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Family differences on triploid induction, sexual maturation and its contribution to sea cage performance of Atlantic cod, *Gadus morhua*

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ABSTRACT

Early maturation has been one of the biological bottlenecks of commercializing Atlantic cod culture. To overcome the bottleneck, production of sterile fish through triploidy and other molecular techniques have been suggested and attempted. Although studies have been carried out on triploid performance of Atlantic cod, no studies have been conducted to see the performance of triploid fish at family level. We produced 29 triploid sibling families using standard hydrostatic pressure technique of newly fertilized eggs with parallel, untreated diploid families. Larvae were reared in separate tanks using standard rearing protocols until reaching 20 g and were PIT tagged. PIT tagged juveniles were transferred to sea cages in duplicate. At 34 months post-hatch, all the fish were sampled and body weight, liver weight and gonadal weight were recorded. Results showed that significant family differences exist between diploid and triploid families in gonadal development, especially for the females. Fish from triploid families had significantly smaller gonadosomatic index than fish from diploid families, but diploid families were heavier than the triploid families. Our result highlight the need for considering a parallel strategy for triploid family selection within the conventional diploid breeding program to exploit the existing variation in triploid performance.

1. Introduction

Interest in cod farming has been increasing since early 1990's when the closing the life cycle of cod in captivity was achieved. Commercial entities in several countries in the Atlantic region including Norway, Canada, UK, Iceland and Faroe Island started investing in cod farming in late 1990's which coincided with decline in wild cod catches (Rosenlund and Halldórsson, 2007). However, with the recovery of the wild cod stocks, European economic crisis during 2008–2010 and due to the biological bottlenecks in the production cycle, cod farming has been steadily declining in Norway since 2008. While, stable production of good quality juvenile has been the bottleneck at the early life stages, early maturation at 1-2^{+} years before reaching preferred harvest weight of 3 kg and development of larger livers have been identified as the biological bottlenecks at later stages of life cycle. Production of good quality juveniles has been improved in recent years but early maturation and fatty liver syndrome are still a problem. When fish mature, they use body energy reserves and the nutritional resources for gonadal development which otherwise could have been used for somatic growth (Tveiten et al., 1996). In cod, most males mature at 1 + year and females at 2 + years (Karlsen et al., 1995). During spawning, condition of adult female fish is reduced (higher water content) and lose as much as 25% of their body weight (Fordham and Trippel, 1999). Poor post-spawning body condition and complications arise from spawning lead to increased mortality, especially in females (Hansen et al., 2016). The slow recovery in growth during post-spawning would lengthen the time required to reach desired harvest size and consequently increases the amount of feed needed for the fish to reach the market size (Hansen et al., 2016). Both reduction in body weight and the post-spawning mortality bring a huge economic loss to the

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commercial fish farmers. Thus, delaying or inhibiting early sexual maturation has become one of the highest priority in cod commercial production.

Uses of artificial lights and manipulating the feeding patterns (ration, feed nutritional profile) have been tried in both tanks and sea cages to delay the maturation with limited success (Karlsen et al., 1995, 2006a, 2006b; Yoneda and Wright, 2005; Taranger et al., 2006). Using delayed sexual maturation as a selection trait in cod breeding programs has also been suggested, but available information shows poor genetic correlation, thus leaving with very little hope (Kolstad et al., 2006; Drangsholt et al., 2014). Thus, developing technologies to produce reproductively sterile fish is becoming increasingly important in aquaculture. New technologies such as germ cell elimination are evolving; however, it is still in its infancy (Wong and Zohar, 2015). Sterility may also be achieved by the induction of polyploidy, particularly triploidy.

Triploidy is a state where the cells have three sets of chromosomes while common diploids have two sets of chromosomes (see Benfey, 1999). In aquaculture practices, triploidy can be induced using chemicals, temperature or pressure shock (See Benfey, 1999). In theory, the triploid fishes should have a suppressed gonadal development and should not reproduce as the normal diploid fishes. This may allow the triploid fishes to invest the metabolic energy and nutritional resources into somatic growth rather than directing it for the development of sexual characteristics and reproduction. Thus, triploid fish may grow faster and convert food more efficiently than diploids, especially during the age of sexual maturation. Further, due to the suppression of the gonadal development, the fish may not mature early which is a major problem in cod aquaculture. In addition, because triploids are sterile, they are incapable of reproducing, and any potential genetic impact on wild fish populations could be minimized. However, in practice, growth performance of triploid fish has shown contradicting results compared to the diploid counterparts; either higher (Leclercq et al., 2011; Oppedal et al. 2003), equal (Galbreath and Thorgaard, 1995) or lower (Withler et al., 1995) to the diploid counterparts. Further the survival of different stages (eggs, larvae, juveniles) has also been reduced (Happe et al., 1988) and showed higher vertebral deformities (Sadler et al., 2001) and poor response to stress (Hyndman et al., 2003); however, improvements have been made in the recent past (Taylor et al., 2011)

Several studies are available on the triploid production of triploid salmonid species since 1970's (Lincoln et al., 1974; Benfey, 1999, 2001) but only limited research has been done in Atlantic cod starting from last decade (Opstad et al., 2013; Otterå et al., 2016; Feindel et al., 2011; Peruzzi et al., 2007, 2013; Trippel et al., 2008, 2014). Further, while few studies available on salmonids comparing the performance of triploid and diploid sibling families (Johnson et al., 2004; Chiasson et al., 2009; Taylor et al., 2013), no studies are available on Atlantic cod. Thus, the aim of this study was to compare performance parameters of triploids with their diploid siblings to see any family trends in the growth performance and gonadal development.

