Vol. 535: 197–211, 2015
doi: 10.3354/meps11406

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published September 15

Spawning success of cultured and wild male Atlantic cod *Gadus morhua* does not differ during paired contests

Brendan F. Wringe[1,2,3,*], Ian A. Fleming[1,3], Craig F. Purchase[1,2]

[1]Fish Evolutionary Ecology Research Group, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, A1C 5S7, Canada

[2]Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, A1B 3X9, Canada

[3]Department of Ocean Sciences, Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, A1C 5S7, Canada

ABSTRACT: Culture of Atlantic cod *Gadus morhua* L. has been proposed as a means of diversifying the aquaculture industry in Canada and other countries within its native range. Lessons gleaned from aquaculture of salmonids suggest that escapes and interactions with wild fish are inevitable. Here, we studied the reproductive interactions of individual cultured and wild male cod in the presence of a cultured female using a series of spawning trios. The spawning success of cultured males, in terms of both overall proportion of eggs fertilized, and number of spawns in which they fertilized the larger proportion of eggs, did not differ from that of wild males. This equality was likely brought about, at least in part, by multiple paternity with appreciable proportions of eggs fertilized by the presumed satellite male. In a subset of spawning events for which behavioural data were available, neither wild nor cultured males were found to be behaviourally dominant during the night of spawning across all spawning events. The spawning success of the males was not influenced by their size or by their agonistic behaviour, but was influenced by their courting behaviour. The courting behaviour of the wild males had a negative influence on their success, while the courting behaviour of the cultured males was found to increase their success. To our knowledge, this is the first study to detect spawning success equality between wild and cultured male cod in competition.

KEY WORDS: Wild/farmed interactions · Mating behaviour · Genetic introgression · Fertilization success · Aquaculture escapes

INTRODUCTION

The waning of fish stocks worldwide has helped spur the development of aquaculture programmes to meet the demand for fish products (Svåsand et al. 2000, Naylor & Burke 2005, Dauer et al. 2009), and this has led to increases in the unintentional release of cultured fish into the wild (Jensen et al. 2010). Exposure to the unnatural culture environment, intentional and unintentional selection ('domestication selection'), founder effects, genetic drift and small effective population sizes (N_e) are likely to cause cultured fish to diverge from wild fish populations, both genetically and phenotypically (Fleming & Einum 1997, Gross 1998, Thorstad et al. 2008). In fact, captivity has been shown to cause rapid phenotypic and genetic changes in cultured relative to wild fish, and there is evidence that escapees from aquaculture may not be as fit as their wild-born counterparts, especially in terms of successfully mating (Fleming et al. 1996, 2000, Meager et al. 2009, 2010). However, while cultured fish may not be as success-

*Corresponding author: bwringe@mun.ca

ful in attaining mates, interbreeding between wild fish and fish that have escaped from aquaculture has been well documented for Atlantic salmon *Salmo salar* L. (Lura & Sægrov 1991, Webb et al. 1993, Fleming et al. 2000, Glover et al. 2013), and evidence exists that such interbreeding and genetic introgression can reduce the fitness of wild stocks (Fleming et al. 2000, McGinnity et al. 2003, Skaala et al. 2012).

Whilst historically aquaculture production and research efforts focused primarily on salmonid species, culture of other marine fishes, such as Atlantic cod *Gadus morhua* L., has been attempted at various times as a means of diversifying the industry. Although the current scale of cod aquaculture is much lower than that of salmonids, the potential for escapes and subsequent interbreeding between wild and escaped cod may be higher. Atlantic cod have been shown to have a greater motivation to escape net pens than do salmonids, and to escape at a greater relative rate than salmonids (Moe et al. 2007, Hansen et al. 2008, Zimmermann et al. 2012). Moreover, cod, and other marine broadcast spawners readily spawn within sea cages, releasing fertilized eggs into the surrounding ocean (Jørstad et al. 2008, Uglem et al. 2012, Somarakis et al. 2013). Like escaped salmon, escaped cod have been found to occupy the same habitat as their wild conspecifics (Zimmermann et al. 2013), even to the extent of having been found among wild fish in spawning aggregations (Wroblewski et al. 1996, Uglem et al. 2008, Meager et al. 2010). Nevertheless, simply being present in a spawning aggregation does not guarantee spawning success.

