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Effects of reproduction on growth and survival in Atlantic cod, *Gadus morhua*, assessed by comparison to triploids

Edward A. Trippel [a,*], Ian A.E. Butts [b], Amanda Babin [c], Steven R.E. Neil [a], Nathaniel J. Feindel [a,d], Tillmann J. Benfey [d]

[a] Fisheries and Oceans Canada, Biological Station, 531 Brandy Cove Rd., St. Andrews, NB E5B 2L9, Canada
[b] DTU Aqua, National Institute of Aquatic Resources, Section for Marine Ecology, Technical University of Denmark, Charlottenlund Slot, Jægersborg Allé 1, 2920 Charlottenlund, Denmark
[c] Department of Biology and Centre for Coastal Studies and Aquaculture, University of New Brunswick, Ganong Hall, P.O. Box 5050, Saint John, NB E2L 4L5, Canada
[d] Department of Biology, University of New Brunswick, P.O. Box 4400, Fredericton, NB E3B 5A3, Canada

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ABSTRACT

Despite increasing interest in optimal life history theory and the associated physiological, ecological and evolutionary processes, little information exists on gonad–soma tradeoffs and longevity of individuals over long time periods. We examined somatic and survival costs of reproduction in captive iteroparous, batch-spawning Atlantic cod (*Gadus morhua*), utilizing diploids and triploids, knowing that triploid females invest little to no energy into gametogenesis. Based on annual specific growth rate, there was no evidence for a somatic cost of reproduction at ages 2 (virgin year) and 4 years, but there was at age 3 years. At age 2 years, low investment in reproduction likely accounted for the lack of a somatic cost of reproduction, whereas at age 4 the absence was associated with heightened growth post-spawning enabling mature fish to catch up to immature fish. At age 3, compensatory growth during post-spawning was below that of immature fish. Survival represented a significant component of the cost of reproduction. Laboratory experiments examining the cost of reproduction have traditionally focused on shorter time periods, commonly spanning several months, whereas ours spanned nearly four years. Although previously done for bivalves, to our knowledge, this is the first time the cost of reproduction has been evaluated using triploid fish as a comparator.

1. Introduction

Triploidy induction is an effective tool for rendering fish infertile (Piferrer et al., 2009). Hence, the costs of current reproduction, growth reduction and heightened adult mortality (Calow, 1979; Reznik, 1992), all of which influence future reproduction, could be assessed by monitoring the life history traits of immature triploids relative to mature diploids. A somatic cost of reproduction was observed in a triploid–diploid comparative study in the Sydney rock oyster, *Saccostrea glomerata* (Honkoop, 2003). Triploidy may be especially suited to studying maternal costs of reproduction in fish, as oogenesis is more greatly suppressed than spermatogenesis in triploids (Piferrer et al., 2009), including Atlantic cod, *Gadus morhua* (Derayat et al., in press; Feindel et al., 2011). Moreover, early puberty in cod in captivity has hindered the ability, under controlled conditions, to evaluate the potential long-term growth benefits of maintaining immaturity for this long-lived, iteroparous, broadcast spawner as captive males and females often mature in their first and second years of life, respectively (Karlsen et al., 1995).

Although the effects of reproduction on growth are commonly examined (see Poizat et al., 1999; Rowe and Thorpe, 1990; Skjæraasen et al., 2010), survival compared to somatic costs of reproduction can greatly diminish lifetime reproductive output and therefore requires further attention (Kotiaho and Simmons, 2003; Stearns and Koella, 1986). When spawning progresses normally, survival costs of reproduction are incurred as animals typically draw heavily on stored energy reserves for the final stages of ovarian maturation and mating, leading to a lowered nutritional state post-spawning that may increase susceptibility to starvation, predation, and disease (Descamps et al., 2009; Dutil and Lambert, 2000; Lambert and Dutil, 2000). Survival costs may be heightened in batch spawners as repeated ovulations without the release of ova can lead to a rotund "egg bound" state and, in extreme cases, death (Árnason and Bjørnsson, 2012).