2. Materials and methods

Experiments were conducted at the Centre for Marine Aquaculture Research (CMAR) in Tromsø. Eggs and sperm were stripped from 3-year-old broodstock (F2 generation) and externally fertilized using standard method to produce full sibling triploid and diploid families. Soon after fertilization, eggs were divided into two portions (3:2) and the larger portion was used for triploid induction. Eggs reserved for triploid induction were subjected to a hydrostatic pressure shock of 5.86×10^4 kPa (8500 psi) for 5 min starting at 180° minutes after fertilization (Trippel et al., 2008). Both triploid induced eggs and the untreated egg (diploid) were incubated separately in conical upwelling incubators until hatch at 4 °C with a flow rate of 1.5 L mins^{-1}. Forty-one families were produced within 10 days and the temperature during this period was very stable at 3.5 °C. After 100% hatching 39 of these full-sibling families were transferred to first feeding tanks while two families had > 99% mortality during incubation period and were discarded. Larvae were reared with the standard rearing protocol (rotifers, *Artemia* at first feeding and later weaned into dry feed) used at the NCBC (Hansen et al., 2016). Thirty-four families survived through weaning and metamorphosis and among these 34 families, 5 families did not have enough juveniles (set a minimum number of 25 fish and these 5 families had 5–12 fish) to continue the experiment to sea cages and only juveniles from 29 families were individually PIT (Passive Integrated Transponder; Sokymat, Switzerland) tagged and transferred to sea cages.

3. Sea cage rearing

PIT tagged juveniles were transferred to four sea cages (7.5 m × 7.5 m × 5 m); 2 for triploids and 2 for diploid families. All cages had 780 fish from 29 families and each family consisted of 25–33 individuals. Once a month, 30 fish from each cage were caught and weighed. Fish were fed with dry feed (Amber Neptun/ Optiline cod; Skretting AS, Stavanger, Norway) at 2% body weight 5 times a week using a feed table provided by the feed manufacturer. Once a month, 30 fish from each cage were caught and weighed and feeding was adjusted according to the average weight (this weight is not reported here because it is an average weight of all 29 families). Any dead fish was removed twice a week and the PIT tag number was recorded. At 34 month post-hatch, all the fish from the triploid and diploid cages were humanely killed and blood sample (for ploidy verification), length, whole body weight, gutted weight, liver weight, gonad weight and fish sex were recorded. From these data, hepatosomatic index (HSI = liver weight/whole body weight) and gonadosomatic index (GSI = gonad weight/whole body weight) were calculated. Percentage slaughter yield was calculated using the whole and gutted weight ([gutted weight/ whole weight] × 100). Further, all the fish were also externally examined for presence of any major skeletal deformities (lordosis, scoliosis, kyphosis, lower and upper jaw and depressed/upward head bone – star gazer). Three different categories were used to assess the deformities, none, mild and severe.

4. Ploidy verification

Ploidy was verified for each fish based on blood cell size (Hartel et al., 2005). To evaluate the occurrence of triploidy in each family, blood samples were drawn from all the presumed triploid fish sampled in February 2014 (34 months post-hatch) and 4 fish from the diploid group. Blood samples were taken from the caudal vein of newly killed fish using a syringe. Subsequently, a blood smear was prepared on a glass slide and air-dried at ambient temperature. Later the blood smears were placed under a compound microscope (Nikon 80i) at 40 × magnification and photographed using a 5 Mp camera (Micropublisher 5 RTV from QImaging) resulting in a picture with a resolution of 10.38 pixels/μm. Two none-overlapping pictures were taken from each blood smear. The pictures were automatically particle analysed using the open source image analysis program ImageJ (http://rsb.info.nih.gov/ij) with the ObjectJ plugin and a slightly modified version of the ObjectJ project "Elliptical oocytes" (https://sils.fnwi.uva.nl/bcb/objectj/). Only blood smears of good quality that resulted in at least 30 measured blood cells were accepted.

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5. Data analysis

Differences in weight and gutted weight between the diploids and triploids were tested by analysing the variance at each sampling date. Results are presented as mean ± SD. Prior to analyses, datasets were checked for normality according to the Kolmogorov–Smirnov test and for homogeneity using Levene's test. Percentages were transformed with arcsine formulas when needed. Two-way mixed model analysis of variance (ANOVA) was used to detect treatment (fixed effect) and family (random) effects on performance measurements. We have also included sex as a third factor along with family and ploidy to see the if sex impact any significant difference on the fish performance. Post-hoc tests were carried out using Bonferroni multiple comparisons. IBM SPSS software v25 was used for basic descriptive statistics and comparisons using a significance level of 5% (P = .05).

6. Results

6.1. Ploidy verification

The results from the particle analysis was used to make size frequency histograms of average red blood cell size (silhouette area) both for the pressure threated fish (805 fish) and control fish (4 fish). For the pressure treated fish, two normal distributions appeared that did not overlap. The lower distribution had full overlap with the histogram of the control fish. Blood cells from the upper distribution were on average 38% larger (area) than those from the lower distribution. Based on the results a size of 103.9 μm2 was chosen as a threshold for separating between diploid and triploid fish. Based on this, the overall triploidization rate was 65% while the rest were diploid. Among the 29 families transferred to the sea cages, eight families had 100% triploid while one family had 0% triploid and the rest of the 20 families had both triploid and diploid fish at different proportions (Fig. 1). Among the 20 mixed families, seven families had > 90% triploid fish and these families were included along with the eight 100% triploid families for further analysis on the effect of triploidization on fish performance especially on growth and sexual maturation. Among these seven mixed families, only triploid fish were included for further analysis on fish performance.

6.2. Fish performance

6.2.1. Comparison of diploid and triploid fish

There were differences among families from diploid (2 N) and triploid (3 N) on average whole body weight, gutted weight, gonadal weight, GSI, liver weight and HSI (Figs. 2, 3 and 4). When comparing the whole-body weight of the 2 N and 3 N families, fish from all 2 N families, except families 3 and 4, were significantly heavier than the corresponding triploid families (Fig. 2A). Ranking of fish performance indicators showed that fish from different families of 2 N and 3 N performed differently. While fish from families 4 and 5 were the heaviest and lightest, respectively in terms of whole body weight and gutted weight in both 2 N and 3 N, ranking of body weight and gutted weight of other families were not in the same order (Fig. 2A and B). When comparing the gutted weight, eight of the 2 N families were significantly heavier than the corresponding 3 N families (Fig. 2B). The difference in whole weight exhibited between fish from 2 N and 3 N of any given family has been reduced when visceral contents were removed, i.e. gutted weight (Fig. 2A and B). Fish from family 4 in 2 N were not only the heaviest but also had heavier gonads and higher GSI (Fig. 3A). Fish from all the 3 N families had significantly lower gonadal weight comparted to fish from corresponding 2 N families (Fig. 3A). Among 2 N families, the lowest and highest averages of gonad weight recorded were 274 g (Family 1) and 480 g (Family 4), respectively while in 3 N families, the lowest and highest averages of gonadal weight recorded were 59.5 g (Family 2) and 227.8 g (Family 12), respectively (Fig. 3A). Further, the ranking of families based on gonadal weight and GSI were different between 2 N and 3 N. Similar to gonadal weight, except fish from family 7 and 12, all families from 3 N had significantly lower GSI compared to 2 N families (Fig. 3B). Except family 3, all other families from 3 N had significantly lower liver weight compared to the corresponding 2 N families (Fig. 4A). Such differences between families of 2 N and 3 N disappeared for the HSI and only 5 families from 2 N had significantly higher HSI compared to 3 N families (Fig. 4B).

