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Sperm characteristics and competitive ability in farmed and wild cod

Jon E. Skjæraasen[1,*], Ian Mayer[1,5], Justin J. Meager[1], Geir Rudolfsen[2], Ørjan Karlsen[3], Thomas Haugland[2], Oddmund Kleven[4]

[1]Department of Biology, University of Bergen, 5020 Bergen, Norway
[2]Department of Evolution and Ecology, University of Tromsø, 9037 Tromsø, Norway
[3]Institute of Marine Research, Austevoll Research Station, 5392 Storebø, Norway
[4]National Centre for Biosystematics, Natural History Museum, University of Oslo, 0318 Oslo, Norway
[5]*Present address:* Norwegian School of Veterinary Science, 0033 Oslo, Norway

ABSTRACT: The development of cod aquaculture has raised concerns about its effect on wild stocks. One risk is hybridisation between escapees and wild cod, causing genetic introgression, and, potentially, fitness depressions in wild populations. The potential for hybridisation depends on escapee success in mating competition with wild fish. Cod have a complex mating system, with males likely to adopt either dominant or sub-dominant roles, the latter typically achieving reproductive success through sperm competition. Studies on salmonids indicate that domesticated males predominantly adopt sub-dominant roles. We therefore analysed sperm characteristics of wild and farmed cod *Gadus morhua* L. around the onset and end of the natural spawning season. Wild and farmed males were also paired in *in vitro* crosses to assess reproductive success in sperm competition. In the early spawning season, wild males had higher sperm velocity, percentages of motile and progressive cells, and spermatocrit. Sperm velocity was the main determinant of fertilisation success in *in vitro* sperm competition and, accordingly, wild males had higher reproductive success. At the end of spawning, the percentages of motile or progressive cells and spermatocrit were similar between wild and farmed males, but wild males maintained higher sperm velocity. Our results indicate that farmed males have limited reproductive success in sperm competition with wild male cod. This presumably reduces the risk of genetic introgression from escapees. We hypothesise that impaired sperm quality and lower reproductive fitness of farmed cod are due to inhibition of the full behavioural repertoire, lack of social structure under culture conditions, and/or nutritional deficiencies.

KEY WORDS: *Gadus morhua* · Escapees · Sperm competition · Mating system · Reproductive roles

INTRODUCTION

Aquaculture is an important industry encompassing a large number of species world-wide. However, in many regions, aquaculture has had severe impacts on wild populations of culture stock species, often exacerbating the effects of heavy fishing pressure (Naylor et al. 2000, 2005). The reasons for this are diverse and include a range of factors from pollution of water bodies, transfer of diseases and parasites, and ecological and behavioural interactions between farmed escapees and wild fish (Naylor et al. 2005, Bekkevold et al. 2006, Jonsson & Jonsson 2006). Development of a cod *Gadus morhua* L. aquaculture industry in the North Atlantic has therefore raised concerns about its potential impacts on wild stocks. Most cod are intensively farmed in net-pens in coastal areas used as habitat by local coastal cod. These wild cod populations are commonly genetically differentiated (Ruzzante et al. 2000, Sarvas & Fevolden 2005) with varying life histories (Salvanes et al. 2004, Olsen et al. 2008). Many of the coastal populations are presently at historically low abundances or close to endangered levels (Hutchings & Baum 2005). For example, the standing biomass of

*Email: jon.skjaeraasen@bio.uib.no

spawning Norwegian coastal cod north of 62° N was estimated at only ca. 51 000 t in 2005 (ICES 2007). Experience with salmon *Salmo salar* shows that it is virtually impossible to stop fish escaping from net-pens as a result of damage caused by storms, predators, operational accidents or vandalism; cod are even more proactive at escaping than salmon (Moe et al. 2007). As cod aquaculture expands, escapees will therefore be a common occurrence in adjacent coastal waters. According to the Norwegian Directorate of Fisheries, ca. 290 000 and 67 000 farmed cod escaped from Norwegian net-pens in 2006 and 2007, respectively. Among the main risks associated with these escapees is hybridisation with wild stocks, causing genetic introgression and, potentially, fitness depression (e.g. Bekkevold et al. 2006 and references therein).

The likelihood of hybridisation will depend on a number of factors, notably the reproductive behaviours of farmed and wild fish (Fleming et al. 1996, Weir et al. 2004), potentially mediated by sperm traits. A common denominator of most mating systems is that males can assume either behaviourally dominant or sub-dominant reproductive roles (Andersson 1994). Sub-dominant males can partly compensate for their behavioural inferiority by enhanced sperm characteristics (e.g. Birkhead & Møller 1998, Birkhead & Pizzari 2002, Locatello et al. 2007). An extreme example of this is found among salmonids, in which males have diverged into 2 distinctly different phenotypic morphs. Large hooked-jaw males dominate behaviourally through active courtship of females and aggressive interactions towards other competing males (Magurran 1992, Koseki & Maekawa 2000). Small precocious males on the other hand depend solely on a sneaking strategy, and rush in at the time the female sheds her eggs. Precocious salmonid males typically have higher sperm velocity and spermatocrit than behaviourally dominant males; through sperm competition sub-dominant males can achieve quite high reproductive success (Hutchings & Myers 1988, Koseki & Maekawa 2000). For example, in controlled experiments, the combined reproductive success of precocious males has been as high as 90% (Moran et al. 1996). Sperm competition occurs when sperm of 2 or more males compete to fertilise the eggs of a female (Parker 1970), and this competition is a potent agent for directional sexual selection in externally fertilising fish (Gage et al. 2004, Casselman et al. 2006).

For marine broadcast spawners like Atlantic cod, in which females release large numbers of gametes that are externally fertilised, sperm competition certainly occurs. In laboratory studies, satellite males have been observed to rush in around a mating pair as the female releases her eggs (Brawn 1961, Rowe & Hutchings 2006), and DNA fingerprinting analyses reveal that multiple males may fertilise eggs from a single batch (Rakitin et al. 2001, Bekkevold et al. 2002). The longevity of cod sperm (Trippel & Morgan 1994) and eggs (Kjørsvik & Lønning 1983) may also increase the importance of sperm competition. However, laboratory studies have also demonstrated that cod have a highly complex reproductive system, involving visual and auditory courtship displays (Brawn 1961, Hutchings et al. 1999, Rowe & Hutchings 2004) and frequent agonistic interactions between males (Brawn 1961, Hutchings et al. 1999). It has been argued that this behavioural repertoire provides a basis for female choice (Hutchings et al. 1999, Rowe et al. 2007). Consistent with this argument is the observation that a large mating skew and high variance in reproductive success occurs among males (Rowe et al. 2008). Taken together, these data support the view that male cod can take on either dominant or sub-dominant reproductive roles.

