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Competitive Spawning of Male Triploid Atlantic Cod (*Gadus morhua*) and the Early Life History Performance of their Offspring

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Abstract

Farmed Atlantic cod (*Gadus morhua*) escaping or spawning directly in sea cages poses a continuous risk to the natural environment, similar to that recognized for the salmon aquaculture industry. Cod culture is in its infancy, providing the industry and scientific community the opportunity to develop cod culture in an environmentally sound and profitable manner. Preliminary research has shown that triploid females do not produce hydrated oocytes, while male triploids undergo spermatogenesis. Although this precludes the opportunity for within-cage mating of triploid females and males, it does pose a possible problem if male triploids were to escape. The purpose of this study was to examine if male triploid Atlantic cod are capable of outcompeting male diploids for spawning access to female partners, and to evaluate the viability of their offspring. Fertilization rates, daily embryonic survival, hatch rates and daily, unfed larval survival were compared by manually fertilizing eggs with sperm from males of each ploidy. Data were collected from ten replicate trios, with each trio comprised of a male of each ploidy and a diploid female. No significant difference was found between fertilization rates using milt stripped from triploids and diploids. A significant difference was found for hatch rates and survival, with offspring from diploid males being superior to those of triploids. Trios of fish were also placed in eight tanks and permitted to undergo spawning to determine whether a triploid male was able to gain access to a female in a competition setting with a diploid male. Egg batches were collected from tanks and microsatellite DNA makers used to determine proportion of embryos sired by each male.

Introduction:

Atlantic cod (*Gadus morhua*) is a candidate species for diversification of marine finfish aquaculture in North Atlantic countries which have historically focused on Atlantic salmon (*Salmo salar*) farming. Preliminary trials have identified sexual maturation of farmed cod as an issue which needs to be addressed if full scale commercial cod culture is to take place. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has listed certain cod stocks in the North Atlantic as endangered. Cod spawning within sea cages or their escape from sea cages and spawning in the wild could have negative impacts on wild cod stocks (Bekkevold et al., 2006; Jorstad et al., 2008). The development of cod aquaculture can benefit from parallel concerns with Atlantic salmon escapees, which have been documented to spawn with wild salmon (Webb et al., 1991) resulting in the genetic introgression of farmed traits into wild gene pools (Crozier, 1992) and a lowered overall fitness of wild stocks (Utter, 1998). Youngson (2001) discussed the potential effects of farmed salmon on wild salmon populations, which are similar to those for gadoids (Bekkevold et al., 2006). It has been shown that cod are more prone to escape from sea cages than salmon as a result of marine accidents, holes in nets and damage to sea cages due to storms (Moe et al., 2007). Cod will also bite at holes in nets to enlarge them to facilitate their escape (Moe et al., 2009) and unlike salmon, cod will spawn directly in sea cages, resulting in embryos being released directly into the natural environment (Jorstad et al., 2008). Pre-harvest sexual maturation is also of direct concern to farmers, as mature fish have lower fillet quality and, hence, lower economic value (Trippel et al, 2008).

Triploidy induction suppresses gonadal development in many fish species (Piferrer et al., 2009) and could potentially solve the problems of sexual maturation in Atlantic cod. Theoretically, triploidy leads to higher growth rates due to more energy being directed towards somatic growth rather than gonadal development (Piferrer et al., 2009). This diversion of energy leads to certain species of triploids outperforming their diploid counterparts and vice versa (Benfey 1999; Piferrer et al., 2009). The effect of triploidy induction on gonadal suppression tends to be greater for females than for males, and in a number of species such as Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*), triploid males produce functional sperm (Benfey and Sutterlin, 1984; Benfey et al., 1986; Benfey 1999; Piferrer et al., 2009). This is the case for cod, where previous research has shown that triploid males develop to a spawning state and produce functional sperm (Trippel et al., 2008; Peruzzi et al., 2009). However, any offspring sired by triploid males will likely be aneuploid and will therefore not survive past embryonic or larval stages (Benfey 1999; Peruzzi et al., 2009). Triploidy induction can thus act as a control for the genetic introgression of cultured genes into wild gene pools and also potentially solve the problem of pre-harvest sexual maturation with regard to females.

This paper assesses the spawning capacity of male triploid Atlantic cod in an *in-vivo* competitive spawning experiment as well as in a number of *in-vitro* experiments. The objectives of this research were to determine whether triploid males cod can compete with diploid males for

spawning access to diploid females, and to conduct an *in-vitro* analysis of fertilization success, sperm morphology and sperm motility.