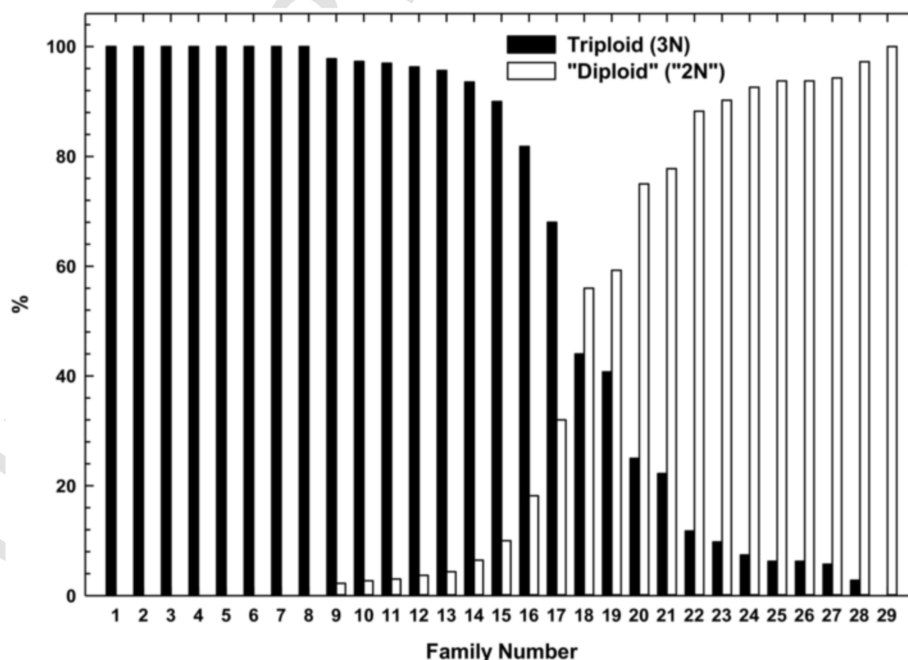

Fig. 1. Percentage of true triploid Atlantic cod (3 N) and failed triploid Atlantic cod ("2 N") among the 29 families used in the sea cage study.

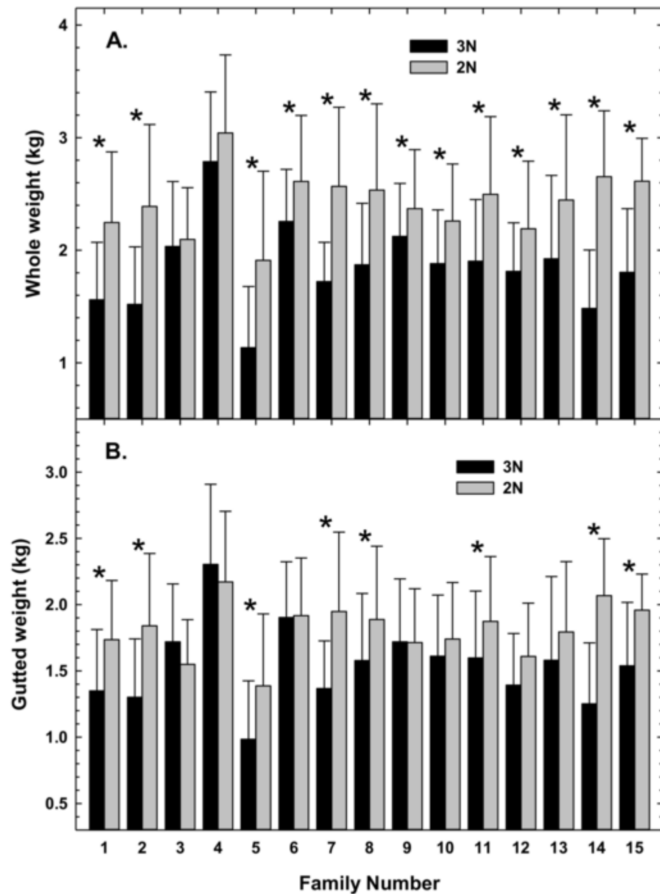

Fig. 2. The whole and gutted weight in the fifteen families of 2 N and 3 N Atlantic cod adults. The error bars represent ± SD. P-value was set at 0.05.

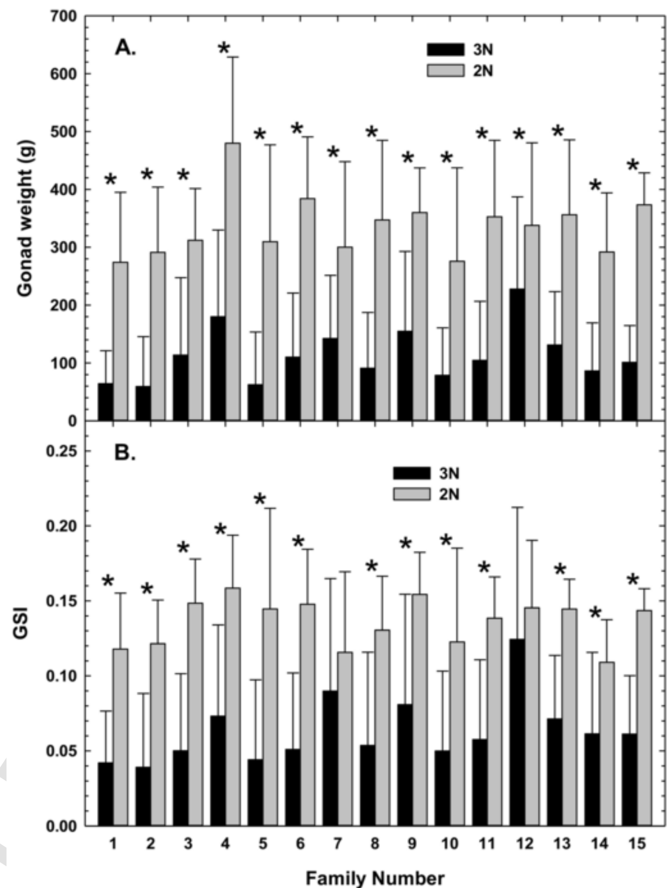

Fig. 3. The gonadal weight and the gonado-somatic index (GSI) in the fifteen families of 2 N and 3 N Atlantic cod adults. The error bars represent ± SD. P-value was set at 0.05.