Studies on mating between farmed and wild salmonids have generally found that domesticated males are inferior to their wild counterparts in mating competition (Fleming et al. 1996, Weir et al. 2004). Escapee cod males may very well take on satellite roles and achieve reproductive success through sperm competition, resulting in hybridisation and genetic introgression. Given the large numbers of escapees that might find their way to local coastal cod spawning grounds, there is a substantial risk of hybridisation and introgression. Similarly, even though considered behaviourally inferior, escaped salmoinds have caused fitness depressions in a number of wild populations (e.g. McGinnity et al. 2003). Thus, differences in sperm competition performance between farmed and wild cod will be fundamental in determining the risk of hybridisation. In the present study we compared sperm characteristics and reproductive success in *in vitro* crosses of farmed and wild cod at around the (1) onset and (2) towards the end of the spawning period after fish had spawned freely in mixed groups. We also tested whether males adjust their sperm traits according to potential physiological or morphological correlates of reproductive success and whether variance in sperm traits increases over the spawning period as this is indicative of the existence of different reproductive roles (e.g. Rudolfsen et al. 2006).

MATERIALS AND METHODS

History of fish. All fish used in the present study originated from local coastal cod catches in the vicinity of Bergen, Norway. Specifically, the bulk of the wild cod (n = 51) were caught in November and December 2005 in the Øygarden area (60° 29′ N 4° 53′ E), at depths from 6 to 20 m. After capture, fish were initially kept in

a large 800 m^3 marine holding pen (13 m diameter, 6 m deep) until January 2006 when they were transported to the Institute of Marine Research (IMR) facility at Austevoll (60° 05′ N, 5° 15′ E) and placed in a 28 m^3 holding tank. An additional 24 wild cod were caught in early January 2006 west of Herdla (60° 34′ N 4° 56′ E) from 10 to 15 m depth. These cod were kept in submerged cages (1 m long, 60 cm wide, 1 m deep) for approximately 2 wk before transport to IMR, where they were placed in the 28 m^3 holding tank with the other fish. Wild fish were fed a mixture of shrimp and fish during their captivity.

Farmed cod were obtained from a population maintained under standard commercial conditions at IMR. These cod were either repeat spawners hatched in spring 2003, or recruit spawners hatched in spring, 2004; fish were the progeny of local wild cod caught west of Parisvannet, again at Øygarden (60° 37′ N, 4° 48′ E). The fish were initially start-fed in large plastic bags using filtered natural zooplankton at Parisvannet, Øygarden. They were then moved to 20 m^3 tanks at the same site the summer after hatching, and to IMR during the following autumn. At IMR, fish were reared at a density of approximately 4 m^{-3} in 5 × 5 × 5 m seapens, and fed daily with commercial cod pellet feed.

Data collection. Early in the spawning season in 2006 (20 and 22 February), 16 wild and 16 farmed males were sedated with Metacaine (0.5 g l^{-1}), tagged and measured for total length and whole body weight. The pelvic fin, a secondary sexual characteristic (Skjæraasen et al. 2006), was also measured with calipers from the base of the pelvic fin to the tip of the longest pelvic-fin ray. After carefully drying the gonadal pore with tissue paper to avoid seawater contamination, males freely extruded sperm following application of gentle pressure on their ventral sides. The sperm was collected into 50 ml vials. Eggs were also collected from 8 wild and 8 farmed females for later *in vitro* crosses. A blood sample was taken from the caudal vein of all fish. The fish were then placed in 2 mixed spawning groups: Tank 1 (60 m^3) contained 20 wild and 20 farmed cod, and Tank 2 (30 m^3) contained 12 wild and 12 farmed cod. The sex ratio in both tanks was 1:1. The cod were allowed to spawn freely for 31 d. On 26 March, all fish were sacrificed by a lethal dose of anaesthetic, and whole body, gonad and liver weights were measured individually. At this time, sperm was again taken from all males still producing milt (a total of 16 farmed and 11 wild males), and a blood sample was also taken from all cod. Additionally, the drumming muscle, a cod secondary sexual characteristic (Engen & Folstad 1999), was removed with forceps from each fish and dried at 60°C for 3 d to obtain dry weight to the nearest 0.0001 g.

Sperm analyses. Sperm quality parameters were quantified immediately following male milt stripping. Recording of sperm followed the method of Rudolfsen et al. (2005, 2008), i.e. an aliquot of undiluted sperm was placed on a pre-cooled (4.5 to 7.0°C) standard counting microscope slide with a 20 µm deep chamber (Leja products). Immediately thereafter, we added 4.5 µl of pre-cooled seawater to activate sperm and recorded motility using a Sony CCD video camera (XC-ST50CE PAL) mounted on an negative phase-contrast microscope (Olympus CH30), with a 10× objective. Recordings were stored on videotapes and later analysed using computer-assisted sperm analysis (HTM-CEROS sperm tracker, CEROS version 12, Hamilton Thorne Research). The image analyser was used with the following settings: frame rate 50 Hz, number of frames 25, minimum contrast 8 and minimum cell size 10 pixels. For each male, we quantified sperm motility 30 s post activation, and each motility measurement lasted 0.5 s. The parameters assessed were: mean average path velocity (VAP, mm s^{-1}), mean straight line velocity (VSL, mm s^{-1}), mean curvilinear velocity (VCL, mm s^{-1}), percent motile cells (MOT) and percentage progressive sperm (PPC, percentage of all sperm that moved with STR > 80 and VAP > 25 µm s^{-1}, STR = VSL/VAP). Cells with VAP < 20 µm s^{-1} and a VSL < 10 µm s^{-1} were considered to be static. After measurements, sperm samples were stored in vials kept on ice for subsequent use in the *in vitro* crosses (see '*In vitro* crosses' below).