Materials and Methods:

Animals

Atlantic cod used in these experiments were maintained at the St. Andrews Biological Station (Fisheries and Oceans, St. Andrews, NB) and were offspring of wild broodstock captured from the Bay of Fundy. Following fertilization, eggs were divided into two groups: one used to produce triploids and the other retained as sibling diploid controls. Triploids were created in 2005 using hydrostatic pressure (5 min at 58,600 kPa, beginning 180°C min post-fertilization; for details see Trippel et al., (2008). Mixed-ploidy populations of these fish were maintained in two 7000 L circular tanks and fed a Marine Growers diet produced by EWOS Canada Limited (Surrey, BC). Different wild broodstock, also from the Bay of Fundy, were used to create the diploid populations from which females were obtained for the spawning experiments described below. These fish were maintained in a separate tank, but treated in the same manner in relation to husbandry practices.

Ploidy Verification

All triploids and diploids used in the current experiments were verified as being of the correct ploidy by flow-cytometric measurement of erythrocyte DNA content. Five 5 µl of whole blood were added to 1 mL of propidium iodide (50 mg/L in 0.1% sodium citrate), mixed on a vortex mixer and refrigerated overnight. The following day, the samples were again mixed, 100 µl of dimethylsulfoxide was added, they were mixed again, and then frozen until later analysis at the Dr. Everett Chalmers Hospital (Fredericton, NB). Similarly prepared samples from a single brook charr (*Salvelinus fontinalis*) were used as a standard.

***In-vivo* Competitive Spawning Experiment**

Six circular tanks were used for the experiment (1.83 m diameter and 1.17 m deep). Each tank was equipped with two egg collectors, one on the surface of the water and the other over the drain. The surface collector was a floating apparatus with the front box shaped and funnelling down to a pipe that lead to another box where the eggs were collected and removed for sampling (Thorsen et al., 2003). The drain collector was a mesh shaped tube which was placed over the drain but still enabled water to run through it and into the outflow pipe. Any eggs that exited the tank through the drain were captured by the drain collector.

The experiment was conducted from January to April 2008, with water temperatures maintained between 5 °C and 8 °C. The 18 experimental fish were transferred from their holding tanks into the 6 experimental tanks on January 24, 2008. One diploid female, 1 triploid male and 1 diploid male were transferred to each of the experimental tanks to create a trio of fish per tank. Upon

being placed in a tank, each dam/sire was fin-clipped and the piece of fin preserved in 95 % ethanol. Males were brothers and were paired to be matched by weight (± 240 g), length (± 2.1 cm) and Fulton`s condition factor (K), where $K= (W/L^3) \times 100$, with a difference no greater than ± 0.1 between males to avoid any size/shape factors affecting dominance. Males within a trio came from different holding tanks to avoid prior exposure to each other. Females were from a separate family to avoid inbreeding and were paired with males within ± 13 % of their body length (Rakitin et al., 2001)(Refer to Table 1 for trios). Once all the trios were placed in their tanks, the egg collectors were monitored twice daily for the occurrence of spawning. Once spawning had occurred, three samples of eggs were then taken from the surface collector, the numbers of fertilized, unfertilized and dead eggs was counted, and the fertilization rate for that particular spawning was determined. Fertilized eggs were then incubated for 3 days to allow for sufficient embryonic genetic material for DNA microsatellite analysis. The fin-clips collected from each parent at the initiation of the experiment were used for identification of unique microsatellite markers and the microsatellites of the sampled embryos compared to them to determine which male was the sire.

Data were analyzed using Minitab Statistical Software (LEAD Technologies, Inc., Version. 15). Data were represented by percentage of eggs fertilized by diploid or triploid male for individual tanks. The eggs from each spawning in an individual tank were pooled for that tank. A paired t-test was then performed on the percentages of eggs fertilized by the diploid and the triploid. A log-likelihood goodness of fit G test was used to determine whether there was a significant difference between the proportions of eggs sired by triploid or diploid male (Table 2). Yates correction was used where no larvae were sired by one of the males within a tank (Sokal and Rohlf, 1995). To determine whether the proportion of eggs sired by triploids and diploids was homogenous between batches within a tank, a heterogeneity G test was applied to tanks with more than one batch produced over the duration of the spawning experiment (Table 2).

