feed (29-36 dph) was observed in the triploid group (Fig. 3B) without any increase in the total number of fast fibres (Fig. 5), indicating a halt in hyperplasia during the temporary growth stagnation (DWI % of 2.9% and 0.31% for diploids and triploids respectively). Our observation of reduced hyperplasia related to somatic growth is in accordance with early findings in first-feeding diploid cod larvae (Galloway et al., 1999). Galloway et al. (1999) found that larvae with high somatic growth showed a higher hyperplasia contribution to axial fast muscle growth compared to slower-growing larvae.

The total mean number (~ 350) and mean diameter (9 μm) of fast fibres reported in the present study, regardless of ploidy, differ from previous observations in newly hatched cod larvae showing lower numbers of fibres (~ 250) and with smaller diameter (~6 μm) (Galloway et al., 1999; Johnston and Andersen, 2008). Such discrepancy might be related to genetic differences between the larvae used in our experiment (issued from F2 generation selected broodstock) and those employed in previous studies.

The use of probability density curves provides a clearer picture of the differences and similarities between diploid and triploid fibre size distributions. The diploid and triploid larvae selected from each sampling point with the exception of 19 dph (Fig. 6A-B) showed similar size distributions. During endogenous feeding and the first days of exogenous feeding (1 – 8 dph), muscle growth hypertrophy was the main growth mechanism. From 1 to 8 dph (Fig. 5), the number of fast muscle fibres remained constant while the mean fast fibre diameter increased from 9 to 11.6 μm (Fig. 3A). In contrast, a distinct stratified hyperplasia was observed in the two ploidy groups at 19 dph as denoted by a left-shift in the distribution of muscle fibre diameter compared to the other sampling points (Fig. 6A-B). New fibres were recruited on the periphery of the myotomal epaxial and hypaxial apices while fibres undergoing hypertrophy were located in the center of the myotomal muscle (Fig. 4). An increase in the number of muscle fibres < 5 μm compared to earlier stages contributed to the substantial somatic growth observed at 19 and 29 dph, indicating that hyperplasia was the major mechanism of muscle growth during that period. The shift from

hypertrophy to stratified hyperplasia and the proliferative zones of newly recruited fibres observed in this study are in accordance with previous findings reported for the same species by Galloway et al. (1999) and Johnston and Andersen (2008).

Considering that the differences found at 19 dph were not related to ploidy status, our results suggest that triploidization has no effect on muscle growth in cod during early larval stages. This is in line with work on early stages of Atlantic salmon where no clear differences were found between diploids and triploids in diameter, number and size distribution of fast fibres during the freshwater period (Johnston et al., 1999). However, a possible effect of triploidization on muscle growth might depend of developmental stage as reported for species such as Atlantic salmon and rainbow trout (Suresh and Sheehan 1998; Johnston et al., 1999). In seawater stages, diploid Atlantic salmon showed significantly greater fibre recruitment than their triploid counterparts (Johnston et al., 1999). Suresh and Sheehan (1998) found differences in fibre size distribution between diploid and triploid rainbow trout of 100 – 300 mm length. In the latter work, diploids showed a higher proportion of fibres < 20 μm whereas triploids had higher proportion of larger fibres (> 20 μm), indicating a reduced hyperplasia within triploids. However, in both cases, the mean diameter of fast fibres did not differ between ploidies.

The lack of triploidy effect on muscle cellularity between diploids and triploids at larval stages found in our study may be explained by the rapid growth

experienced during larval stages. This growth pattern is characteristic for many fish species including Atlantic cod to cope with activities linked to active foraging and predators' avoidance. For example, Alami-Durante et al. (1997) reported a daily muscle mass increase of 28 % during the period 4-18 dph in the common carp *Cyprinus carpio* L. Considering that the growth potential depends on several aspects, such as environmental conditions and nutrition (Valente et al., 2013), these factors may therefore hinder any potential effect of triploidization on muscle cellularity during early stages. Nevertheless, the trial lasted 44 days and any potential differences in myotomal growth pattern could occur after this rearing period.

In conclusion, muscle growth and cellularity of Atlantic cod during early stages was not affected by triploidization. However, the markedly stagnant growth observed in triploids during the transition from *Artemia* to dry feed might suggest a possible deficiency in digestion and absorption capacity of triploids compared to diploids at that developmental stage. Larval growth is affected by numerous environmental and nutritional factors, thus any potential impact of triploidization on muscle characteristics might have been overshadowed. Therefore, further research needs to be addressed to develop larval rearing protocols adapted to triploids and to investigate in more detail factors influencing early growth performance.

