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Performance of triploid Atlantic cod (*Gadus morhua* L.) in commercial aquaculture

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ABSTRACT

The use of triploid fish can potentially reduce two of the major obstacles in Atlantic cod aquaculture; early sexual maturation that causes reduced profitability, and avoiding introgression between farmed cod and native populations, by preventing spawning in the netpens or that escapees reproduce.
Cod eggs were pressure treated to produce triploid fish. However, we observed that the pressure treatment produced a mixture of triploid and diploid individuals. The diploid and triploid fish were reared together; in a seawater pond during their larval and juvenile period, and in a commercial cod farm from juveniles to slaughter. Two experiments were performed on two separate year-classes of cod: in the first experiment, fish were reared under artificial continuous light, while in the second experiment the fish were reared under ambient light conditions.
Triploid cod had lower gonadosomatic index at all measurements compared to the diploids. However, from a production point of view, triploidisation alone only slightly reduced the gonadosomatic index and had to be combined with traditionally employed artificial light in order to be effective. Triploid fish were generally lighter than the diploids, but this difference disappeared towards time-of-harvest in the year-class without artificial light, possibly because of the lower maturation rate among the triploids. The triploid fish had a higher incidence of skeletal deformities compared to the diploids.
Statement of Relevance: The use of sterile fish by means of triploidisation is a promising method to reduce sexual maturation and the same time eliminate potential interbreeding of escaped cod with wild cod. In this paper we compare growth, maturation and deformities between triploid and diploid cod reared in common garden under commercial condition.

1. Introduction

Atlantic cod is farmed in netpens along the coast in countries around the North Atlantic, including Norway. In captivity, cod generally reach sexually maturity at 2 years old; however, some males can reach maturity even as 1 year olds (e.g. Karlsen et al., 1995). This early maturation of farmed cod is probably related to the favourable nutritional and growth conditions on the farms, and this causes some problems from an aquaculture point of view. The weight loss associated with maturation and spawning can be considerable due to reduced appetite related to spawning (Fordham and Trippel, 1999; Skjæraasen et al., 2004), and a reallocation of feed energy from somatic to gonad growth (Kjesbu et al., 1991; Karlsen et al., 1995; Schwalme and Chouinard, 1999). In addition, increased mortality of mature cod has been reported, particularly for females failing to release eggs (eggbound) (Árnason and Björnsson, 2012). Furthermore, farmed cod spawn in the netpens releasing fertilised eggs into the surrounding environment (Jørstad et al., 2008). Since it seems like the coastal cod population is composed of many local genetically distinct spawning stocks (Ruzzante et al., 2000; Knutsen et al., 2003), release of viable eggs from cod farms may pose a threat to the genetic composition of these local cod stocks. Escaped or released farmed Atlantic cod have also been observed at local spawning grounds (Svåsand et al., 1990; Meager et al., 2009; Jørstad et al., 2013), and there is therefore a risk that cod escapees will hybridise with local cod.

Sexual maturation is therefore considered to be one of the most important factors hampering an economically and environmentally feasible Atlantic cod farming industry. Even though the use of manipulated photoperiods to delay the age of puberty can reduce these problems (Taranger et al., 2006), the use of sterile fish would be a clear advantage. At present the only available method to produce sterile cod is

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triploidisation. Methods to produce triploid Atlantic cod are available (Peruzzi et al., 2007; Trippel et al., 2008). However, male triploid cod still develop gonads (Feindel et al., 2010; Feindel et al., 2011) and escaped triploid cod may potentially interbreed with wild (diploid) females. However, as the spermatozoa of triploid cod are aneuploid these offspring will suffer severe deformities and die before the onset of exogenous feeding (Peruzzi et al., 2009; Feindel et al., 2010). Triploid female cod also develop small gonads, but do not mature (Feindel et al., 2011). Since the weight loss due to spawning may exceed 20% (Karlsen et al., 1995; Karlsen et al., 2006), the use of sterile fish could therefore improve production performance as they would invest less energy in gonad growth. One interesting approach is to masculinise gynogenetic females and then use sperm from these fish to produce all female populations (Haugen et al., 2011; Otterå et al., 2011; Karlsen et al., 2013). Hence, use of all-female fish in combination with triploidisation may be an efficient means to mitigate both genetic mixing with wild populations and improve production performance in cod farming.

An initial study of the performance of juvenile triploid Atlantic cod has shown a far higher frequency of deformed triploids than diploid controls (Opstad et al., 2013). These experiments were based on intensively farmed cod juveniles produced in indoor tanks with continuous light and fed enriched rotifers and *Artemia*, where significant deformities are also often seen in diploid cod (Kolstad et al., 2006; Fjelldal et al., 2009). In contrast, a lower frequency of deformed cod were observed when the larvae were fed natural zooplankton in more extensive rearing systems (Imsland et al., 2006; Fjelldal et al., 2009). Hence, it may be that first-feeding triploid cod with natural zooplankton can reduce, or avoid, the deformities observed in the intensive first-feeding system.