Hormonal analyses. Plasma concentrations of the steroids testosterone (T) and 11-ketotestosterone (11kT) were measured by radioimmunoassay (RIA) according to Schulz (1985). In brief, steroids were extracted from 200 µl plasma with 4 ml diethylether. The aqueous phase was frozen on dry ice, after which, the organic phase was transferred to a glass tube, evaporated in a water bath, and then reconstituted with 600 µl assay buffer. Samples were assayed in duplicate.

***In vitro* crosses.** *In vitro* sperm competition trials were performed, with minor modifications, according to the procedure of Rudolfsen et al. (2005). Two sexually mature male cod, 1 farmed and 1 wild, were randomly selected and paired. A volume of milt was collected such that the total quantity of sperm cells from each male equalled, in terms of volume, 62.5 µl. The total milt volume (i.e. sperm plus seminal fluid) required from each male was calculated from the measured spermatocrit values (e.g. Rudolfsen et al. 2005, 2006, 2008, Liljedal et al. 2008). Rakitin et al. (1999a) found that spermatocrit was positively and significantly correlated with spermatozoa density and hence number. More importantly, Rakitin et al. (1999a) also documented that spermatozoa size remained unchanged throughout the sampling period, indicating that any observed differences in spermatocrit values

resulted not from differences in spermatozoa size per se, but rather from sperm number differences.

The females picked for the experiment were of roughly the same size and were chosen from a pool of wild and farmed females with a length range of 55 to 70 cm for both female types. By applying gentle pressure on the ventral side of each female, fish releasing eggs could be identified and selected for use in the *in vitro* crosses. After collection, eggs from different females were stored in separate vials kept on ice until further use. *In vitro* crosses were performed within 8 h of sperm and egg collection. However, the collection of sperm and eggs from wild and farmed cod, the pairing of individual males, and the order in which crosses were done were completely randomised to avoid the influence of any systematic time effect between groups on the results of our crosses.

The sperm was first mixed in 0.4 l of seawater for 10 s to activate the male gametes. The sperm solution was then added to a 0.5 l plastic container containing 1 ml (~500 eggs) of stripped eggs. This container was gently mixed and placed in a 10°C incubator. Subsequently, dead eggs were removed daily, and the water was changed every other day until hatching (ca. 10 d at 10°C). Hatched larvae were preserved in 96% ethanol for later paternity determination. Although we obtained eggs from several farmed and wild females, technical problems led to the loss of numerous crosses. Finally, DNA fingerprinting results were obtained from 21 *in vitro* crosses involving 10 different male pairs crossed against 5 farmed and 1 wild female.

Paternity determination. We extracted DNA from whole larvae and fin tissue of adults with the E.Z.N.A.® DNA Tissue Kit (Omega Bio-Tek). Paternities of larvae were determined using 1 to 3 polymorphic microsatellite markers (all with tetranucleotide repeat motifs) previously developed for cod (*Gmo8*, *Gmo19* and *Gmo37*, Miller et al. 2000). For each fertilization experiment, we only used the marker(s) that allowed us to unambiguously assign paternity of the larvae to one of the 2 competing males. Microsatellite loci were amplified by polymerase chain reaction (PCR). Each 10 µl reaction consisted of about 30 ng of genomic DNA, 0.5 µl of each primer (forward primers were fluorescently dyed), 0.1 mM dNTP mix (ABgene) and 0.2 units of DNA polymerase (DyNAzyme, Finnzymes) in the manufacturer's buffer (final concentrations of 10 mM Tris HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100). PCR was run on a GeneAmp 9700 Thermocycler (Applied Biosystems). The PCR profile used consisted of an initial denaturing step at 94°C for 5 min, followed by 35 cycles consisting of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s. The PCR profile was terminated with 72°C for 7 min, followed by 4°C for 5 min. PCR products were sized using a capillary automated ABI 3100 sequencer (Applied Biosystems) and allele binning performed with GeneMapper v3.7 analytical software (Applied Biosystems).

Data analyses. We first compared steroid and spermatocrit levels of farmed and wild cod with 2-tailed *t*-tests. We had repeated observations of VCL, MOT and PPC for farmed and wild males in seawater at the onset of spawning, and hence included a random effect term for individual fish in these tests. This linear mixed-effect (LME) model had the form:

$$y_{ij} = \mu_0 + \beta_{0j} + b_{0i} + e_{ij} \quad (1)$$

where y_{ij} is the VCL, MOT or PPC of male i of type j, i.e. farmed or wild. β_{0j} is the effect of male type, μ_0 is general intercept, b_{0i} is the random effect of individual males and e_{ik} is unexplained error. For all models, a Greek letter denotes a fixed effect and a Latin letter a random effect. MOT and PPC were proportions, and were therefore arcsine transformed in all analyses. The same sets of analyses were also performed for all males measured in March.

Spermatocrit, total length, condition, steroid values, pelvic-fin length and drumming muscle size as correlates of sperm characteristics: We used a linear regression approach to examine whether sperm motility (VCL, MOT or PPC) was correlated to male spermatocrit, length, condition, pelvic-fin length, drumming muscle mass or sex steroid values. Regressions included a random effect for individual fish, as indicated by Eq. (1). We controlled for the effects of fish body size on condition, pelvic-fin length and drumming muscle mass by using residuals. For condition, these were obtained by taking the residuals from ANCOVA of weight (g, log-transformed), with length (cm, log-transformed) as the covariate and fish type as the fixed effect (following Rowe & Hutchings 2004). We used the same approach for residual pelvic-fin length, but for residual drumming muscle mass we used body weight as the log-transformed covariate (e.g. Rowe & Hutchings 2004). The regressions were performed by the following LME model, exemplified by spermatocrit:

$$y_{ij} = \mu_0 + \beta_{0j} + b_{0i} + (\beta_{1j} + \mu_1) \times S_i + e_{ij} \quad (2)$$

where β_{1j} is the effect of male type on the slope of the regression line, μ_1 is the general slope value, and S_i is the spermatocrit levels of individual males.