Tank	Sex	Treatment	Length (cm)	Weight (g)	CF
1	M	2n	47.5	1550	1.45
	M	3n	48.3	1545	1.37
	F	2n	47.5	1830	1.71
2	M	2n	44.5	1190	1.35
	M	3n	46	1235	1.27
	F	2n	47.2	1650	1.57
3	M	2n	48.8	1405	1.21
	M	3n	49	1395	1.19
	F	2n	48.5	1455	1.27
4	M	2n	47.8	1720	1.57
	M	3n	48.2	1685	1.5
	F	2n	59.3	1890	0.91
5	M	2n	55.2	2285	1.36
	M	3n	53.1	2045	1.37
	F	2n	52.3	2045	1.43
6	M	2n	51	1570	1.18
	M	3n	51	1690	1.27
	F	2n	53.6	2680	1.74

Table 1. Trios of cod for *in-vivo* competitive spawning experiment. Trios made, based on length, weight and condition factor. Diploid (2n) and triploid (3n) males were siblings. Females are of a separate family.

In-vitro Fertilization/Hatch Rates/Larval Survival

To compare fertilization rates, gametes were stripped from diploid females and diploid and triploid males. To do this, the fish were first anaesthetized using tricaine methanesulfonate (Aqualife TMS, Syndel Laboratories Ltd.). The external genital region was wiped dry and pressure was applied to the abdominal region enabling collection of expressed milt and oocytes. The number of eggs per mL was calculated volumetrically. This enabled the tabulation of the volume of eggs needed for each replicate from an individual female. The eggs were then placed in a 6 °C cold room while milt from males was collected to perform the *in-vitro* fertilization. Once the milt was collected, three samples from each male were centrifuged to determine average spermatocrit (i.e., density of sperm per mL). The amount of milt needed for each replicate was determined using a technique developed by Rakitin et al. (1999). The eggs from one female were then fertilized using the milt from one diploid and one triploid male. Fertilizations were replicated 5 times for each male with an individual female, using 10 different females and 20 different males (10 diploids and 10 triploids), resulting in ten trios (1 diploid male, 1 triploid male, 1 diploid female). A ratio of 100,000 sperm to 1 egg was used for the fertilization trial (Butts et al., 2009). Approximately 300 eggs and 30,000,000 sperm were used for one replicate. The eggs were incubated in 250 mL Pyrex beakers for 12-24 hours, after which the numbers of fertilized, unfertilized and dead eggs were counted. From these counts, fertilization rates were determined for each ploidy. Dead and unfertilized eggs were removed and the fertilized embryos maintained in the beakers to develop. Embryos were followed daily until

they either died or hatched, enabling the estimation of hatch rates. On the day of peak hatch, the larvae were counted, placed in a beaker and followed daily until mortality had occurred. This enabled the comparison of larval survivorship of larvae sired by triploids compared to diploids (time to 100 % mortality).

Comparisons between diploids and triploids were made using a GLM Randomized Block ANOVA design (Minitab). Residuals were plotted to test for normality and standard deviations were compared to assess homogeneity of variances. Larval survival was compared between diploids and triploids using an ANCOVA (Minitab). This was due to unequal densities being placed in the beakers and the triploids having an overall lower number of larvae available to be utilized on peak hatch days. Regression analysis was performed on the larval densities to determine whether density affected larval survivorships. All larvae were pooled and the arithmetic means were estimated to represent the average day to 100 % mortality for diploid and triploid sired offspring, as outlined by Probst et al. (2006).

Sperm Morphology

Milt samples from both diploid (n = 7) and triploid (n = 7) cod were obtained by applying slight pressure on the abdomen, and collecting semen into 50 ml beakers. The initial male ejaculate was discarded, to ensure no blood, urine or feces contamination. Immediately after collection milt samples were covered with parafilm, kept on ice, and then transferred to a 6 °C fridge. For each male, 10 µl of milt was then added to 1 mL of a 1:1 mixture of seawater and 3% sodium citrate (Remel, USA). The solution was then mixed for 30 s to ensure homogenization. A 5 µL aliquot of the milt mixture was smeared over the length of a labelled frosted microscope slide. Slides were allowed to dry for approximately 10 s after smearing and then stained using Hemacolor® (EMD Chemicals, Inc., Gibbstown, NJ; see Tuset et al., 2008 for details). Slides were allowed to air dry and then permanently sealed with Eukitt mounting medium (Kindler & Co., Freiberg, Germany), and topped with a cover slip (22 mm x 40 mm).