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6. References

- Alami-Durante, H.; Fauconneau, B.; Rouel, M.; Escaffre, A.M.; Bergot, P. 1997: Growth and multiplication of white skeletal muscle fibres in carp larvae in relation to somatic growth rate. *J. Fish Biol.* **50**(6), 1285-1302.
- Blanc, J.M.; Poisson, H.; Vallée, F. 2001: Covariation between diploid and triploid progenies from common breeders in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.* **32**, 507-516.
- Benfey, T.J. 1999: The Physiology and Behavior of Triploid Fishes. *Rev. Fish. Sci.* **7**(1), 39-67.
- Benfey, T.J. 2011: The physiology of triploid fish. In: Encyclopedia of fish physiology: from genome to environment. Ed: Farrell AP. Academic Press, San Diego, pp 2009–2015.
- Cal, R.M.; Vidal, S.; Gómez, C.; Álvarez-Blázquez, B.; Martínez, P. and Piferrer, F. 2006: Growth and gonadal development in diploid and triploid turbot (*Scophthalmus maximus*). *Aquaculture.* **251**(1), 99-108.
- Cotter, D.; O'Donovan, V.; Drumm, A.; Roche, N.; Ling, E.N. and Wilkins, N.P. 2002: Comparison of freshwater and marine performances of all-female diploid and triploid Atlantic salmon (*Salmo salar* L.). *Aquac. Res.* **33**(1), 43-53.

- Devoto, S.H.; Melancon, E.; Eisen, J.S. and Westerfield, M. 1996: Identification of separate slow and fast muscle precursor cells in vivo, prior to somite formation. *Development*. **122**(11), 3371-80.
- Feindel, N.J.; Benfey, T.J. and Trippel, E.A. 2011: Gonadal development of triploid Atlantic Cod *Gadus morhua*. *J. Fish Biol.* **78**(7), 1900-1912.
- Fraser, T.W.K.; Fjellidal, P.G.; Skjæraasen, J.E.; Hansen, T. and Mayer, I. 2012: Triploidy alters brain morphology in pre-smolt Atlantic salmon *Salmo salar*: possible implications for behaviour. *J. Fish Biol.* **81**(7), 2199-2212.
- Galloway, T.F.; Kjorsvik, E. and Kryvi, H. 1999: Muscle growth and development in Atlantic cod larvae (*Gadus morhua* L.) related to different somatic growth rates. *J. Exp. Biol.* **202**(15), 2111-2120.
- Hollway, G.E.; Bryson-Richardson, R.J.; Berger, S.; Cole, N.J.; Hall, T.E. and Currie, P.D. 2007: Whole-Somite Rotation Generates Muscle Progenitor Cell Compartments in the Developing Zebrafish Embryo. *Dev. Cell.* **12**(2), 207-219.
- Johnson, R.M.; Shrimpton, J.M.; Heath, J.W. and Heath, D.D. 2004: Family, induction methodology and interaction effects on the performance of diploid and triploid chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*. **234**, 123-142.
- Johnston, I.A. 1999: Muscle development and growth: potential implications for flesh quality in fish. *Aquaculture*. **177**(1–4), 99-115.
- Johnston, I.A. 2006: Environment and plasticity of myogenesis in teleost fish. *J. Exp. Biol.* **209**(12), 2249-2264.