Reproductive success of farmed and wild males in sperm competition: We first employed a 2-tailed binomial test (H_0, p = 0.5) to see whether the number of *in vitro* crosses in which the male with the highest values for sperm velocity or percentage of motile or progressive sperm had the highest reproductive success was different from a random 50:50 distribution. We then compared the reproductive successes of farmed and wild males with a LME model, where individual males were used as a random effect.

RESULTS

Wild and farmed males were of similar lengths and weights (Table 1, 2-tailed *t*-tests, $p > 0.05$). However, the farmed males were in significantly better condition than the wild cod (Table 1, 2-tailed *t*-test, $t = 6.13$, df = 30, $p < 0.001$).

Steroid values, gonad and liver size

In both wild and farmed males, there was a significant decrease in plasma levels of both testosterone (T) and 11-ketotestosterone (11kT) from February to March (Fig. 1). Although wild males tended to have higher steroid values in the February measurements and lower values than farmed males at the termination of the experiment in March (Fig. 1), there was no difference in either T or 11kT levels on either date (2-tailed *t*-tests, $p > 0.05$, Fig. 1). Farmed males had significantly larger gonads and livers than wild fish at sacrifice (2-tailed *t*-tests, $p < 0.01$, Fig. 2).

Sperm characteristics

Wild males had significantly higher spermatocrit levels than farmed males (2-tailed *t*-test with unequal variance, df = 22, $t = 2.39$, $p < 0.05$, Fig. 3), higher VCL (LME model [Eq. 1], df = 29, $t = 2.37$, $p < 0.05$, Fig. 3) and larger MOT (LME model [Eq. 1], df = 29, $t = 2.69$, $p < 0.05$, Fig. 3) and PPC in February (LME model [Eq. 1], df = 29, $t = 2.90$, $p < 0.01$, Fig. 3). At the end of the spawning season in March, spermatocrit (2-tailed *t*-test with unequal variance, df = 15, $p = 0.55$, Fig. 3), MOT (Fig. 3, LME model [Eq. 1], df = 23, $t = 0.08$, $p = 0.94$) and PPC (Fig. 3, LME model [Eq. 1], df = 23, $t = 0.47$, $p = 0.64$) did not differ between wild and farmed males. However, wild males still maintained a higher VCL (Fig. 3, LME model [Eq. 1], df = 23, $t = 2.51$, $p < 0.05$). While variation in VCL, MOT and PPC were higher among farmed than wild cod at the start of the spawning season, variation in all sperm traits were lower in farmed than wild males at the end of the spawning season (Table 2).

Table 1. *Gadus morhua*. Sizes of 16 wild and 6 farmed males used for the study. Means ± SE. Fulton's *K* is calculated as weight × length^{-1} × 100

Cod	Length (cm)	Weight (g)	Fulton's *K*
Farmed	57.7 ± 1.33	2461 ± 152	1.26 ± 0.027
Wild	60.3 ± 1.22	2348 ± 144	1.06 ± 0.021

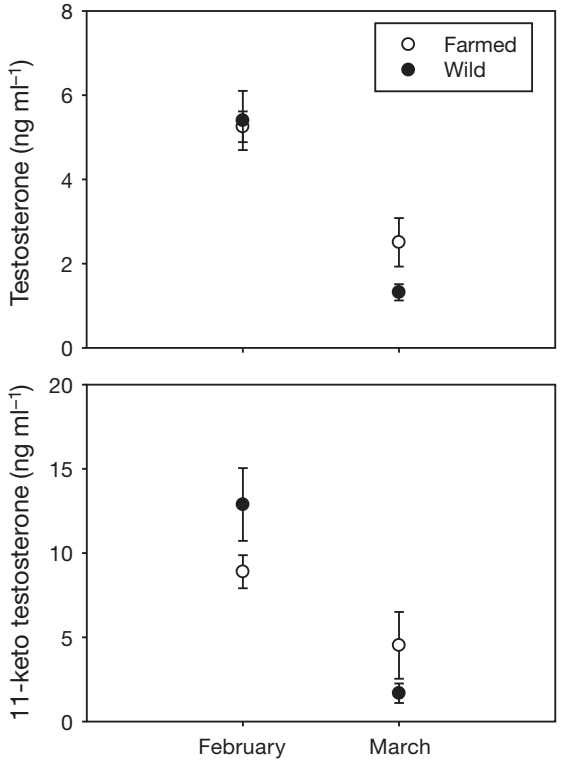

Fig. 1. *Gadus morhua*. Testosterone and 11-keto testosterone concentrations in farmed and wild males at the onset of the experiment in February and at sacrifice in March. Means ± SE

Correlates of sperm characteristics

MOT and PPC were positively correlated with fish length, weight and spermatocrit in farmed (but not wild) males at the onset of spawning (LME model [Eq. 2], $p < 0.01$). No significant correlations were found between sperm characteristics and other morphological and physiological parameters. It is, however, note-

Fig. 2. *Gadus morhua*. Gonadosomatic (GSI: gonad weight × total weight^{-1} × 100; ●,○) and hepatosomatic (HSI: liver weight × (total weight – gonad weight)$^{-1}$ × 100; ■,□) indices at sacrifice in March. Means ± SE