For morphological analyses, images of the sperm heads were recorded using Image Pro Plus imaging software (Media Cybernetics, Inc., USA), a Leica DMLB microscope (Leica, Tokyo, Japan) and a 100 × oil immersion objective. Once images were obtained, they were converted to an 8-bit gray scale to facilitate the measurements of sperm morphological parameters through ImageJ Version.1.41o (National Institute of Health, USA) analysis software. A macro plugin (developed by R.M. Rideout and modified by I.A.E. Butts) was created in ImageJ with set parameters to obtain area (μm^2), perimeter (µm), length (µm) and width (µm) measurements of each sperm cell. Shape indices such as elongation [(L –W)/(L+W)] and elipticity (L/W) were also calculated. Measurements of 100 sperm cells were collected for each male. For each parameter measured, a nested ANOVA was performed to test for significant differences between diploids and triploids using Minitab. To test for ANOVA assumptions, residuals of the data for each parameter were plotted and assessed for normality and Cochran's Test was used to confirm

homogeneous variances. All data met the criteria for normality but only the log-transformed perimeter data had homogeneous variance.

Sperm Motility

Thirteen cod of each ploidy were used to assess the track velocity (VCL) of triploid and diploid sperm. Milt was collected in the same manner as described above. One sample from an individual fish was collected and its motility data collected before the next sample was collected from another fish. Sperm motility was assessed using a two-step activation procedure developed by Rouxel et al. (2008). Diluted, activated sperm was placed in a 10-well multitest glass slide (MP Biomedicals, Irvine, California, USA) and covered with a glass coverslip. Sperm motility was then assessed at 20 and 40 s post-activation using ISAS (Proiser R+D SL, Bunol, Spain) image analysis software. An ISAS compound microscope with a 40 × negative phase objective and a Basler A312Fc camera (Basler Vision Technologies, Germany) were used to capture motile sperm at the specified post-activation times. Frames were then manually inspected to ensure that uninterrupted tracks of sperm were analyzed with no interference due to foreign particles or sperm crossing paths resulting in altered tracks which would result in false calculations of sperm VCLs. Data analysis was carried out using Minitab Statistical Software. A nested ANOVA was applied to test whether there was a significant difference in VCL swimming speeds between triploid and diploid sperm at 20 and 40 s post-activation. Data were normally distributed at both times but only homogeneous for the 20 second post-activation time. All data were therefore pooled by ploidy and a two sample t-test was conducted on the 40 s post-activation data, producing the same results as the nested ANOVA.

Results

In-vivo Competitive Spawning Experiment

Overall, no significant difference was found between the ability of diploids and triploids to sire batches of eggs produced by a diploid female in a one-on-one (diploid male vs. triploid male) competitive setting ($p > 0.05$). However, log-likelihood goodness of fit G tests performed on individual batches within tanks revealed significant differences in proportions of larvae sired for some batches but not others (Table 2). Heterogeneity G tests also revealed significant differences in the proportion of larvae sired by particular fish within a tank over numerous batches in some, but not all tanks (Table 2).

Tank	Batch	2n	3n	G	G_H
1	Feb 1/08	24	0	28.41***	0
1	Feb 11/08	19	0	21.71***	
2	Feb 19/08	24	1	23.31***	n/a
3	Mar 2/08	2	11	5.29*	73.47***
3	Mar 6/08	0	21	24.39***	
3	Mar 12/08	0	6	4.88*	
3	Mar 18/08	23	9	27.07***	
4	Feb 21/08	0	14	24.39***	n/a
5	Feb 27/08	0	14	15.09***	10.68
5	Mar 6/08	0	11	11.18***	
5	Mar 10/08	5	30	18.08***	
5	Mar 18/08	0	11	11.18***	
5	Mar 24/08	0	11	11.18***	
5	Mar 30/08	0	15	16.41***	
6	Mar 9/08	9	22	4.77*	7.68**
6	Mar 12/08	0	14	15.09***	

Table 2. Number of embryos sired by 2n and 3n fish sampled from a specific spawning event. 16 spawning events in total were analyzed with some tanks having one or more events in total. G is the log-likelihood goodness of fit G test to assess whether there was a significant difference in proportion of larvae sired within a batch. G_H is the heterogeneity G test used to assess whether batches within a tank were homogeneous in the proportion of larvae sired among all the batches in that specific tank. Significance levels: *p<0.05, **p<0.01, ***p<0.001

In-vitro Fertilization/Hatch Rates/Larval Survival

Analysis of the 10 *in-vitro* fertilization trials revealed no significant difference in fertilization rate between diploid (44.1 % ± 9.7) and triploid males (38.9 % ± 9.3) (Fig. 1). There was, however, a significant difference in hatch rates of their offspring (43.1 % ± 5.6 and 19.5 % ± 4.0, respectively) (Fig. 2). Significant differences were also found for larval survival time to 100 % mortality (13.0 ± 1.4 and 5.0 ± 1.4 days, respectively). Overall survivorship performance of triploid sired larvae was inferior to that of their diploid counterparts (Fig. 3).