- Johnston, I.A. and Andersen, Ø. 2008: Number of muscle fibres in adult Atlantic cod varies with temperature during embryonic development and pantophysin (PanI) genotype. *Aquat. Biol.* **4**(2), 167-173.
- Johnston, I.A.; Strugnell, G.; McCracken, M.L. and Johnstone, R. 1999: Muscle growth and development in normal-sex-ratio and all-female diploid and triploid Atlantic salmon. *J. Exp. Biol.* **202**(15), 1991-2016.
- Maxime, V. 2008: The physiology of triploid fish: current knowledge and comparisons with diploid fish. *Fish Fish.* **9**(1), 67-78.
- McGeachy, S.A.; Benfey, T.J. and Friars, G.W. 1995: Freshwater performance of triploid Atlantic salmon (*Salmo salar*) in New Brunswick aquaculture. *Aquaculture.* **137**(1–4), 333-341.
- Opstad, I.; Fjelldal, P.G.; Karlsen, Ø.; Thorsen, A.; Hansen, T.J. and Taranger, G.L. 2013: The effect of triploidization of Atlantic cod (*Gadus morhua* L.) on survival, growth and deformities during early life stages. *Aquaculture.* **388–391**(0), 54-59.
- Osse, J.W. and VandenBoogaart, J.G.M. 1997: Priorities during early growth of fish larvae. *Eur. J. Morphol.* **35**(1), 52-53.
- Peruzzi, S., Hagen, Ø. & Jobling, M. (2014) Gut morphology of diploid and triploid Atlantic salmon (*Salmo salar* L.). *Aquacult. Int.* 1-4. In press. DOI: 10.1007/s10499-014-9867-2.
- Peruzzi, S.; Kettunen, A.; Primicerio, R.; Kaurić, G. 2007: Thermal shock induction of triploidy in Atlantic cod (*Gadus morhua* L.). *Aquac. Res.* **38**, 926-932.
- Piferrer, F.; Beaumont, A.; Falguiere, J.-C.; Flajshans, M.; Haffray, P. and Colombo, L. 2009: Polyploid fish and shellfish: Production, biology and

applications to aquaculture for performance improvement and genetic containment. *Aquaculture*. **293**(3-4), 125-156.

Radaelli, G.; Poltronieri, C.; Simontacchi, C.; Negrato, E.; Pascoll, F.; Libertini, A.; Bertotto, D. 2010: Immunohistochemical localization of IGF-I, IGF-II and MSTN proteins during development of triploid sea bass (*Dicentrarchus labrax*). *Eur. J. Histochem.* **54**, 74-80.

Rescan, P.Y. 2005: Muscle growth patterns and regulation during fish ontogeny. *Gen. Comp. Endocr.* **142**(1-2), 111-116.

Rescan, P.Y. 2008: New Insights Into Skeletal Muscle Development and Growth in Teleost Fishes. *J. Exp. Zool. Part B - Molecular and Developmental Evolution.* **310B**(7), 541-548.

Stellabotte, F. and Devoto, S.H. 2007: The teleost dermomyotome. *Dev. Dyn.* **236**(9), 2432-2443.

Suresh, A.V.; Sheehan, R.J. 1998: Muscle fibre growth dynamics in diploid and triploid rainbow trout. *J. Fish Biol.* **52**, 570-587.