In general, fish from 3 N families were significantly higher percentage ($p < .0001$) slaughter yield than the fish from 2 N families (Fig. 5). Except for fish from family 7 and 12, all 3 N families had significantly higher percentage slaughter yield than 2 N families.

Average weight of the "2 N" families (failed triploidization in families 16–29) was 2.45 kg after removing all the true 3 N from the data. The parallel 2 N families (16–29) had similar weight (2.47 kg) as the "2 N" families.

6.3. Deformities among 2N and 3N families

Fish from 3 N families had significantly higher skeletal deformities compared to fish from 2 N families ($p < .002$; Fig. 6). Overall, fish from 3 N had 35.3% skeletal deformities and fish from 2 N had 20.1% skeletal deformities. Except for fish from families 9 and 14, all 3 N families had higher skeletal deformities compared to their 2 N counterparts (Fig. 6). The major type of deformity was the 'star gazer' in which the head of the fish was somewhat either flat (level 1, mild) and had a clear upward bending/depression (level 2, severe) in both 3 N and 2 N (Fig. 7A and B). Interestingly, only 28.8% of the skeletal deformities in 3 N were severe while 54% of the skeletal deformities in 2 N were severe. Among the different types of skeletal deformities, 'star gazer' and lower jaw deformities were the dominant types in both 2 N and 3 N (Fig. 7A). Again, 74% of the 'star gazer' and 56.7% of the lower jaw deformities in 3 N were mild while 2 N had 54% and 29% mild deformities, respectively (Fig. 7B).

6.4. Comparison among 3 N families

Comparison of whole and gutted weight of the fish among the 3 N families revealed that significant family differences exist (Table 1). Triploid fish from family 4 were significantly heavier than fish from all other triploid families while fish from family 5 weighed significantly lesser than fish from most of the families (in total 9 families; Table 1; Fig. 2A and B). Comparison of gonadal weight of the fish among the triploid families revealed that significant family differences exist (Table 2). Gonads of triploid fish from family 12 weighed significantly more than fish from 9 of the triploid families while significant differences among other families were minimal (Table 2). Fish from family 2 had the lightest and smallest average gonadal weight and GSI, respectively (Fig. 3A and B). The average lowest and highest GSI were recorded in Family 2 (0.039) and 12 (0.124) among 3 N families (Fig. 3B). Comparison of liver weight and HSI of the fish among the 3 N families also revealed significant family differences exist but in only few families (Table 3). Fish from family 4 had the heaviest liver (which had the highest gutted weight) while fish from family 5 had the lightest liver (Fig. 4A).

6.5. Comparison of gonadal weight of 3 N male and female

Sex of the triploid fish had significant effect on gonadal weight, GSI, whole body weight and gutted weight ($p < .0001$ for all performance indices). Except for family 12, female fish had lower gonadal weight compared to males of the corresponding family (Fig. 8A). Further, females from 12 of the families had significantly smaller gonads

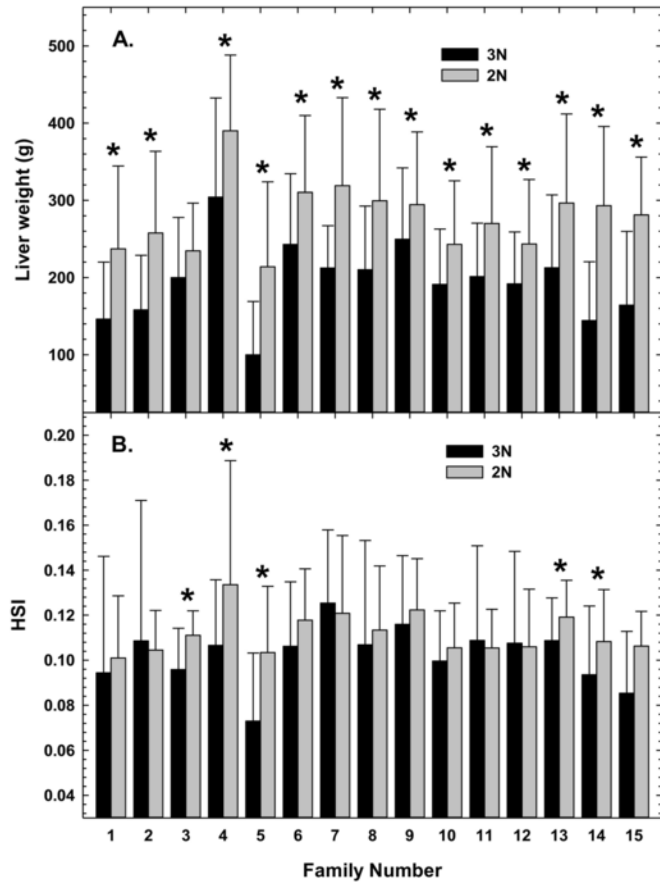

Fig. 4. The liver weight and the hepato-somatic index (HSI) in the fifteen families of 2N and 3N Atlantic cod adults. The error bars represent ±SD. P-value was set at 0.05.