Figure. 1. Fertilization success for diploids (2n) and triploids (3n) (± SE) for the ten replicated *in-vitro* fertilization trials.

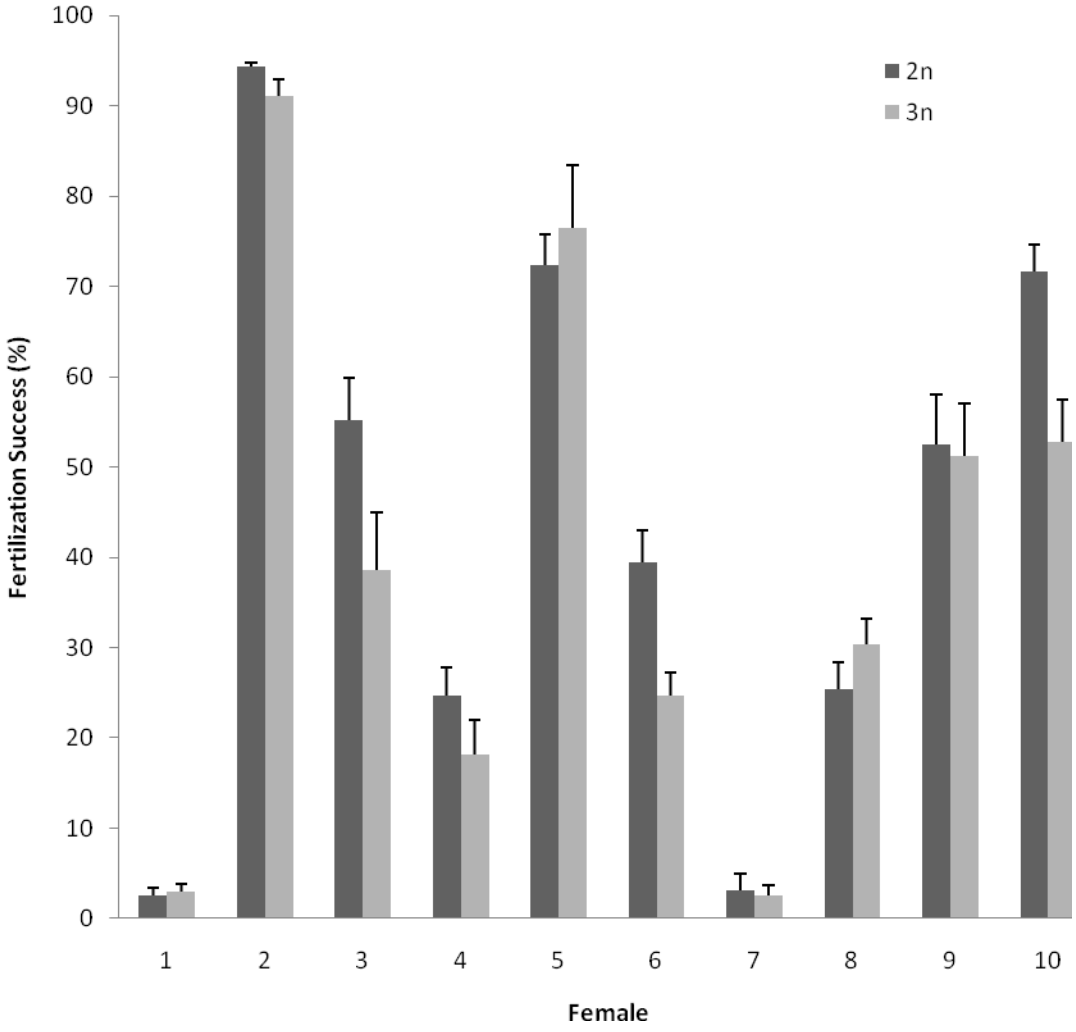


Figure. 2. Hatching success for diploid (2n) and triploid (3n) sired offspring (± SE) for the ten replicated trios resulting from the fertilization trials.