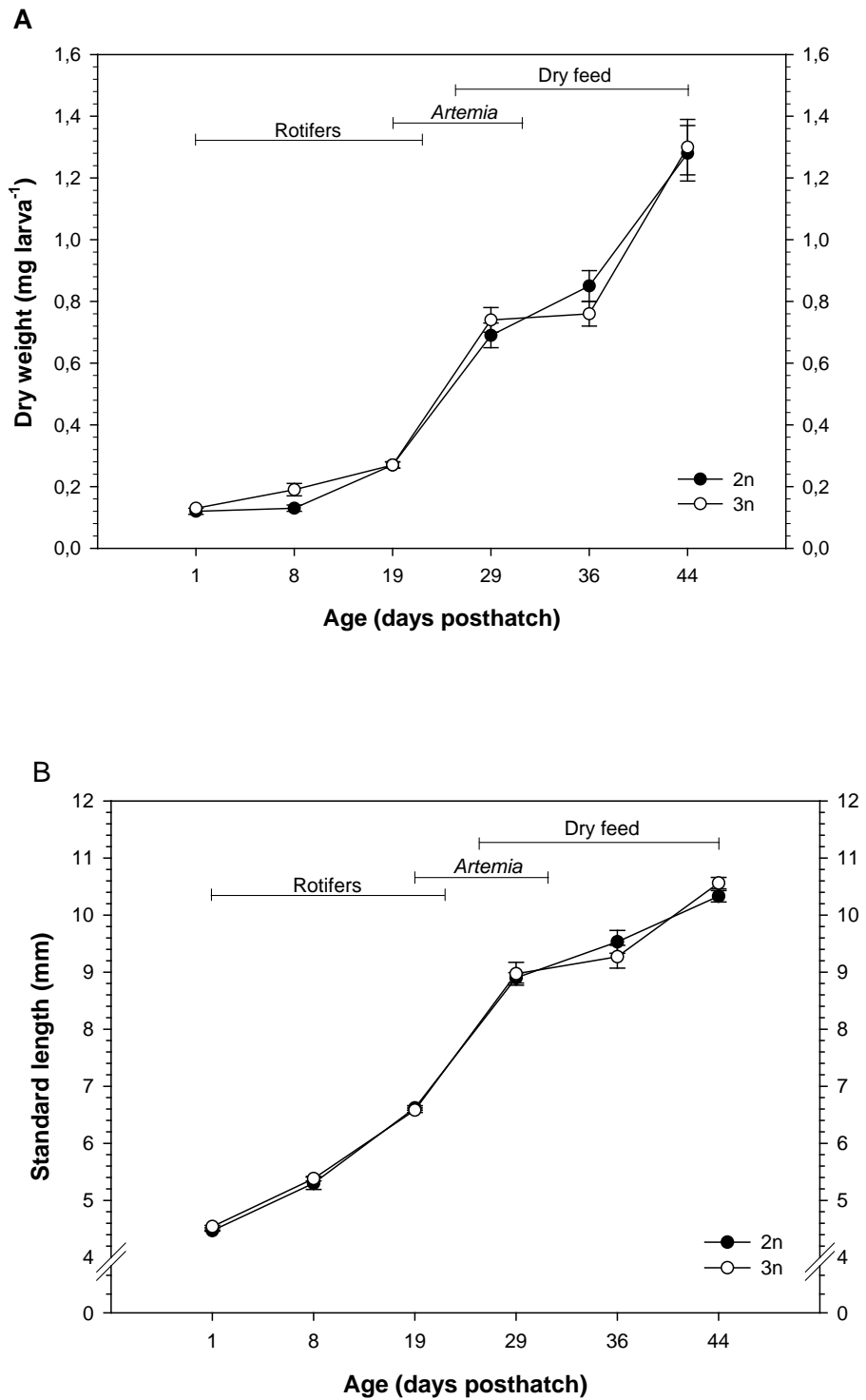
Taylor, J.F.; Preston, A.C.; Guy, D. and Migaud, H. 2011: Ploidy effects on hatchery survival, deformities, and performance in Atlantic salmon (*Salmo salar*). *Aquaculture*. **315**(1–2), 61-68.

Taylor, J.F.; Sambras, F.; Mota-Velasco, J.; Guy, D.R.; Hamilton, A.; Hunter, D.; Corrigan, D. and Migaud, H. 2013: Ploidy and family effects on Atlantic salmon (*Salmo salar*) growth, deformity and harvest quality during a full commercial production cycle. *Aquaculture*. **410–411**(0), 41-50.

Tiway, B.K.; Kirubakaran, R.; Ray, A.K. 2004: The biology of triploid fish. *Rev. Fish Biol. Fisher.* **14**, 391-402.

- Trippel, E.A.; Benfey, T.J.; Neil, S.R.E.; Cross, N.; Blanchard, M.J.; Powell, F. 2008: Effects of continuous light and triploidy on growth and sexual maturation in Atlantic cod, *Gadus morhua*. *Cybium*. **32**, 136-138.
- Valente, L.M.P.; Moutou, K.A.; Conceição, L.E.C.; Engrola, S.; Fernandes, J.M.O. and Johnston, I.A. 2013: What determines growth potential and juvenile quality of farmed fish species? *Rev. Aquacult.* **5**, S168-S193.
- Vargas, C.C.; Hagen, Ø.; Solberg, C.; Jobling, M. and Peruzzi, S. 2014: Growth and gut morphology of diploid and triploid juvenile Atlantic cod (*Gadus morhua*). *Aquac. Res.* n/a-n/a. DOI: 10.1111/are.12603
- Wagner, E.J.; Arndt, R.E.; Routledge, M.D.; Latremouille, D.; Mellenthin, R.F. 2006: Comparison of hatchery performance, agonistic behavior, and poststocking survival between diploid and triploid rainbow trout of three different Utah strains. *N. Am. J. Aquacult.* **68**, 63-73.