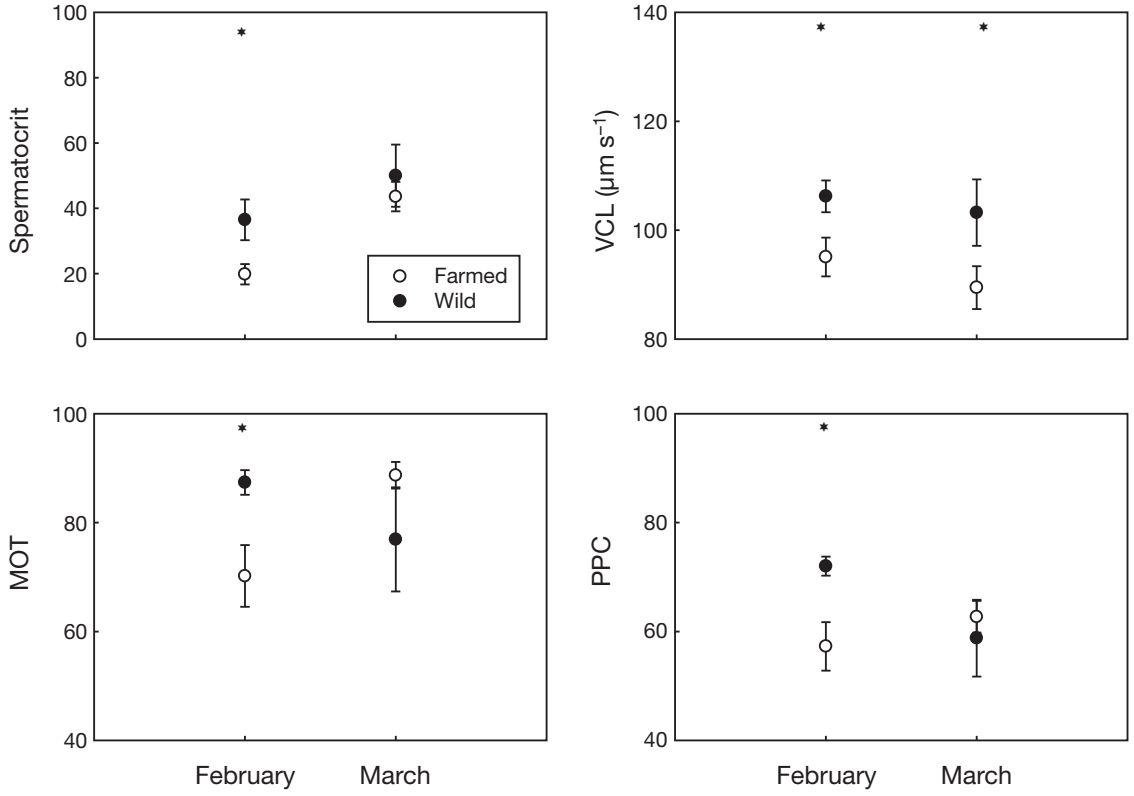

Fig. 3. *Gadus morhua*. Spermatocrit, curvilinear velocity (VCL) and percentage of motile (MOT) and progressive (PPC) cells in February and in March. * Significant difference ($p < 0.05$) found in linear mixed effect models. Means ± SE

worthy that known correlates of male cod reproductive success, male total and pelvic-fin lengths (Rowe et al. 2008), and male aggression, i.e. values for T and 11kt) tended to have negative relationships with VCL, MOT and PPC on the final sampling date for wild, but not farmed males.

Sperm traits as proxies for reproductive success

In 19 out of 21 crosses, the male with the highest sperm velocity had the highest fertilisation success ($p < 0.001$, binomial test). The male with the highest MOT and PPC had the highest reproductive success in 15 out of 21 crosses ($p > 0.05$). Along with their overall higher sperm velocity, wild males had significantly higher reproductive success than farmed males in the *in vitro* crosses (df = 11, $t = 3.10$, $p < 0.05$, Fig. 4).

Table 2. *Gadus morhua*. Coefficients of variation in February and March for sperm traits measured. VCL: sperm curvilinear velocity; MOT: % of motile sperm; PPC: % progressive sperm cells; n = 16, except where given in parentheses

	Spermatocrit	VCL	MOT	PPC
Farmed				
Feb	0.63	0.15	0.32	0.31
Mar	0.41	0.18	0.11	0.19
Wild				
Feb	0.68	0.11	0.10	0.27
Mar	0.63 (11)	0.19 (10)	0.39 (10)	0.38 (10)

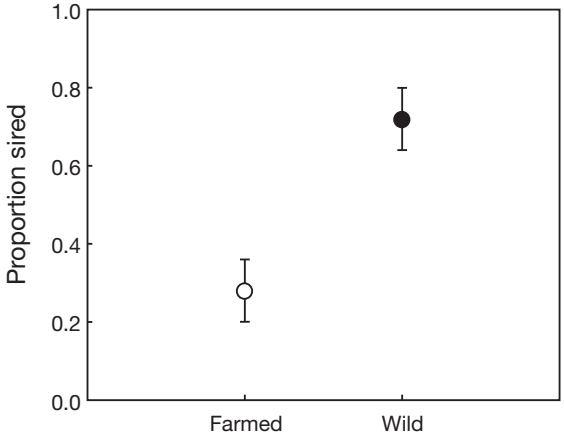

Fig. 4. *Gadus morhua*. Average proportion of offspring sired by farmed and wild cod in the *in vitro* crosses. Means ± SE

DISCUSSION

Early in the spawning season, wild males had sperm with higher VCL, MOT, PPC and spermatocrit compared to farmed males (Fig. 3). Towards the end of the spawning season, there were no differences in spermatocrit, motile sperm or progressive sperm, but wild males maintained a higher sperm velocity at this time (Fig. 3). Sperm velocity was the main determinant of fertilisation success, and although our results need verification from other populations, this implies limited reproductive success for farmed males in sperm competition with wild males. From a risk-management perspective, this may limit the likelihood of hybridisation between male escapees and wild females.

VCL was the best proxy of male fertilisation success. Recently, Rudolfsen et al. (2008) found that the proportion of progressive sperm was positively associated with paternity. They suggested that having a large fraction of faster sperm leaves the average sperm behind in the race towards the egg. Surprisingly, they also found a negative association between sperm velocity and paternity. This contradicts not only theoretical predictions of sperm-egg encounter rates in broadcast spawners (e.g. Levitan 2000, Riffell & Zimmer 2007), but also the results of numerous other studies across taxa, including Atlantic salmon (Gage et al. 2004), walleye *Sander vitreus* (Casselman et al. 2006), the sepulid polychaeate *Galeoaria caespitose* (Kupriyanova & Havenhand 2002) and the internally fertilising domestic fowl (Birkhead et al. 1999). Theoretically, the probability of a sperm encountering an egg is a function of sperm swimming speed, gamete concentration and egg target area (Levitan 2000, Riffell & Zimmer 2007). Sperm density (spermatocrit) has been shown to positively influence male reproductive success in a number of teleost species, including cod (Rakitin et al. 1999b), bluegill *Lepomis macrochirus* (Stoltz & Neff 2006) and common carp *Cyprinus carpio* (Linhart et al. 2005). Wild males had a higher VCL both early and late in their spawning period, and had significantly higher spermatocrit values early and a tendency towards higher values late in their spawning period (Fig. 3). Thus, in general, wild males outcompeted farmed males in sperm competition measured in both terms of sperm velocity and number. Further, the observation that these proxies of male reproductive success followed a similar pattern on both sampling dates suggests that our results show real differences in sperm traits and competitive ability between wild and farmed populations. However, the fingerprinting results in our experiment were obtained mainly from crosses containing eggs from farmed females. Although we have no reason to believe that the observed pattern of male fertilisation success would have changed with the inclusion of more samples from wild females, we would encourage future studies to examine this further.