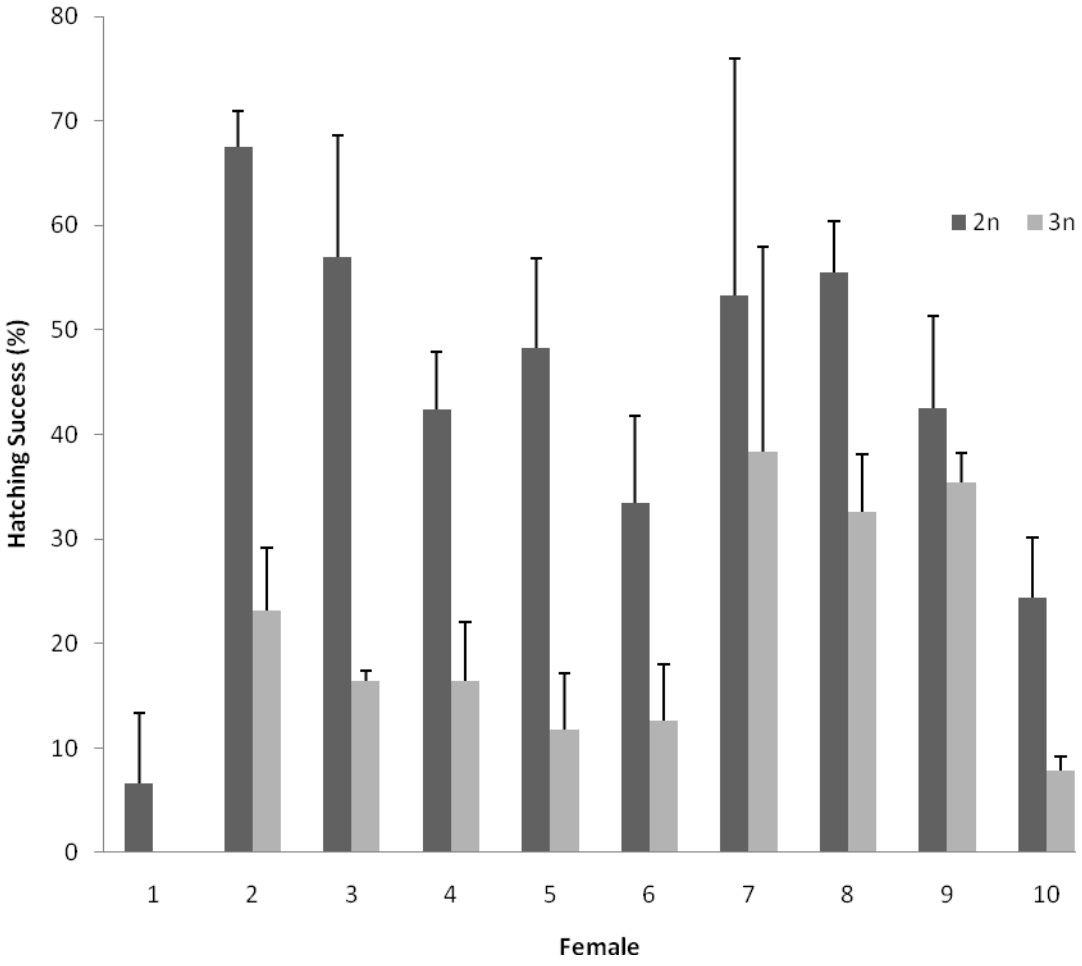
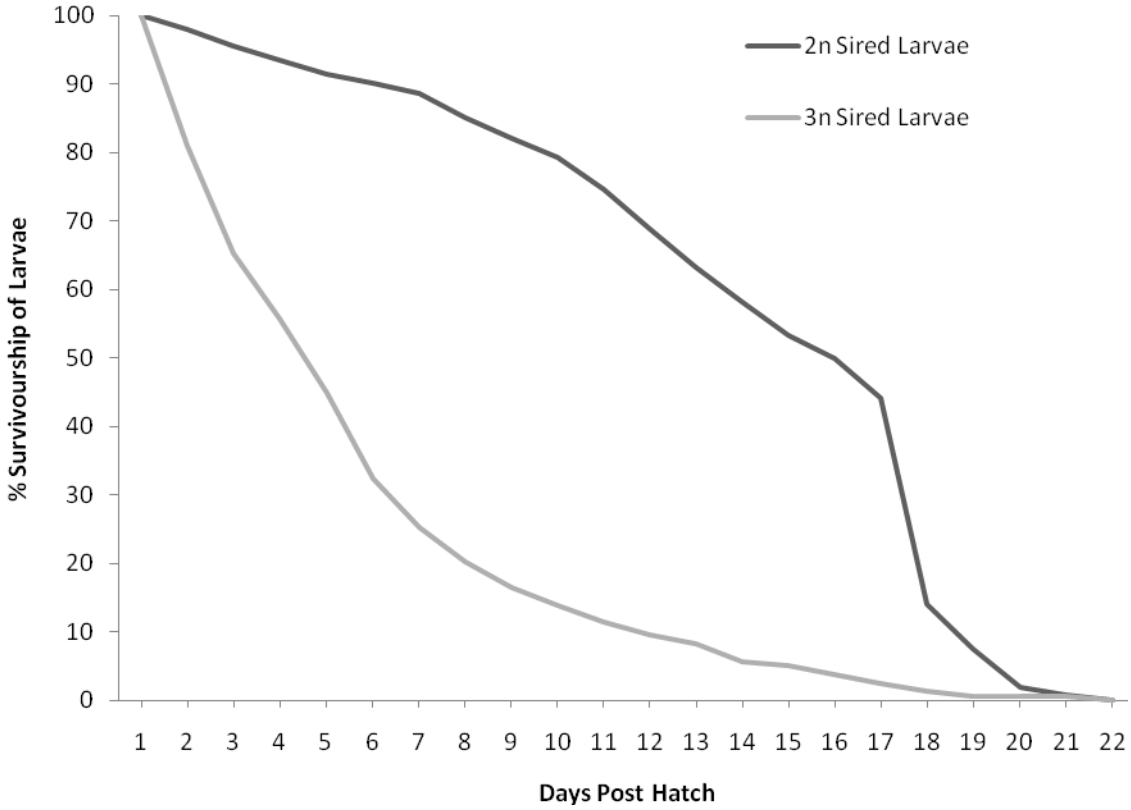