Atlantic cod exhibit lek-like mating aggregations (Hutchings et al. 1999, Rose et al. 2008, Meager et al. 2010), with female mate choice apparently based on both visual and acoustic displays, and broadcast spawning of buoyant, planktonic eggs occurs with the selected male in a ventral mount on the female (Brawn 1961, Hutchings et al. 1999). Within spawning aggregations, male cod form dominance hierarchies based on agonistic interaction, usually with the largest males occupying the highest ranks, and access to females and spawning success being related to this hierarchical position (Hutchings et al. 1999, Bekkevold et al. 2002, Bekkevold 2006). Experimental studies have shown that whilst the most dominant males obtain greater access to females and acquisition of ventral mounts, the majority of the egg batches spawned have some degree of multiple paternity, indicating the importance of satellite spawning in the cod mating system (Rakitin et al. 2001, Bekkevold et al. 2002, Herlin et al. 2008). The spawning success of cultured males in competition with wild males in multi-individual groups has been found to be mixed. Skjæraasen & Hutchings (2010) found that the reproductive success of cultured cod in competition with wild cod was 'essentially nil', but in another study, Skjæraasen et al. (2010) observed that cultured cod fertilized approx. 25% of eggs spawned by wild females, but up to 52% of eggs spawned by cultured females.

Taking into account the apparent importance of male dominance hierarchies, courting behaviours and sperm competition in cod mating, we tested the competitive ability of paired cultured and wild male cod in the presence of individual cultured female cod. We did this to remove the effect of multi-male dominance hierarchies, which would exclude a large number of males from spawning; thus, this design should provide further insight into the inter-individual variation in competitive ability between cultured and wild male cod. The behaviour of the females within the trios was also considered because male success has been observed to be dependent upon the type of female with which they spawned (Skjæraasen et al. 2010). We examined whether females exhibited any behavioural preference for either male type and if so, whether this behavioural preference was also reflected in the male's spawning success. We hypothesized that the wild males would be dominant over the cultured males, both behaviourally and in terms of spawning success. We further hypothesized that male spawning success would be influenced by female behavioural preference.

MATERIALS AND METHODS

Experimental fish

Wild cod were collected from Smith Sound in Trinity Bay, Newfoundland (48° 9′ N, 53° 44′ W) (Fig. 1) on 10 and 20 November 2009, using baited cod pots. The cultured cod were the progeny of wild-caught fish from Bay Bulls, Newfoundland, Canada (47° 18′ N, 52° 48′ W) (Fig. 1), and are members of the same population as the wild fish (Beacham et al. 2002, COSEWIC 2010). Both collection areas are designated Northwest Atlantic Fisheries Management Division 3L. The cultured fish were spawned between 13 December 2006 and 27 February 2007 in the Joe Brown Aquatic Research Building (JBARB) at Memorial University of Newfoundland's Ocean Sciences Centre (OSC) in Logy Bay, Newfoundland