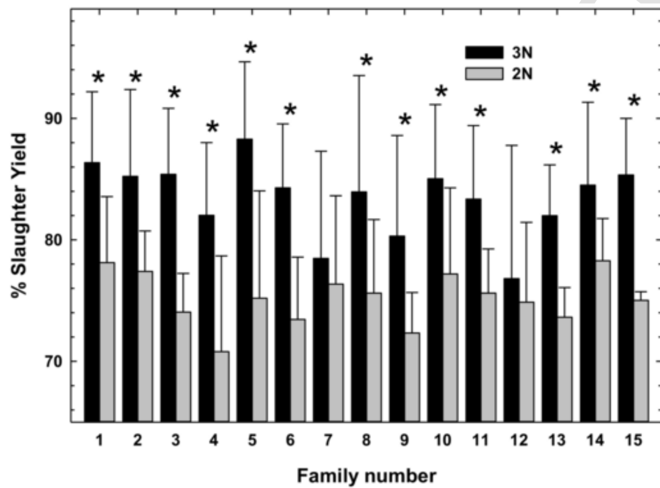

Fig. 5. Slaughter yield in the fifteen families of 2N and 3N Atlantic cod adults. The error bars represent ±SD. P-value was set at 0.05.

compared to the corresponding males (Fig. 8A). Most males in all the 15 families had matured testis (with higher GSI and/or running males) at the time of sampling while most females, except for females from family 12, had undeveloped ovaries (with lower GSI and/or smaller oocytes).

Further, ploidy*sex interaction was significant for GSI, Gonadal weight and gutted weight (p values for GSI 0.0001, Gonad weight

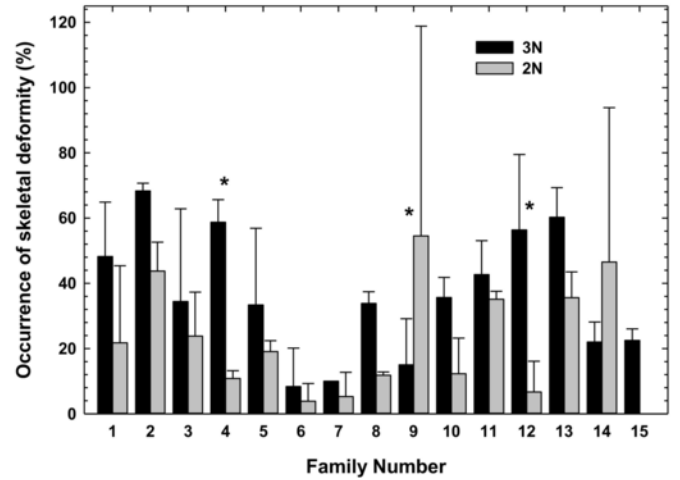

Fig. 6. Occurrence of overall skeletal deformities in 2N and 3N Atlantic cod adults. The error bars represent ±SD. P-value was set at 0.05.

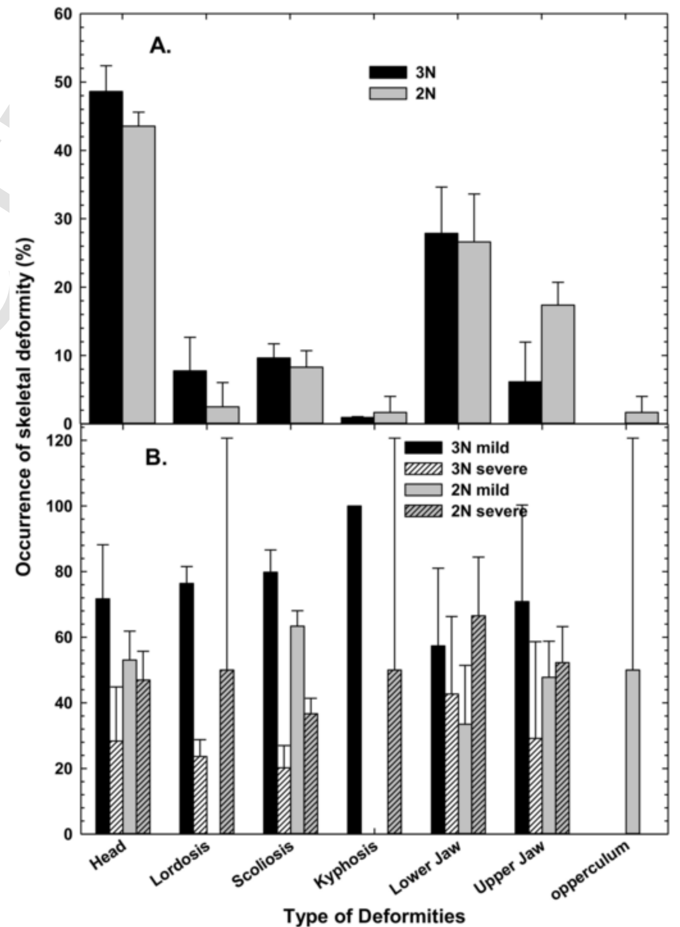

Fig. 7. Occurrence of different types (A) and the severity (B) of skeletal deformities in the fifteen families of 2N and 3N Atlantic cod adults. The error bars represent ±SD. P-value was set at 0.05.

Table 1
Results of the Tukey analysis comparing whole weight (top triangle) and gutted weight (bottom triangle) of adult triploid cod from 15 different families. * indicates significant difference between families (p < .05).

Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	---	1.000	0.186	**0.000***	0.703	**0.000***	1.000	0.765	**0.005***	0.635	0.561	0.947	0.604	1.000	0.998
2	1.000	---	0.122	**0.000***	0.857	**0.000***	0.997	0.628	**0.003***	0.486	0.418	0.873	0.465	1.000	0.991
3	0.430	0.268	---	**0.000***	**0.000***	0.966	0.849	0.999	1.000	0.999	1.000	0.983	1.000	**0.022***	0.999
4	**0.000***	**0.000***	**0.005***	---	**0.000***	**0.010***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***	**0.000***
5	0.753	0.914	**0.002***	**0.000***	---	**0.000***	0.160	**0.007***	**0.000***	**0.003***	**0.002***	**0.023***	**0.004***	0.884	0.238
6	**0.002***	**0.001***	0.986	0.089	**0.000***	---	**0.020***	0.222	0.998	0.132	0.251	0.066	0.544	0.000*	0.573
7	1.000	1.000	0.541	**0.000***	0.694	**0.005***	---	1.000	0.229	0.999	0.997	1.000	0.996	0.964	1.000
8	0.962	0.868	0.999	**0.000***	**0.036***	0.286	0.984	---	0.842	1.000	1.000	1.000	1.000	0.268	1.000
9	0.190	0.097	1.000	**0.000***	**0.000***	0.920	0.287	0.995	---	0.763	0.894	0.516	0.981	0.000	0.942
10	0.795	0.597	1.000	**0.000***	**0.008***	0.357	0.881	1.000	1.000	---	1.000	1.000	1.000	0.140	1.000
11	0.873	0.704	1.000	**0.000***	0.014	0.331	0.933	1.000	0.999	1.000	---	1.000	1.000	0.113	1.000
12	1.000	1.000	0.565	**0.000***	0.499	**0.003***	1.000	0.991	0.271	0.905	0.952	---	1.000	0.569	1.000
13	0.960	0.869	1.000	**0.000***	0.041	0.427	0.982	1.000	0.999	1.000	1.000	0.990	---	0.167	1.000
14	1.000	1.000	**0.049***	**0.000***	0.962	**0.000***	1.000	0.466	**0.005***	0.151	0.238	0.999	0.493	---	0.956
15	1.000	0.996	1.000	**0.005***	0.370	0.763	1.000	1.000	0.999	1.000	1.000	1.000	1.000	0.963	---