Figure. 3. Larval survival in relation to time for diploid (2n) and triploid (3n) sired larvae.



Sperm Morphology

Sperm cells sampled from 14 males (n = 7/ploidy and n = 100 sperm cells/male) revealed significant differences between diploids and triploids in all parameters measured, with triploid cells being larger, more elongated and more elliptical (Table 3).

Table 3. Sperm morphological parameters measured for diploid (2n) and triploid (3n) spermatozoa ± 1 standard deviation.

	Area (µm)	Perimeter (µm)	Length (µm)	Width (µm)	Elipticity	Elongation
2n	4.81 ± 0.801	8.44 ± 0.706	3.05 ± 0.278	2.01 ± 0.214	1.53 ± 0.166	0.21 ± 0.052
3n	6.29 ± 0.896	9.79 ± 0.778	3.60 ± 0.344	2.22 ± 0.203	1.63 ± 0.188	0.24 ± 0.057

Sperm Motility

There was no significant effect of ploidy on sperm motility at 20 and 40 s post-activation ($p > 0.05$). The average VCL for triploids and diploids was 55.3 ± 28.5 µm s^{-1} and 55.6 ± 29.7 µm s^{-1} respectively at 20 s post activation. Average VCL for triploids and diploids at 40 s post activation was 43.7 ± 24.1 µm s^{-1} and 42.7 ± 19.6 µm s^{-1} respectively.

Discussion:

Cod spawning in sea cages or tanks enables the study of different factors influencing spawning success and enables the execution of studies which can be related to both aquaculture raised and wild Atlantic cod. Fordham and Trippel. (1999) observed feeding behaviour of cod in relation to spawning. Rakitin et al. (1999 and 2001) studied spermatocrit and sperm density as well as male body sizes in relation to spawning success. Other studies examine spawning behaviour and mate choice (Hutchings et al., 1999) or communal spawning and inbreeding in gadoid species (Trippel et al., 2009). Peruzzi et al. (2009) showed that when triploid cod are placed in a competitive setting with other triploids, they are able to spawn with diploid females. Therefore, triploids are able to go through the spawning ritual and court females in a similar manner as their diploid counterparts. The current study expands on the in-tank competitive spawning experiment by Peruzzi et al. (2009) by placing a triploid male in a one-on-one competitive spawning situation with a diploid sibling for a diploid female of a separate family. The situation was created to mimic a triploid escaping from a sea cage and competing with a diploid sibling of similar size and condition on the spawning grounds for a diploid female. No difference was found in the ability of the triploids to outcompete their siblings for access to a gravid female.

When cod escape from sea cages, they will most likely be competing with diploids on the spawning grounds for access to females. Large aggregations of cod form on the spawning grounds which would significantly raise competitive levels for triploids to access female mates. Morgan et al. (1997) showed a spawning shoal of cod on the Grand Banks was approximately 5 km wide and 25 km long. Behavioural observations (Hutchings et al., 1998; Bekkevold et al., 2002) have suggested that cod display a lekking mating system (Nordeide and Flstad, 2000). Within large aggregations forming hierarchical ranks, triploids may be at a disadvantage due to them behaving less aggressive than diploids (Benfey, 1999). Theoretically, this will result in triploids being positioned at the lower ranks and increasing the probability of not gaining access to mates on competitive spawning grounds. This may be the case on larger spawning grounds, but inshore spawning grounds may be more at risk than those offshore. Svasand et al. (1990a,b) showed that the majority of cod released from known areas remain in close proximity of the release points. If there is a large escape of fish from a cage, inshore spawning of cod in areas such as Trinity Bay, Newfoundland (Smedbol and Wroblewski, 1997), which would be smaller in size and numbers of fish compared to the Grand Banks, may be overwhelmed and lead to a larger number of triploids gaining access to females. Even though the current study is based on a one-on-one scenario, we can conclude that there is some potential for males to gain access to females and spawn with them.