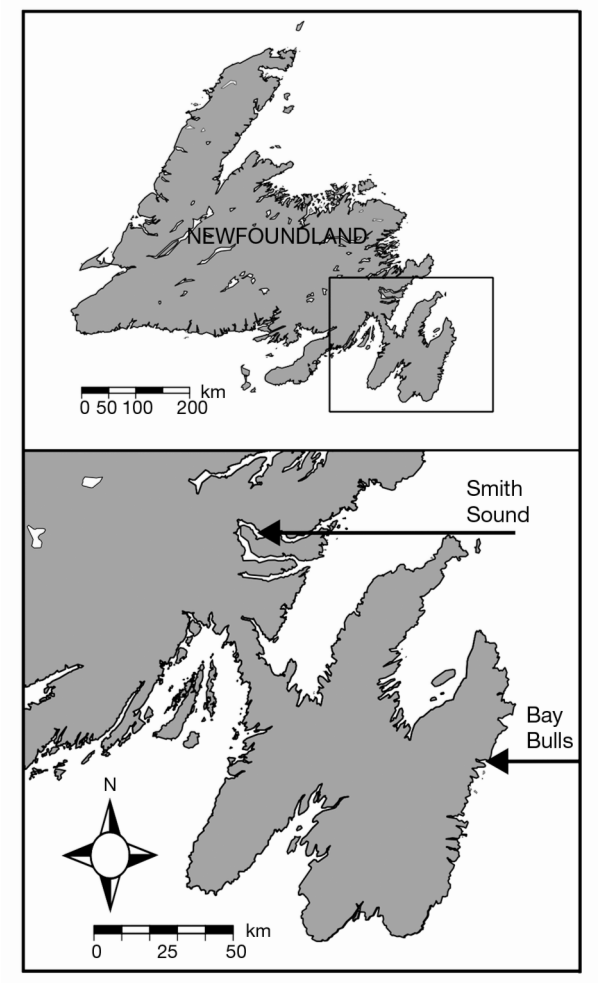

Fig. 1. Locations in Newfoundland, Canada, from which the wild Atlantic cod *Gadus morhua* were captured (Smith Sound; 48° 9′ N, 53° 44′ W), and the cultured cod were obtained (Bay Bulls; 47° 18′ N, 52° 48′ W)

(47° 37′ N, 52° 40′ W), and raised there until they were stocked into sea cages at the Sapphire Sea Farms site in Bay Bulls on the 30 November 2008 (ca. 31 cm total length). On 30 October 2009, cultured cod were collected from the Sapphire Sea Farms cage facility, and transported to the OSC.

The wild and cultured cod were placed in adjacent identical 24.3 m^3 tanks (5.3 m diameter, 1.1 m deep) and acclimated for at least 4 mo; thus, the wild and cultured cod were not exposed to one another prior to the start of experimentation. Both tanks were illuminated with an ambient photoperiod, and supplied with ca. 5–8°C seawater inflows (ca. 1.5–1.8 l s^{-1}) and oxygen supplementation to ensure that oxygen saturation at the outflow was maintained at ≥90%. Approximately 1 wk after the wild cod were collected, passive integrate transponder (PIT) tags (Avid Identification Systems) were inserted into the dorsal musculature under anaesthesia with MS-222 (tricaine methanesulfonate). The cultured cod had been implanted with PIT tags (at ca. 15–20 g body weight) in their abdominal cavities prior to our acquisition of them. All cod were fed a diet consisting primarily of herring *Clupea herengus* L., supplemented with mackerel *Scomber scombrus* L. and squid *Ilex* sp. as available, 3 times per week to satiation. Cultured cod were easily weaned onto this diet over the course of ca. 1 mo.

From mid-February 2010, both the wild and cultured cod were checked weekly for signs of gonad maturation, and the tanks were checked daily for the presence of eggs. Experimentation began once it appeared the majority of fish were in, or near, spawning condition.

Experimental conditions

A trio, consisting of 1 wild male, 1 cultured male, and 1 cultured female, were placed in each of 10 circular experimental tanks; 3 of the tanks were 3.8 m^3 (2.0 m diameter, 1.2 m deep), 3 were 4.6 m^3 (1.8 m diameter, 1.8 m deep) and 4 of the tanks were 1.8 m^3 (1.25 m diameter, 1.5 m deep). Tanks were maintained on natural photoperiod and supplied with ambient flow-through seawater. To increase our sample size, we ran 3 temporal replicates, each using 10 unique trios, giving a total of 30 unique trios (i.e. 1 trio per tank × 10 tanks × 3 temporal replicates). The first temporal replicate began on 18 March 2010, and ran until 13 April 2010. The second temporal replicate ran between 13 and 30 April 2010, and the final replicate ran between 30 April and 27 May 2010.