Figures and legends:



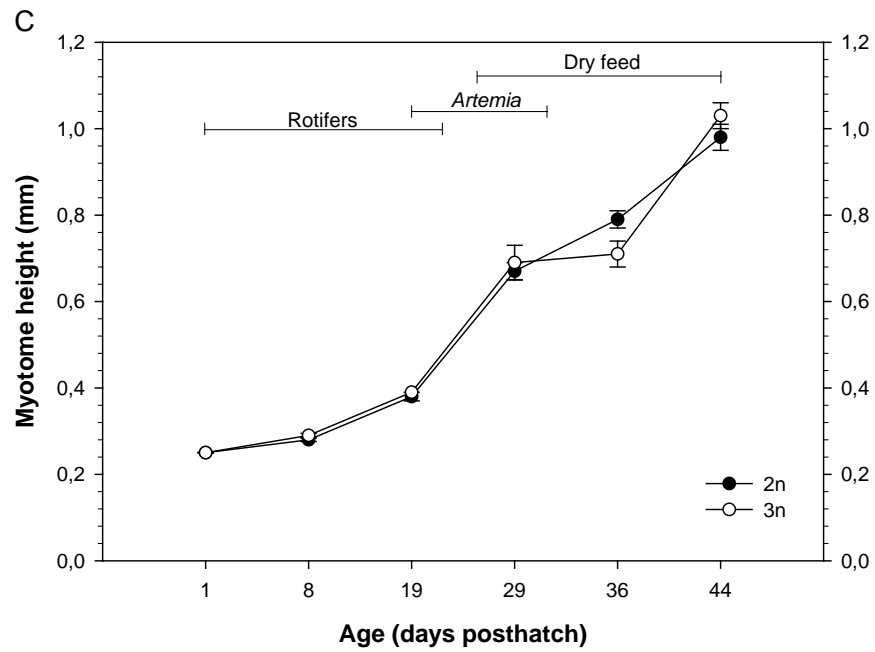


Figure 1. Mean \pm SEM values of dry weight (mg larva^{-1}) (A), standard length (mm) (B) and myotome height (mm) (C) of diploid and triploid cod larvae sampled between 1 – 44 dph.