Previous work has shown that triploid Atlantic cod males develop to a spawning state and produce viable spermatozoa (Trippel et al., 2008; Peruzzi et al., 2009). The current study has

extended this work to show that there is no significant difference in *in-vitro* fertilization success of diploid and triploid spermatozoa. The ability to fertilize eggs has also been shown in triploids of other species such as rainbow trout (Chourrout et al., 1986), grass carp (*Ctenopharyngodon idella*) (van Eenennaam et al., 1990), yellowtail flounder (*Limanda ferruginea*) (Manning et al., 2004) and the barfin flounder (*Verasper moseri*) (Mori et al., 2006). Triploids of species such as the European sea bass (*Dicentrarchus labrax*) (Felip et al., 1999), turbot (*Scophthalmus maximus*) (Cal et al., 2006), and Arctic charr (*Salvelinus alpinus*) (Gillet et al., 2001) are unable to produce sperm at all, eliminating concerns of fish escaping and spawning with wild stocks. A study by Chourrout et al. (1986) showed a decrease in the ability of spermatozoa from tetraploid rainbow trout to fertilize eggs, due to their sperm heads being too large to enter the micropyle of the oocyte. The current study has also shown triploid sperm heads to be significantly larger for triploid cod (head width ranging from 1.6µm to 2.9µm) compared to diploids, although still able to enter the micropyle (approximately 4.5µm in diameter; measurement made from Trippel, 2003). Triploids can have a range of genetic material present in their spermatozoa, from haploid (1n) to diploid (2n) but predominantly aneuploid with an average of 1.5n (Benfey et al., 1986; Peruzzi et al., 2009). This range is one source of variation observed within sperm cell morphology of triploid cod. The increased DNA content would increase the variation of measured sperm cell parameters when compared to their diploid counterparts resulting in non-homogenous variances. Diploids will have a narrower range in which measured sperm cell parameters would exist.

It was expected that sperm VCL would be slower for triploids compared to diploids due to more drag created by their larger head size, but no difference was found for mean VCL at 20 and 40 s post activation. Peruzzi et al. (2009) found a significant difference at 20 s post activation but not at 40 s. This may be due to the small sample size of three fish per ploidy used to assess motility in their study, compared to thirteen per ploidy used for the current experiment. The fact that triploid cod are able to produce sperm capable of fertilizing eggs is not a promising projection for the application of male triploid Atlantic cod for commercial scale farming from an ecological point of view. If triploid fish were to escape and gain access to females in the wild, the embryos which would normally be sired by a diploid would be substituted by aneuploid offspring sired by a triploid. The aneuploid offspring would not survive, contributing no positive production to the wild stocks.

Offspring sired by triploids will be aneuploid and should not develop past the embryonic or larval stages (Benfey, 1999), as was observed in the current study. Poor performance of triploid sired offspring was evident during embryonic development in both this study and that of Peruzzi et al. (2009) for cod, and by Manning et al. (2004) for yellowtail flounder. These results support the fact that triploid males are sterile and if industry or scientific researchers are searching for a method for the control of genetic introgression of genes into the wild gene pool, then triploidy is a method which can be utilized as a genetic containment application.

Overall, the current research on male triploid Atlantic cod informs us that they are able to gain access to females in a competitive setting. They are able to spawn with them and fertilize their eggs, but their progeny will not develop past the larval stages. From the farmers' perspective, the application of triploidy in cod is not beneficial for the suppression of male gonadal development. From a conservation perspective, it is also noteworthy that if triploid males were to escape and

spawn with wild females, they would be displacing diploid males that would normally be fertilizing the eggs and contributing to the overall production of the wild stocks. This could be viewed as a negative impact on wild stocks, although it does still ensure that any offspring sired by aquaculture escapees will not survive, and genetic dilution of the wild gene pool will not occur and genetic variation within the stocks will be maintained. Theoretically, all-female triploid stocks would be the next step in the development of the industry and this warrants more research to be carried out on Atlantic cod.

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