In a first attempt to study the sexual maturation, growth performance and deformity rate in triploid cod first-fed with natural zooplankton, we conducted a large scale study with two year-classes of triploidised cod. These fish were initially reared in a marine pond and subsequently transferred to commercial sea cages in western Norway for on-growth until harvest. Since the triploidisation protocol used resulted in a mixture of diploid and triploid cod (in both year-classes) the diploid fish served as controls in a common-garden approach.

2. Material and methods

2.1. Overview of the experiment

During this experiment we followed two year-classes of cod (2010 and 2012) reared under commercial conditions, and analysed production parameters like growth, sexual maturation and deformity level. Cod eggs were subjected to pressure treatment after fertilisation in order to induce triploidy. However, the triploidisation procedure was not 100% effective, and gave a mixture of triploid- and diploid individuals. We, therefore, had the opportunity to compare the production parameter between triploid- and diploid individuals reared in a common-garden environment. Larval and juvenile rearing took place in an extensive rearing system, while the performance experiment was done on a commercial cod farm. Continuous artificial light was used during the ongrowing period in the first experiment but not in the second experiment.

2.2. Production of triploid juveniles

Cod juvenile production took place at the Institute of Marine Research (IMR) Parisvatnet Biological Station near Bergen, western Norway (60°37N 4°49E). Broodstock used for the experiment were held in sea cages under ambient conditions, with a sea-temperature of around 5 °C during the spawning season. In the first experiment (2010) we used broodstock born in 2004 while the broodstock for the second experiment (2012) was born in 2008. Both 2010 and 2012 broodstocks originated from captured Norwegian coastal cod and were F1 and F3 generations respectively, with no deliberate selection undertaken.

Atlantic cod spawns 10–20 batches of eggs at 2–3 day intervals during the spawning season from February–April (Kjesbu, 1989). At the time of the experiments, milt and ova from mature fish were collected by stripping and transported to the nearby hatchery for fertilisation and triploidisation. We used a total of 8 males and 41 females for the 2010 production, and 10 males and 30 females for the 2012 production.

Eggs and milt were kept at approximately 5 °C, and fertilisation took place within 2 h of stripping. All egg batches obtained on a single day were mixed together and 5–20 mL of milt from at least two males was added. The milt and eggs were gently mixed together and seawater (1 L per L eggs) was added to activate the eggs. The fertilised eggs where then divided into a varying number of batches (maximum of 1 L of eggs per batch), depending on total egg amount that day, and treated one after another in a hydrostatic pressure chamber (TRC-Apv, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada). We used a triploidisation protocol developed for cod by Trippel et al. (2008). Briefly, a hydrostatic pressure of 58.6 MPa (8500 PSI) was applied for 5 min, starting 30 min (180 min-degrees) after fertilisation. This is based on a temperature of 6 °C. We adjusted this to our lower temperature (3.5–5.2 °C) using the same number of min-degrees. In 2010, pressure treatment was started between 35 and 51 min after fertilisation in. In 2012 the protocol was modified slightly as follows: the treatment temperature was kept stable at 6 °C by using a thermostat-controlled water bath, and the pressure treatment was started 5 min earlier (25 min after fertilisation, 150 min-degrees). The latter change was due to preliminary trials indicating that the optimal time to begin the triploidisation treatment was 26 min after fertilisation (Nordhus, 2013).

After the pressure treatment the batches were transferred to the hatchery and incubated in 180 L tanks supplied with seawater at approximately 1 L min^{-1}. In addition air-bubbling was used to prevent the eggs from being sucked into the outlet sieve. The incubation temperature was approximately 5 °C. After 16–17 days the eggs hatched and 1–2 days later the larvae were released into the rearing pond. In 2010 a total of approximately 7.5 million larvae, originating from one week of stripping were released into the pond. In 2012 approximately 3.5 million larvae from 5 days of stripping were released.

The larval rearing took place in a 270,000 m^3/50,000 m^2 enclosed seawater pond where the larvae can feed on the naturally occurring plankton organisms like rotifers, copepod nauplii, and copepodids (Blom et al., 1991). In this semi-natural environment predators had been removed by rotenone treatment, and fertilisers had been added to increase the production of natural phytoplankton, and thereby zooplankton production. This natural feeding was supplemented with formulated feed (Gemma Micro followed by Gemma Wean and Gemma Diamond, www.skretting.com) from 3 weeks of age, and this gradually became the major feed for the juveniles. At an age of approximately 2 months the juveniles were harvested from the pond by dip netting and transferred to net cages floating in the pond. They were then fed formulated feed (Amber Neptun, www.skretting.com) and kept in the cages until transfer to the commercial cod farm. Formulated feed was distributed by automatic feeders during daylight hours both in the pond and in the cages. The juveniles were vaccinated against the common bacterial disease Vibriosis (Alpha marine Vibrio, www.pharmaq.no).

2.3. Commercial performance experiment

The on-growing, from juveniles to harvest, took place at the commercial cod farm Gulen Marine Ltd., situated in Gulen, western Norway (60°56′N 4°57E). In July 2010 approximately 127,000 juveniles were transported from the juvenile facility and released into two sea-cages at the farm. The 2012 year-class was transported to the farm in October 2012, and 48,000 juveniles released into one sea-cage. The juveniles were transported by well-boat.