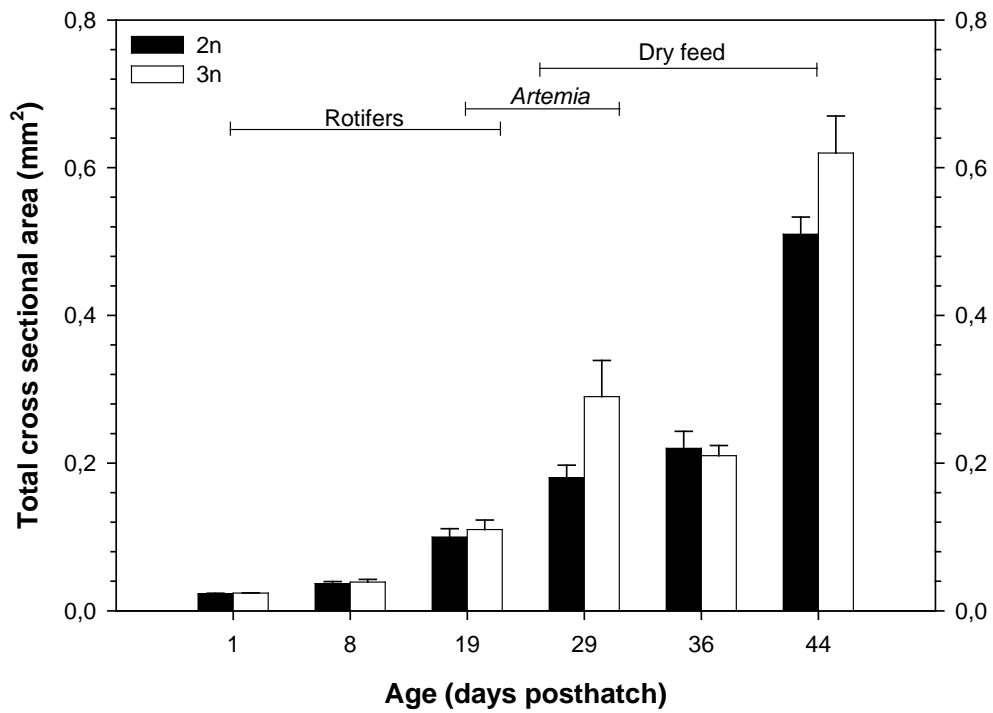
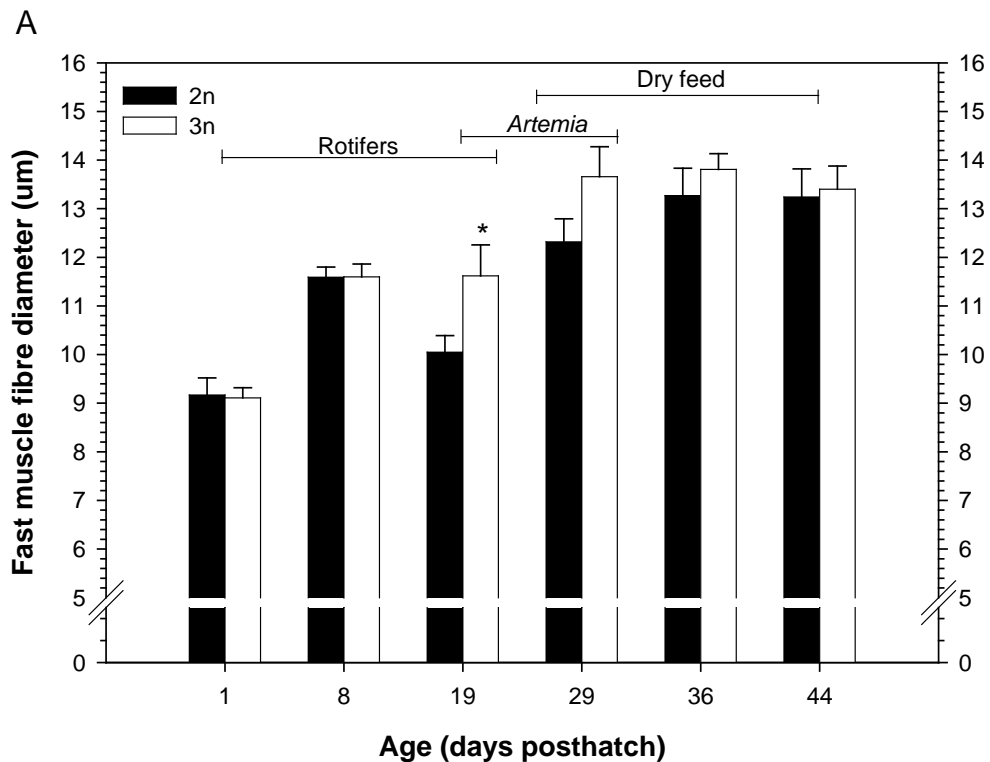


Figure 2. Mean \pm SEM total cross sectional area (mm²) of the myotome of diploid and triploid cod larvae sampled at 1, 8, 19, 29, 36 and 44 dph.