Table 2
Results of the Tukey analysis comparing gonadal weight (top triangle) and GSI (bottom triangle) of adult triploid cod from 15 different families. * Significant difference between families (p < .05).

Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	---	1.000	0.982	**0.040***	1.000	0.972	0.629	1.000	0.132	1.000	0.994	**0.000***	0.812	1.000	1.000
2	1.000	---	0.968	**0.034***	1.000	0.951	0.568	0.999	0.114	1.000	0.987	**0.000***	0.759	1.000	1.000
3	1.000	1.000	---	0.802	0.993	1.000	1.000	1.000	0.988	0.998	1.000	**0.039***	1.000	1.000	1.000
4	0.914	0.866	0.994	---	0.133	0.510	0.999	0.297	1.000	**0.044***	0.432	0.972	0.977	0.139	0.891
5	1.000	1.000	1.000	0.996	---	0.992	0.788	1.000	0.353	1.000	0.998	**0.001***	0.910	1.000	1.000
6	1.000	1.000	1.000	0.984	1.000	---	0.999	1.000	0.892	0.997	1.000	**0.004***	1.000	1.000	1.000
7	0.385	0.325	0.701	1.000	0.794	0.534	---	0.982	1.000	0.768	0.997	0.381	1.000	0.923	1.000
8	1.000	1.000	1.000	0.999	1.000	1.000	0.831	---	0.667	1.000	1.000	**0.002***	0.998	1.000	1.000
9	0.464	0.395	0.822	1.000	0.904	0.607	1.000	0.926	---	0.143	0.827	0.335	1.000	0.393	0.993
10	1.000	1.000	1.000	0.978	1.000	1.000	0.504	1.000	0.574	---	1.000	**0.000***	0.920	1.000	1.000
11	1.000	0.999	1.000	1.000	1.000	1.000	0.847	1.000	0.935	1.000	---	**0.003***	1.000	1.000	1.000
12	**0.000***	**0.000***	**0.002***	0.141	**0.013***	**0.000***	0.826	**0.003***	0.175	**0.000***	**0.002***	---	0.157	**0.000***	0.180
13	0.954	0.921	0.998	1.000	0.998	0.994	1.000	1.000	1.000	0.992	1.000	0.128	---	0.985	1.000
14	0.998	0.995	1.000	1.000	1.000	1.000	0.944	1.000	0.989	1.000	1.000	**0.009***	1.000	---	1.000
15	1.000	1.000	1.000	1.000	1.000	1.000	0.997	1.000	1.000	1.000	1.000	0.278	1.000	1.000	---

0.0001, gutted weight 0.009). However, the ploidy*family*sex interaction was significant only for GSI (p < .002).

6.6. Comparison of gonadal weight of 2N & 3N male and female

Ploidy had a significant effect on the gonadal weight of Atlantic cod (p < .0001). Except for family 12, male fish from 2N had significantly higher testicular weight compared to 3N males of the corresponding family (Fig. 8B). Females from all fifteen 3N families had significantly smaller ovaries compared to 2N females of the corresponding families (Fig. 8C).

7. Discussion

The induction of triploidy was not successful in all families although similar protocols were used for ploidy induction across all families (Fig. 1). Often, induction of ploidy is not 100% due to several reasons such as variation in the pressure intensity used, developmental stage of the eggs when stripped, quality of the egg and maternal effects (Diaz et al., 1993; Felip et al., 1997; Huergo and Zaniboni-Filho, 2006). Johnson et al. (2004) also reported that differences in 3N induction among different families of Chinook salmon (*Oncorhynchus tshawytscha*) using heat and pressure shock. In our experiment, triploid families were produced over a week, however even families produced

Table 3
Results of the Tukey analysis comparing liver weight (top triangle) and HSI (bottom triangle) of adult triploid cod from 15 different families. * Significant difference between families (p < .05).

Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	---	1.000	0.737	**0.000***	0.964	**0.002***	0.406	0.357	**0.000***	0.814	0.542	0.864	0.355	1.000	1.000
2	0.994	---	0.961	**0.000***	0.830	**0.025***	0.774	0.752	0.006	0.987	0.899	0.991	0.736	1.000	1.000
3	1.000	0.998	---	**0.003***	0.051	0.853	1.000	1.000	0.626	1.000	1.000	1.000	1.000	0.569	0.999
4	0.998	1.000	0.999	---	**0.000***	0.237	**0.024***	**0.007***	0.384	**0.000***	**0.001***	**0.000***	**0.018***	**0.000***	**0.002***
5	0.994	0.522	0.986	0.564	---	**0.000***	**0.014***	**0.010***	**0.000***	0.054	**0.019***	0.081	**0.010***	0.958	0.904
6	0.996	1.000	0.999	1.000	0.468	---	0.992	0.969	1.000	0.326	0.749	0.510	0.991	**0.000***	0.408
7	0.229	0.975	0.305	0.906	**0.016***	0.803	---	1.000	0.944	1.000	1.000	1.000	1.000	0.244	0.983
8	0.954	1.000	0.979	1.000	0.288	1.000	0.988	---	0.843	1.000	1.000	1.000	1.000	0.189	0.984
9	0.569	1.000	0.688	0.999	0.053	0.995	1.000	1.000	---	0.114	0.446	0.248	0.933	**0.000***	0.249
10	1.000	1.000	1.000	1.000	0.866	1.000	0.345	0.995	0.750	---	1.000	1.000	1.000	0.627	1.000
11	0.979	1.000	0.993	1.000	0.348	1.000	0.942	1.000	1.000	0.999	---	1.000	1.000	0.326	0.998
12	0.994	1.000	0.998	1.000	0.482	1.000	0.926	1.000	1.000	1.000	1.000	---	1.000	0.726	1.000
13	0.991	1.000	0.997	1.000	0.464	1.000	0.968	1.000	1.000	1.000	1.000	1.000	---	0.198	0.979
14	1.000	0.981	1.000	0.990	0.994	0.980	0.111	0.877	0.319	1.000	0.930	0.978	0.970	---	1.000
15	1.000	0.947	1.000	0.966	1.000	0.955	0.221	0.853	0.519	0.999	0.904	0.947	0.934	1.000	---