If farmed males are able to compete with wild males behaviourally, they may still achieve high reproductive success, as male size and aggressive behaviour were found to be the main correlates of male reproductive success in large-scale mesocosm studies on wild cod (Rowe et al. 2008). To our knowledge, there are no published results on the outcome of spawning competition between wild and farmed cod. However, experiments on domesticated and wild salmonids show that consistent reproductive behavioural differences exist, such that domesticated males are generally inferior to their wild counterparts in spawning competition (Fleming et al. 1996, Weir et al. 2004). Whether this is also the case for farmed cod remains to be seen. Further, even if farmed males are dominated by their wild opponents, hybridisation may still occur through farmed females. This pattern has, for example, been demonstrated in Atlantic salmon (Fleming et al. 2000). At present we are examining the results of mating competition between farmed and wild cod in mixed spawning shoals.

This is among the first studies to examine the effect of farming on sperm traits. Rideout et al. (2004) reported no difference in spermatocrit or sperm motility between wild and cultured haddock *Melanogrammus aeglofinus*. Wild sea trout *Salmo trutta* were found to have higher sperm concentrations than reconditioned males, whereas sea-reared males had higher sperm concentrations than wild males in one year and lower concentrations in another year (Poole & Dillane 1998). Studies on penaeid prawns have found a negative effect of rearing on several sperm traits (Leungtrujillo & Lawrence 1987, Rendon Rodriguez et al. 2007). Even though our results need to be tested in other farmed and wild cod populations, we propose 2 possible explanations for how farming could lead to impaired sperm quality and reduced success in sperm competition for farmed cod. Firstly, we suggest that the differences arise from the contrast in social dynamics in the farm and wild environments. Typically, enhanced sperm traits are a result of clear dominance hierarchies with males occupying different reproductive roles (e.g. Birkhead & Møller 1998, Birkhead & Pizzari 2002, Locatello et al. 2007). The rearing of fish for aquaculture purposes typically occurs at very high densities in large land-based tanks or outdoor seapens devoid of structure; this environment imposes social conditions very different from those experienced by their wild counterparts. The diminished reproductive success of farmed salmon has been attributed to behavioural deficits resulting from such tank

and cage conditions and the failure of farmed males to establish proper dominance hierarchies (Fleming et al. 1996, Weir et al. 2004). Hence, a similar lack of social structure among farmed cod might explain the overall decrease in sperm motility, spermatocrit and the competitive success of sperm from farmed compared to wild males in our study. Tentatively congruent with this explanation is the observation that the coefficients of variation (CV) of the measured sperm traits generally increased for wild males and decreased for farmed males across the spawning period (Table 2). If males occupy dominant and sub-dominant reproductive roles, this is expected to increase variance in sperm traits (e.g. Rudolfsen et al. 2006). Hence, the results for wild males may stem from some form of social structure, whereas this does not seem to be the case for farmed males (Table 2). The observation that known correlates of male reproductive success (e.g. Rowe et al. 2008) tended to produce negative relationships with sperm traits in wild, but not farmed cod towards the end of the spawning period also supports such an explanation. However, as increased sperm competition reduces variation in sperm traits (e.g. Kleven et al. 2008), it could also indicate differences in sperm competition levels. Recently, Herlin et al. (2008) published the results of a study on correlates of reproductive success and mating skew in a spawning group of cod kept at farming densities. No correlation between male length and reproductive success was found. This indicates a lack of social structure compared to wild cod, in which there is a general positive correlation between male length and reproductive success (Hutchings et al. 1999, Rowe et al. 2008). However, a large mating skew was demonstrated between different males in the study of Herlin et al. (2008), indicating some form of male correlate to reproductive success. Thus, the results were inconclusive as to whether social structures exist in cod farms. However, offspring from only one spawning day were examined in this study (op. cit).

Another possible explanation for the differences in gamete quality, and in turn reproductive success, between wild and farmed fish is difference in nutrition. To date, a major problem facing the cod farming industry is mortality during spawning, which, particularly for farmed females, far exceeds that reported for wild cod (Ø. Karlsen pers. obs.). It has been suggested that dietary-induced nutritional deficiencies could in part explain this spawning defect. Similarly, even though farmed males had higher condition factors (Table 1) and larger livers than wild males (Fig. 2), it is possible that micro-nutrient and vitamin imbalances in the diet of the farmed cod may have had detrimental effects on sperm quality traits. Bell et al. (1996) found that farmed seabass *Dicentrarchus labrax* had different ratios of fatty acids in their sperm compared to wild fish. Although the functional significance of their findings remains uncertain, this shows that a pellet diet can affect sperm characteristics. For this reason, the wild cod used in the present study were fed a varied diet after capture, i.e. shrimps and fish, in order to mimic as closely as possible the nutritional status of wild fish. During the experiment itself, fish were not fed as appetite is generally very low during the spawning period (Fordham & Trippel 1999, Skjæraasen et al. 2004, Michalsen et al. 2008).

Finally, differences in sperm quality between farmed and wild males could be a consequence of subtle differences in hormone profiles. In the present study, both male types had high plasma levels of T and 11kT early in the spawning season, similar to levels previously measured in mature male cod (Norberg et al. 2004, Meier et al. 2007). While both these androgens, especially 11kT, are involved in the control of spermatogenesis in teleost fish (Schulz & Miura 2002), it is now known that final sperm maturation and release are specifically under the control of the maturation-inducing hormone MIS (Vizziano et al. 2008), which, in most fish, has been found to be the C-21 steroid 17, 20β-dihydroxy-4-pregnen-3-one (17,20βP). Unfortunately, the MIS in Atlantic cod has yet to be identified. The fact that only low plasma levels of 17,20βP were measured in spawning cod supports the view that this progestin is not the major MIS in Atlantic cod (Kjesbu et al. 1996). The future identification of the MIS in Atlantic cod would allow us to determine whether the differences in sperm quality observed between farmed and wild males were indeed due to endocrine factors.