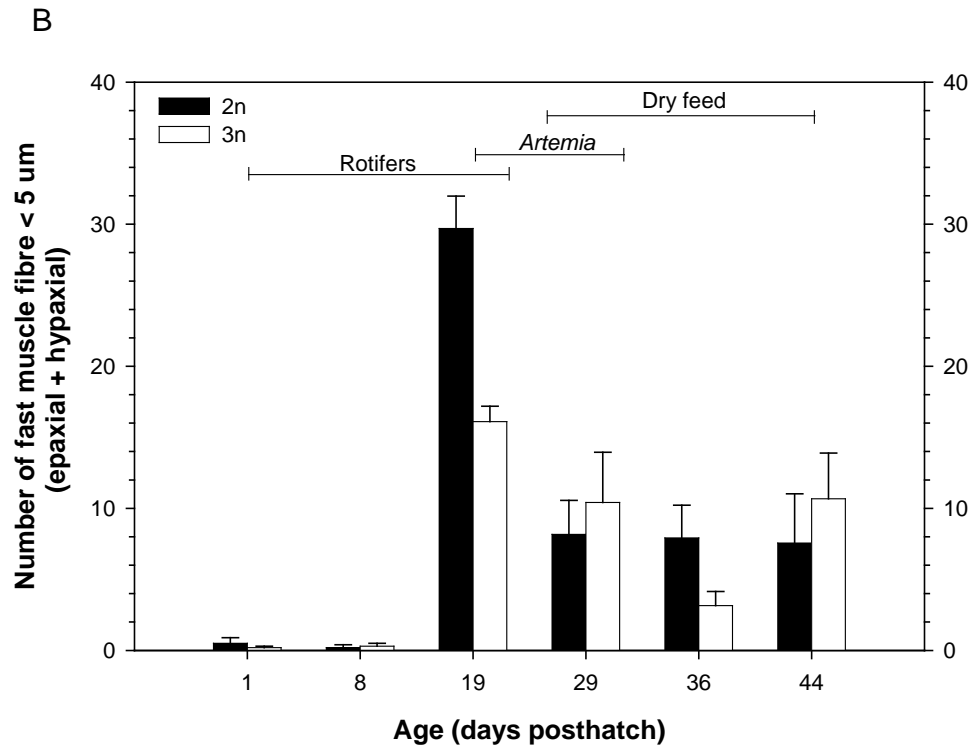


Figure 3. Mean fast fibre diameter (μm^2) (A) and number of fast fibre with diameter < 5 μm in left epaxial and hypaxial quadrants of the myotome (B) in diploid and triploid cod larvae sampled at 1, 8, 19, 29, 36 and 44 dph. Significant differences (ANCOVA, $P < 0.05$) are indicated with (*).

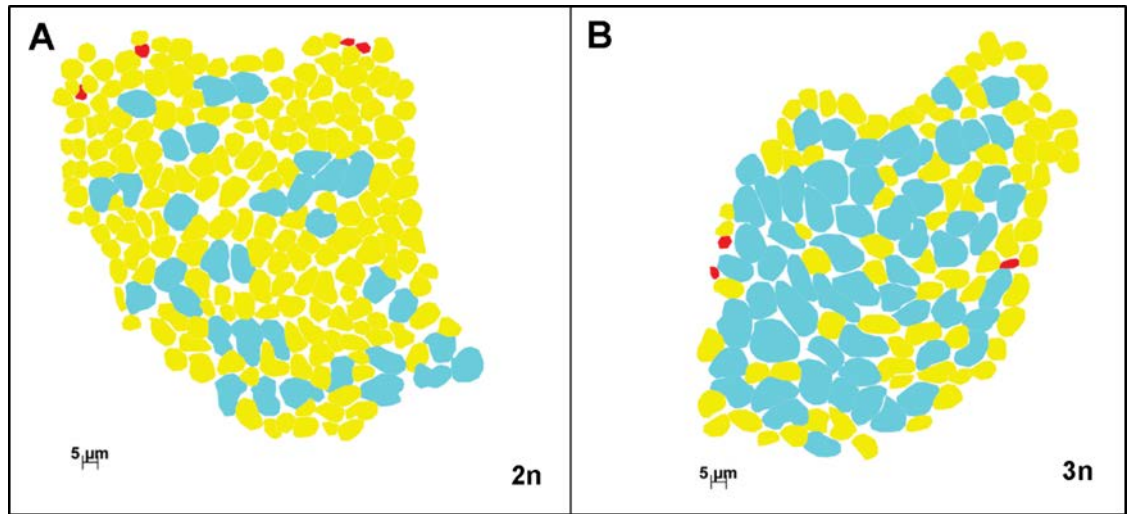


Figure 4. Fast muscle fibres sampled from the same location (left hypaxial) from one diploid (A) and one triploid (B) 19 dph larva of identical length (6.1 mm) were color coded and divided into size classes. Red = < 5 μm , yellow = 5-10 μm and cyan = > 10 μm .