in the same day showed huge variation in triploidization (ranging from 4 to 100%). Further, the temperature was very stable during the egg pressure treatment. The average fertilization success for 3 N was 81.5% (range: 68.4–92.5%) and for 2 N was 82.9% (Range: 65.8–95.1%) which were not different between two ploidies. However, normal cleavage for 3 N was 60% (Range: 41.1–73%) and for 2 N was 71.4% (Range: 60.5–85.4%) which subsequently affected the average embryonic mortality was higher for 3 N (71%) while 2 N had lower egg mortality (43.6%). Thus, triploidization procedures did not affect the fertilization rates but it seems to have affected the further development of the embryo. Individual variation among egg batches has been shown to affect the egg quality and fertilization rates in fish (Brooks et al., 1997). Each family used in this experiment was produced using different females, thus effects of individual female differences in gamete quality cannot be ruled out (Brooks et al., 1997).

Significant family differences existed among the 3 N families on the final slaughter weight, gutted weight, gonadal weight, GSI and HIS (Tables 1, 2 and 3). Similar differences on slaughter weight among 3 N families in Atlantic salmon, *Salmo salar* (Friars et al., 2001) and, Chinook salmon (Johnson et al., 2004) have also been reported. As in our study (Fig. 2A and B), other studies have also found that variability in growth performance is consistently higher among triploid families compared to their diploid counterparts (Friars et al., 2001; Johnson et al., 2004). Xu et al. (2013) have suggested that triploidization can be an additional source of variability along with genetic diversity within 2 N. Such significant differences among 3 N families in important traits indicate that it is possible to do a separate and parallel selection along with 2 N selective breeding programs. This would allow selecting diploid bloodstock that are optimal for triploid production based on the performance of their triploid sib-relatives. However, in a typical breeding program, this requires performance recording from triploid sib-relatives from large number of families to predict average family breeding values for the diploids to select as parents.

In our experiment, fish from triploid families weighed significantly less compared to fish from corresponding diploid families (Fig. 2). Similar results have also been reported from various experiments examining the growth performance of triploid and diploid fish (Derayat et al., 2013). Triploid fish are expected to show a better growth performance, especially when diploid counterparts become sexually mature. During sexual maturation, energy reserves are used for gonadal development which otherwise diverted to somatic growth (Piferrer et al., 2009). Nevertheless, female triploid fish had significantly smaller ovary compared to female diploid fish in our experiment. Such advantage of having small gonads did not translate to higher growth in triploid fish in the third year of their life. The 2 N fish have invested large amount of resources into gonadal development in the third year compared to 3 N fish. Trippel et al. (2014) showed that the 3 N fish that were reared for more than four years had similar or even higher body weight than the 2 N fish, especially soon after every spawning period. Because of the low investment on gonadal development by 3 N fish, they would have outperformed or at least catch up with 2 N growth in the following year (Feindel et al., 2011).

Although fish from 3 N families had significantly lower weight compared to the fish from 2 N families, 3 N fish had significantly higher percentage slaughter yield (Fig. 5). This shows that the percentage slaughter weight of fish could be used as selection criteria in calculating breeding values for selecting 2 N parents for the production of 3 N siblings. Similar higher percentage slaughter yield was also reported in rainbow trout (Muller-Belecke et al., 2006) and higher carcass weight at slaughter in 3 N Channel catfish (Chrisman et al., 1983).

Triploid cod have significantly smaller gut length and fewer number of pyloric caeca compared to the diploid fish (Peruzzi et al., 2013) which may result in lower efficiency in digestion and/or absorption of nutrients. Further, it should be noted that our fish were sampled when they were 30 months old and the diploid fish were going through their first sexual maturation. The potential of having undeveloped smaller gonads in triploid fish at 30 months post hatch could have been realized in growth in the fourth year of the life as shown other two studies involving Atlantic cod (Feindel et al., 2011; Trippel et al., 2014). It is also shown that triploid fish have inferior behavioural traits such as lower competitive abilities, which could also have affected the performance of triploids when reared together with diploids counterparts. O'Flynn et al. (1997) in their study with Atlantic salmon recommended separate rearing of the 2 N and 3 N mainly because 2 N fish were aggressive and more competitive for food and space. Considering this phenomenon, in our experiment, we did raise the 2 N and 3 N sepa-