In conclusion, we have found differences in sperm traits between wild and farmed males that negatively influenced fertilisation success of farmed males in sperm competition trials with wild males. From a risk-management perspective, this presumably limits the likelihood of hybridisation between male escapees and wild females as the sperm from wild males may outcompete sperm from farmed males. Although the mechanisms mediating sperm traits in farmed cod are currently unclear, our findings provide impetus for future research in light of the increasing numbers of farmed cod escapees occurring in coastal habitats today.

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LITERATURE CITED

Andersson M (1994) Sexual selection. Princeton University Press, Princeton, NJ

Bekkevold D, Hansen MM, Loeschcke V (2002) Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.). Mol Ecol 11:91–102

Bekkevold D, Hansen MM, Nielsen EE (2006) Genetic impact of gadoid culture on wild fish populations: predictions, lessons from salmonids, and possibilities for minimizing adverse effects. ICES J Mar Sci 63:198–208

Bell MV, Dick JR, Thrush M, Navarro JC (1996) Decreased 20:4n–6/20:5n–3 ratio in sperm from cultured sea bass, *Dicentrarchus labrax*, broodstock compared with wild fish. Aquaculture 144:189–199

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic Press, London

Birkhead TR, Pizzari T (2002) Postcopulatory sexual selection. Nat Rev Genet 3:262–273

Birkhead TR, Martínez JG, Burke T, Froman DP (1999) Sperm mobility determines the outcome of sperm competition in the domestic fowl. Proc R Soc Lond B Biol Sci 266: 1759–1764

Brawn VM (1961) Reproductive behaviour of the cod (*Gadus callarias* L.). Behaviour 18:177–198

Casselman SJ, Schulte-Hostedde AI, Montgomerie R (2006) Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can J Fish Aquat Sci 63: 2119–2125

Engen F, Folstad I (1999) Cod courtship song: a song at the expense of dance? Can J Zool 77:542–550

Fleming IA, Jonsson B, Gross MR, Lamberg A (1996) An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). J Appl Ecol 22:893–905

Fleming IA, Hindar K, Mjolnerod IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. Proc R Soc Lond B Biol Sci 267:1517–1523

Fordham SE, Trippel EA (1999) Feeding behaviour of cod (*Gadus morhua*) in relation to spawning. J Appl Ichthyol 15:1–9

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA (2004) Spermatozoa traits and sperm competition in Atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. Curr Biol 14: 44–47

Herlin M, Delghandi M, Wesmajervi M, Taggart JB, McAndrew BJ, Penman DJ (2008) Analysis of the parental contribution to a group of fry from a single day of spawning from a commercial Atlantic cod (*Gadus morhua*) breeding tank. Aquaculture 274:218–224

Hutchings JA, Baum JK (2005) Measuring marine fish biodiversity: temporal changes in abundance, life history and demography. Phil Trans R Soc Lond, B 360:315–338

Hutchings JA, Myers RA (1988) Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. Oecologia 75:169–174

Hutchings JA, Bishop TD, McGregor-Shaw CR (1999) Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. Can J Fish Aquat Sci 56:97–104

ICES (International Council for the Exploration of the Sea) (2007) Report of the arctic fisheries working group, Vigo, Spain, 18–27 April 2007. ICES C.M. 2007/ACFM:16. ICES, Copenhagen

Jonsson B, Jonsson N (2006) Cultured Atlantic salmon in nature: a review of their ecology and interaction with wild fish. ICES J Mar Sci 63:1162–1181

Kjesbu OS, Kryvi H, Norberg B (1996) Oocyte size and structure in relation to blood plasma steroid hormones in individually monitored, spawning Atlantic cod. J Fish Biol 49: 1197–1215

Kjørsvik E, Lønning S (1983) Effects of egg quality on normal fertilization and early development of the cod, *Gadus morhua* L. J Fish Biol 23:1–12

Kleven O, Laskemoen T, Fossøy F, Robertson RJ, Lifjeld JT (2008) Intraspecific variation in sperm length is negatively related to sperm competition in passerine birds. Evolution 62:494–499

Koseki Y, Maekawa K (2000) Sexual selection on mature male parr of masu salmon (*Oncorhynchus masou*): Does sneaking behavior favor small body size and less-developed sexual characters? Behav Ecol Sociobiol 48:211–217

Kupriyanova E, Havenhand JN (2002) Variation in sperm swimming behaviour and its effect on fertilization success in the serpulid polychaete *Galeolaria caespitosa*. Invertebr Reprod Dev 41:21–26

Leungtrujillo JR, Lawrence AL (1987) Observations on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. Aquaculture 65:363–370

Levitan DR (2000) Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. Proc R Soc Lond B Biol Sci 267:531–534

Liljedal S, Rudolfsen G, Folstad I (2008) Factors predicting male fertilization success in an external fertilizer. Behav Ecol Sociobiol 62:1805–181

Linhart O, Rodina M, Gela D, Kocour M, Vandeputte M (2005) Spermatozoal competition in common carp (*Cyprinus carpio*): What is the primary determinant of competition success? Reproduction 130:705–711

Locatello L, Pilastro A, Deana R, Zarpellon A, Rasotto MB (2007) Variation pattern of sperm quality traits in two gobies with alternative mating tactics. Funct Ecol 21: 975–981

Magurran AE (1992) Individual differences an alternative behaviours. In Pitcher TJ (ed) Behaviour of teleost fishes, 2nd edn. Chapman & Hall, London, p 441–478

McGinnity P, Prodohl P, Ferguson K, Hynes R and others (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proc R Soc Lond B Biol Sci 270:2443–2450

Meier S, Andersen TE, Norberg B, Thorsen A and others (2007) Effects of alkylphenols on the reproductive system of Atlantic cod (*Gadus morhua*). Aquat Toxicol 81:207–218