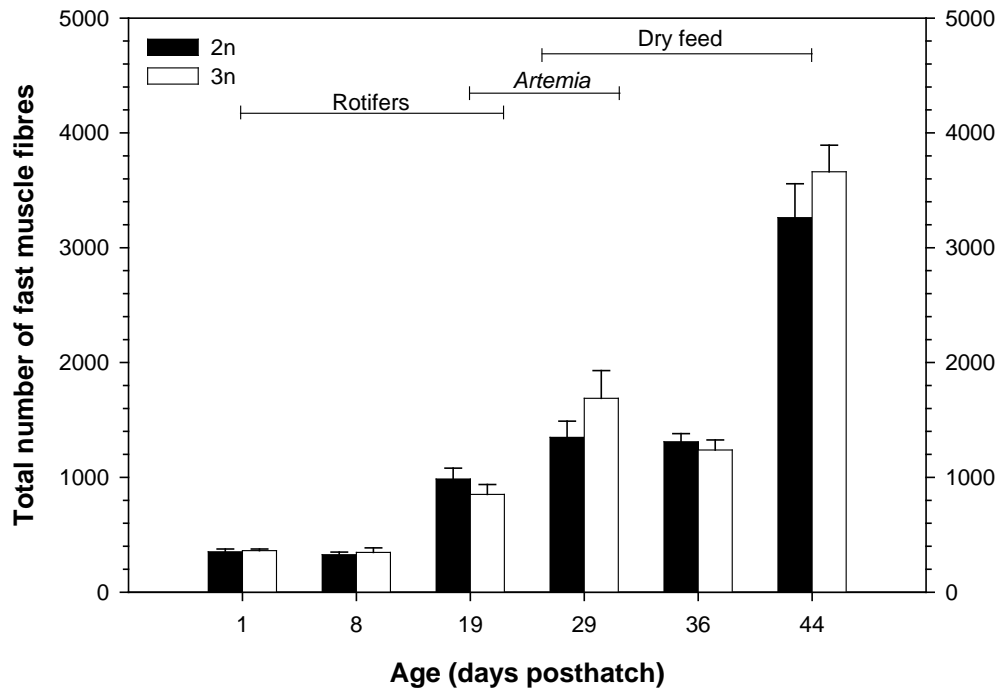


Figure 5. Total numbers of fast fibres (mean \pm SEM) in diploid and triploid cod larvae at 1, 8, 19, 29, 36 and 44 dph (n= 9 – 15 larvae per treatment per sampling point).

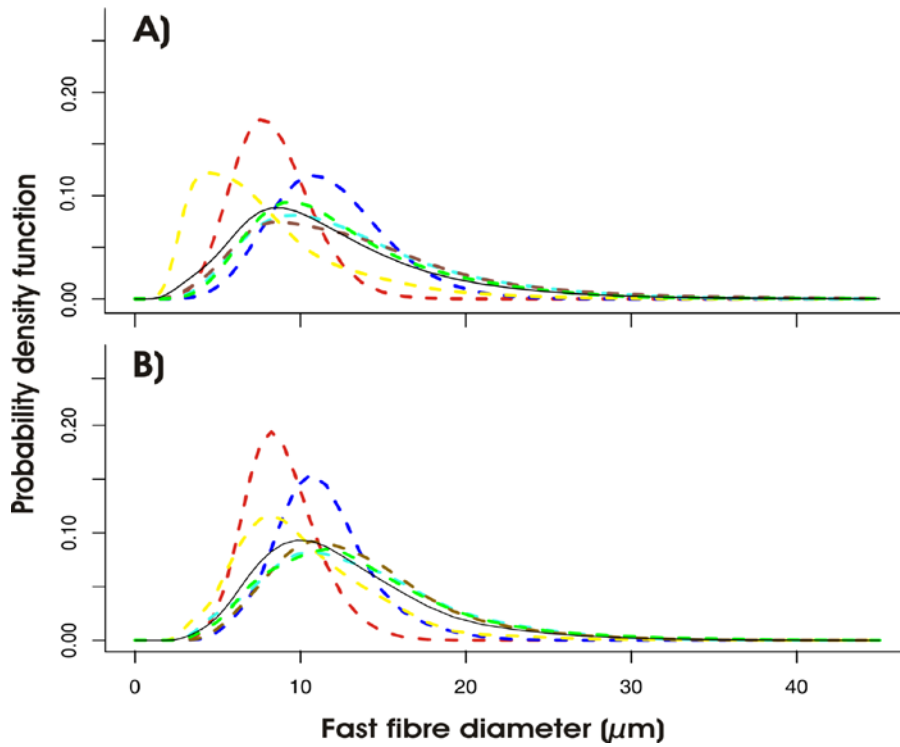


Figure 6. Non-parametric probability density functions fitted to measurements of fast fibre diameter of one average sized larva per sampling point for the diploid (A) and triploid (B) larval groups. Dotted lines of different colors indicate the age of the larva, 1 (red), 8 (blue), 19 (yellow), 29 (green), 36 (brown) and 44 dph (cyan), while the black solid line represent (1 – 44 dph).