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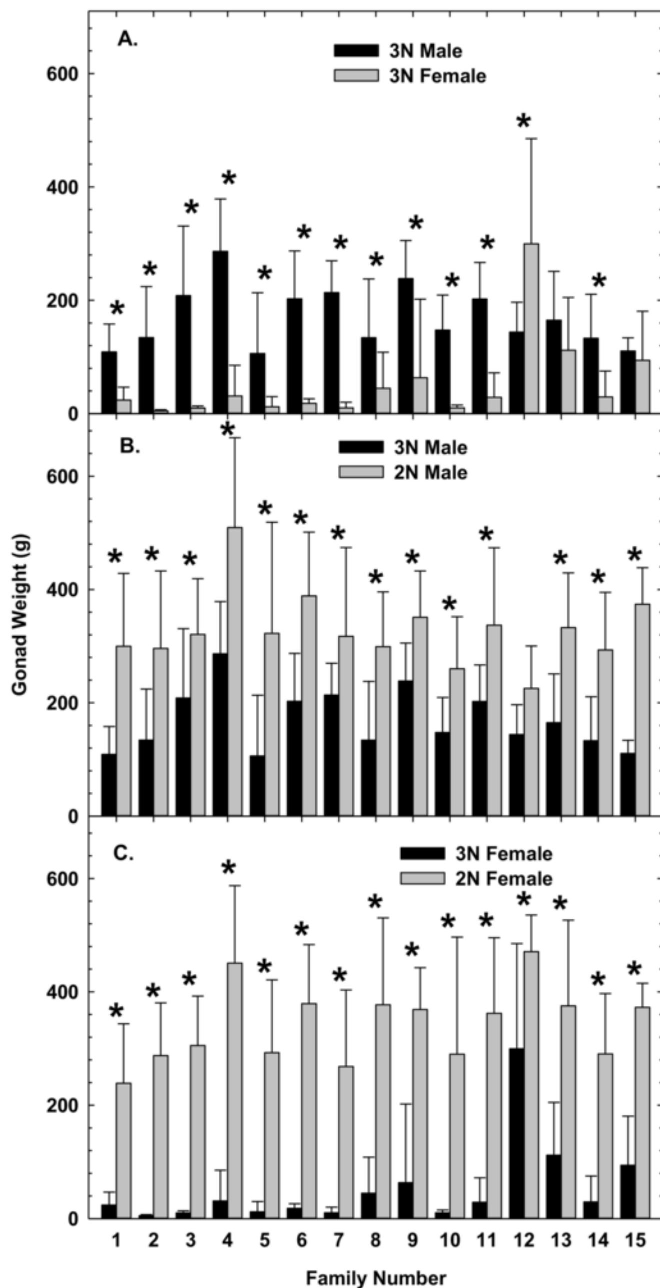

Fig. 8. Comparison of the gonadal weight of (A) male and female 3 N; (B) 2 N and 3 N male and (C) 2 N and 3 N female Atlantic cod. The error bars represent ± SD. P-value was set at 0.05.

rately, however due to the differences in triploidy induction (Fig. 1) we ended up with a mix group of '2 N' (families 16–29) and 3 N (families 1–15) in our triploid only cages. 2 N and 3 N fish may be one species, but are with different biologies. Thus, taking together morphological (especially the digestive system), and behavioural shortcomings of triploid fish, 3 N fish should not only be reared separately but also treated as a separate 'species' with different feed and environmental conditions than 2 N fish.

Several studies have shown that triploidy induction results in increased skeletal deformities in Atlantic cod (Opstad et al., 2013; Otterå et al., 2016), in Atlantic salmon (Taylor et al., 2011, 2013). As discussed earlier, 3 N fish have different gut morphology and all these studies have used diets formulated for diploid fish on the assumption that triploids have the same dietary requirements as their diploid counterparts. However, use of higher levels of phosphorus in the triploid diet reduced the skeletal deformities in Atlantic salmon and this confirms that triploids have different nutritional requirements than diploids (Feindel et al., 2011). In our study, we also used the same diet for 2 N and 3 N and this may have affected not only the deformity but also growth. Interestingly in our study, most of the skeletal deformities of 3 N fish were very mild but fish from 2 N had higher proportion of severe skeletal deformities. Further, Benfey (2001) and Sadler et al. (2001) reported a higher incidence of lower jaw deformities in triploid Atlantic salmon compared to diploids. In our study, 3 N fish not only had lower incidence of lower jaw deformities but these lower jaw deformities were also less severe (43 vs 71%) compared to 2 N families.

Although the triploid fish were smaller in our experiment, smaller gonads and lower GSI in females are very promising for cod aquaculture because this will potentially reduce mortalities in females due to post-spawn egg-bound problems (Hansen et al., 2016). Cod is a batch spawner and releases several batches of smaller eggs during a spawning season and these tiny eggs can easily pass through the sea cage nets and could create a genetic interaction with the wild eggs (Jørstad et al., 2008). In salmon, only the escaped fish could interact with the wild fish but in cultured matured cod kept in sea cages can release eggs and the eggs can disperse outside the sea cage due to their smaller size. Although the severity of this genetic interaction is not well understood (Varne et al., 2015), but precautions should be taken to minimize it. Studies have also showed that the aneuploid spermatozoa of triploid males can be considered functionally sterile because 100% mortality of triploid-sired embryos occurs during embryonic or larval stages of development (Peruzzi et al., 2009; Piferrer et al., 2009). Thus, smaller undeveloped ovaries as shown in our study and functionally sterile spermatozoa of triploid females and males, respectively, are positive outcome of triploidization in Atlantic cod and could be a solution to minimize the 'genetic interaction'.

Triploidy induction suppresses gonadal development in many fish species (Piferrer et al., 2009) and this could potentially solve the problems associated with pre-harvest sexual maturation in Atlantic cod. In our study, suppressed gonadal development was more evident in females than in males (Fig. 8). It was shown by the significant ploidy*sex interaction where sex had a significant effect on the performance indices (GSI, gonad weight, gutted weight) of 2 N and 3 N. Several other studies have also shown that the effect of triploidy induction on gonadal suppression tends to be greater for females than males (Benfey, 1999; Piferrer et al., 2009). Although studies have shown that male sperm could be functionally sterile (Peruzzi et al., 2009), testicular development in males is not suppressed. Due to these differences in gonadal development between males and females, male triploid fish were less heavy than triploid female counterparts. Production of all female triploid could be solution to this but so far, use of all female triploid did not provide greater growth performance compared to all female diploids (Cotter et al., 2002).

In general, our results showed that growth performance of triploidization in Atlantic cod did not improve the growth at slaughter compared to the diploid counterparts. Further, there are several problems with regard to the performance of triploid fish in aquaculture, which can include a reduced ability to withstand chronic stress and an increased incidence of deformities. However, our results showed that differences exist in many important traits (such as weight, gonadal development) among triploid cod families and in between corresponding diploid families. Considering the differences in performances between diploid and triploid families, it would be of great interest to select diploid broodstock that are optimal for triploid production based on the performance of their triploid offspring. Thus, maximising the performance of triploid fish requires a long-term commitment to selective breeding based on triploid production characteristics and a clearer understanding of their unique biology.

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Piferrer et al., 2000

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