Michalsen K, Johannesen E, Bogstad B (2008) Feeding of mature cod (*Gadus morhua*) on the spawning grounds in Lofoten. ICES J Mar Sci 65:571–580

Miller KM, Le KD, Beacham TD (2000) Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Mol Ecol 9:238–239

Moe H, Dempster T, Sunde LM, Winther U, Fredheim A (2007) Technological solutions and operational measures to prevent escapes of Atlantic cod (*Gadus morhua*) from sea cages. Aquacult Res 38:91–99

Moran P, Pendás AM, Beall E, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. Heredity 77:655–660

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N and others (2000) Effect of aquaculture on world fish supplies. Nature 405:1017–1024

Naylor R, Hindar K, Fleming IA, Goldburg R and others (2005)

Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. Bioscience 55:427–437

Norberg B, Brown CL, Halldorsson O, Stensland K, Björnsson BTh (2004) Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid profiles in Atlantic cod (*Gadus morhua*). Aquaculture 229: 451–467

Olsen EM, Knutsen H, Gjøsaeter J, Jorde PE, Knutsen JA, Stenseth NC (2008) Small-scale biocomplexity in coastal Atlantic cod supporting a Darwinian perspective on fisheries management. Evol Appl 1:524–533

Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. Biol Rev Camb Phil Soc 45: 525–567

Poole WR, Dillane MG (1998) Estimation of sperm concentration of wild and reconditioned brown trout, *Salmo trutta* L. Aquacult Res 29:439–445

Rakitin A, Ferguson MM, Trippel EA (1999a) Spermatocrit and spermatozoa density in Atlantic cod (*Gadus morhua*): correlation and variation during the spawning season. Aquaculture 170:349–358

Rakitin A, Ferguson MM, Trippel EA (1999b) Sperm competition and fertilization success in Atlantic cod (*Gadus morhua*): effect of sire size and condition factor on gamete quality. Can J Fish Aquat Sci 56:2315–2323

Rakitin A, Ferguson MM, Trippel EA (2001) Male reproductive success and body size in Atlantic cod *Gadus morhua* L. Mar Biol 138:1077–1085

Rendon Rodriguez S, Macías Regalado E, Calderón Pérez JA, Núñez Pastén A, Solis Ibarra R (2007) Comparison of some reproductive characteristics of farmed and wild white shrimp males *Litopenaeus vannamei* (Decapoda: Penaeidae). Rev Biol Trop 55:199–206

Rideout RM, Trippel EA, Litvak MK (2004) Relationship between sperm density, spermatocrit, sperm motility and spawning date in wild and cultured haddock. J Fish Biol 65:319–332

Riffell JA, Zimmer RK (2007) Sex and flow: the consequences of fluid shear for sperm–egg interactions. J Exp Biol 210: 3644–3660

Rowe S, Hutchings JA (2004) The function of sound production by Atlantic cod as inferred from patterns of variation in drumming muscle mass. Can J Zool 82:1391–1398

Rowe S, Hutchings JA (2006) Sound production by Atlantic cod during spawning. Trans Am Fish Soc 135:529–538

Rowe S, Hutchings JA, Skjæraasen JE (2007) Non-random mating in a broadcast spawner: mate size influences reproductive success in Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 64:219–226

Rowe S, Hutchings JA, Skjæraasen JE, Bezanson L (2008) Phenotypic and behavioural correlates of individual variation in male Atlantic cod reproductive success. Mar Ecol Prog Ser 354:257–265

Rudolfsen G, Figenschou L, Folstad I, Nordeide JT, Søreng E (2005) Potential fitness benefits from mate selection in Atlantic cod (*Gadus morhua*). J Evol Biol 18:172–179

Rudolfsen G, Figenschou L, Folstad I, Tveiten H, Figenschou M (2006) Rapid adjustments of sperm characteristics in relation to social status. Proc R Soc Lond B Biol Sci 273: 325–332

Rudolfsen G, Figenschou L, Folstad I, Kleven O (2008) Sperm velocity influences paternity in the Atlantic cod (*Gadus morhua* L.). Aquacult Res 39:212–216

Ruzzante DE, Wroblewski JS, Taggart CT, Smedbol RK, Cook D, Goddard SV (2000) Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. J Fish Biol 56:431–447

Salvanes AGV, Skjæraasen JE, Nilsen T (2004) Sub-populations of coastal cod with different behaviour and life-history strategies. Mar Ecol Prog Ser 267:241–251

Sarvas TH, Fevolden SE (2005) The scnDNA locus Pan I reveals concurrent presence of different populations of Atlantic cod (*Gadus morhua* L.) within a single fjord. Fish Res 76:307–316

Schulz R (1985) Measurement of 5 androgens in the blood of immature and maturing rainbow trout *Salmo gairdneri* (Richardson). Steroids 46:717–726

Schulz RW, Miura T (2002) Spermatogenesis and its endocrine regulation. Fish Physiol Biochem 26:43–56

Skjæraasen JE, Salvanes AGV, Karlsen O, Dahle R, Nilsen T, Norberg B (2004) The effect of photoperiod on sexual maturation, appetite and growth in wild Atlantic cod (*Gadus morhua* L.). Fish Physiol Biochem 30:163–174

Skjæraasen JE, Rowe S, Hutchings JA (2006) Sexual dimorphism in pelvic fin length of Atlantic cod. Can J Zool 84: 865–870

Stoltz JA, Neff BD (2006) Sperm competition in a fish with external fertilization: the contribution of sperm number, speed and length. Evol Biol 19:1873–1881

Trippel EA, Morgan MJ (1994) Sperm longevity in Atlantic cod (*Gadus morhua*). Copeia 1994:1025–1029

Vizziano D, Fostier A, Loir M, Le Gac F (2008) Testes development, its hormonal regulation and spermiation induction in teleost fish. In: Alavi SMH, Cosson JJ, Coward K, Rafiee G (eds) Fish spermatology. Alpha Science International, Oxford, p 103–140

Weir LK, Hutchings JA, Fleming IA, Einum S (2004) Dominance relationships and behavioural correlates of individual spawning success in farmed and wild male Atlantic salmon, *Salmo salar*. J Anim Ecol 73:1069–1079

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