Despite their importance in evolutionary ecology and biological production models, growth and mortality, which are key components of the cost of reproduction, are often difficult to measure in wild populations (Belk and Tuckfield, 2010; Beverton et al., 2004; Lester et al., 2004). Consequently, experimental long-term rearing of fish provides an opportunity to more closely assess whether immaturity improves growth and survival. Conspecific triploids are a unique model for conducting such research. In the present study, using two experiments, we examined the cost of reproduction in captive-bred cod by comparing mature diploids and immature triploids. The shorter of the two had the fish separated by ploidy and therefore included tank replicates, where

* Corresponding author. Tel.: +1 506 529 5937; fax: +1 506 529 5862.
E-mail address: edward.trippel@dfo-mpo.gc.ca (E.A. Trippel).

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the longer one used a 'common garden' approach with all fish reared in a single tank. Specifically, we examined: (i) growth and reproduction during the year preceding their first spawning (as well as comparing immature and mature diploid growth), (ii) annual growth in four successive years, (iii) seasonal growth during pre-spawning, spawning and post-spawning phases, and (iv) survival in days and spawning seasons. The reason for examining temporal growth patterns at this level was to aid in understanding how cod of each sex and ploidy performed seasonally in relation to annual differences in growth. Because triploids often exhibit higher rates of deformities than diploids (Piferrer et al., 2009), including in Atlantic cod (Derayat et al., in press), we included an examination of the effect of deformities on growth rate to ensure that this was not a confounding factor in our analyses.

2. Materials and methods

2.1. Fish

The experimental fish were maintained at the St. Andrews Biological Station (Fisheries and Oceans Canada, St. Andrews, NB, 45° 07′N, 67° 05′W) and were offspring of wild broodstock captured from the Bay of Fundy (Northwest Atlantic Fisheries Organization Division 4X). Gametes of three fish per sex were used to generate offspring for Experiment 1 on March 15, 2008, and two fish per sex for Experiment 2 on March 9, 2005. The spawning period of captive cod from this region spans January to April. In each year, following fertilization, eggs were divided into two groups: one to produce triploids and the other retained as sibling diploid controls. Triploids were created using hydrostatic pressure (5 min at 58,600 kPa, beginning 180 °C min post-fertilization, using a TRC-APV™ high pressure chamber [www.trchydraulics.com]; Trippel et al., 2008). Larvae and juveniles were reared under standardized procedures (Brown et al., 2003).

2.2. Experiment 1

For Experiment 1, on December 18, 2008, following individual tagging with passive integrated transponders (PIT-tags) and measurement of total length (±0.1 cm) and body weight (±1 g), 300 diploid (2n) and 300 triploid (3n) 9-month-old juveniles were distributed among six 3000 L circular tanks (diameter = 2.0 m, depth = 1.5 m, flow = 26 L/min, oxygen saturation > 95%), such that 100 fish of a given ploidy were placed into each tank. Distribution was according to 2n and 3n, with three replicates per ploidy (treatment). Fish were fed daily to satiation via feeding belt delivery with a Marine Grower diet produced by EWOS Canada Limited (www.ewos.com). Tanks were covered with light tight, black vinyl dome tops. The source of light for each tank was a centrally located 100 W incandescent bulb suspended 1.5 m above the water's surface (100 lx at water's surface). Simulated natural photoperiod (45° N) was administered with 30 min ramping of sunrise and sunset. Water temperature followed local seasonal changes (range = 3 °C in March to 12 °C in September). Body measurements were recorded at approximately four week intervals and included assessment of morphological deformities (i.e., asymmetry) in four body regions: (i) mouth/jaw (upper and lower), (ii) head/upper body by anterior end of spine bent neck or "stargazer", (iii) body (e.g., scoliosis), and (iv) tail. Body and tail deformities were rare in all groups (<3%) and thus excluded from further analyses. Approximately 50% of the fish remaining in each tank were killed on January 26, 2010 (age 22 months) for gonad measurements and the remaining fish were retained for another study. Of the sacrificed fish, sex was determined, gonads removed, weighed (±0.1 g) and assessed macroscopically for developmental stage based on descriptions by Tomkiewicz et al. (2002). Gonadosomatic index:

$$\mathrm{GSI}\,(\%) = [(\text{gonad weight})/(\text{body weight})] \times 100$$

was calculated for each fish. Specific growth rate (SGR; Busacker et al., 1990) over the 13-month period was estimated as:

$$\mathrm{SGR} = 100 \times (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_2 and W_1 are body weights (g) at days t_2 and t_1, respectively (also performed for body length).

2.3. Experiment 2

For Experiment 2 on April 26, 2006, following individual tagging with PIT tags and measurements of total length and body weight, a mixed-ploidy (2n and 3n) population of two hundred and twenty eight 412-day-old fish was maintained in an 18,000 L covered outdoor circular tank (diameter = 4.0 m, depth = 1.5 m, flow = 45 L/min, turnover time = 6.6 h, oxygen saturation > 95%). Fish were maintained under ambient temperature regimes and photoperiod (20 cm diameter hole in black vinyl dome tank cover) and fed to satiation with a pellet Marine Grower diet. Over a 3.7-year period (1360 days), incidence of mortalities was recorded monthly and body measurements at monthly or longer intervals were recorded, such that 27 sampling events were achieved. On January 15, 2010, the remaining fish were killed and processed in the same manner as in Experiment 1. Specific growth rate (weight) was estimated annually and during pre-spawning, spawning, and post-spawning periods. Mean life span per ploidy and sex were estimated (days and potential reproductive seasons). A potential reproductive season is defined as a spawning season (January–April) where a fish survived through, regardless of reproductive state (commencing at age 21 months, January 2007).

2.4. Ploidy verification

Fish were verified as being of the correct ploidy by flow-cytometric measurement of erythrocyte DNA content. Briefly, 5 μL of whole blood was added to 1 mL of propidium iodide (50 mg/L in 0.1% sodium citrate). Samples were mixed on a vortex mixer for 5 s and refrigerated overnight at 6 °C. The following day (~15 h later), the samples were mixed, 100 μL of dimethylsulfoxide was added, they were mixed again, and then stored at −20 °C until a later flow-cytometric analysis at the Dr. Everett Chalmers Hospital (Fredericton, NB). Randomly-selected sub-samples from each presumptive 3n replicate were confirmed to be all triploid (n = 30 and 132 for Experiments 1 and 2, respectively).

2.5. Statistical analyses

All data were analyzed using SAS statistical analysis software (v. 9.1; SAS Institute Inc., Cary, NC, USA). Residuals were tested for normality (Shapiro–Wilk test) and homogeneity of variance (plot of residuals vs. predicted values). Data were transformed to meet assumptions of normality and homoscedasticity when necessary. The Kenward–Roger and Satterthwaite procedures were used to approximate the denominator degrees of freedom for unbalanced and balanced data, respectively (Spilke et al., 2005). Treatment means were contrasted using the Tukey–Kramer method.

2.5.1. Experiment 1

In these analyses mean values per tank were considered the replicates. We investigated the effects of sex (male vs. female) and ploidy (2n vs. 3n), as well as all accompanying interactions, on percent maturity, SGR (weight), GSI, and mouth and head deformities using a series of two-way factorial ANOVA models. Linear regression analyses were employed to examine if accumulation of reproductive tissue, as represented by the GSI (y-axis), was negatively related to SGR for weight (x-axis) during the 13 months preceding the 2010 spawning period. Data collected on December 17, 2008, were analyzed using a Student's