For each temporal replicate, trios were assembled by haphazardly selecting from their respective holding tanks, the first 10 wild male, 10 cultured male, and 10 cultured female cod that were found to be in, or near spawning condition (i.e. when male's semen was freely released following gentle pressure to ventral surface, and females had developed soft, distended bellies). Subsequently, one of each type was randomly assigned to each of the 10 experimental tanks using a randomization script written in R (R Development Core Team 2011). The females were added to the experimental tanks first, followed by the simultaneous introduction of the paired males approx. 5 min later. Due to low maturation rates, a number of the cultured females and wild males had to be used in more than one round of experimentation (see Table S1 in the Supplement at www.int-res.com/articles/suppl/

m535p197_supp.pdf). In cases where fish were used in more than a single round of experimentation, it was ensured that trios were created using fish which had not previously competed, and that each trio was placed in a tank in which none of the fish had previous experience. Unfortunately, no female wild cod were detected to be in or near spawning condition during the experiment, so the experiment was conducted using only cultured females. In total, of 110 wild cod collected only 4 females were found in spawning condition.

Prior to being added to the experimental tanks, the selected fish were sedated with MS-222, scanned for PIT tag number, weighed (to a precision ± 0.1 g), and measured for total body length (± 0.5 cm) and pelvic fin lengths (fin origin to tip of the longest fin ray [± 0.01 cm]) using digital callipers. Wild males were tagged sinistrally to the origin of their third dorsal fin, and the cultured males dextrally to the origin of their first dorsal fin with 5 cm long yellow T-bar tags (Floy Tag) for visual identification on video (females were not tagged). Although not all trios were filmed, for consistency, all males were tagged. Fish were not observed to interact with these tags during the course of the experiment, and the tags did not appear to cause any stress.

All tanks were affixed with egg collectors, consisting of a surface-skimming drain that emptied into a fine-meshed aquarium net suspended in a 19 l bucket. These egg collectors were checked daily between 10:00 and 12:00 h, and when eggs were detected they were transferred into labeled 1 l beakers. A subsample of the eggs collected from each spawning event was examined under a dissecting microscope to verify that the eggs were at a developmental stage consistent with having been spawned during the preceding 24 h period (Hall et al. 2004). Once verified, eggs were then transferred in their 1 l beakers to a climate-controlled room with the temperature set to 4°C (± 1°C of that of the spawning tanks), and a 12:12 h light:dark cycle. After settling for ca. 15 min, non-viable eggs (i.e. those that had sunk) were discarded, while the viable eggs, which were floating, were retained. Viable eggs were transferred to a new 1 l beaker, and the beaker was filled with ca. 800 ml of filtered seawater. Eggs were attended to daily, and any that sunk to the bottom were removed using a pipette and discarded. Then around half of the water in the beaker was removed and replaced with fresh, filtered seawater. Following ca. 72 h of development, all floating eggs, up to a max. 5 ml, were collected and preserved in 95% ethanol, which was subsequently exchanged twice.

DNA extraction and amplification

DNA was extracted from 25 preserved fertilized eggs from each spawning event and from fin clips from each potential parent, using Promega Wizard SV 96 Genomic DNA Purification kits (Promega catalogue number A2371) following the manufacturer's protocol. Extracted DNA was amplified via polymerase chain reaction (PCR) using the multiplex protocol of Wesmajervi et al. (2006), with some modification: based on preliminary analysis of parents, which were genotyped in duplicate, the gadoid microsatellite *Tch11* (O'Reilly et al. 2000) was dropped from our multiplex as it failed to amplify consistently. Thus our multiplex consisted of the fluorescently end-labelled markers *Gmo8*, *Gmo19*, *Gmo35*, and *Gmo37* (Miller et al. 2000) (Table S2 in the Supplement).

The multiplex PCR mixture consisted of 5 µl Qiagen Multiplex PCR Master Mix (Qiagen), 1 µl 5X Q-Solution (Qiagen), 0.4 µl primer master mix (Table S2 in the Supplement), and 4.8 µl extracted DNA, for a total reaction volume of 10 µl. The thermocycler conditions were: an initial denaturation step of 95°C for 15 min, followed by 40 cycles consisting of 94°C for 35 s, 57°C for 60 s, and 72°C for 30 s. The reaction was terminated by a final extension at 72°C for 10 min, followed by incubation at 4°C.

PCR products were sized on an ABI 3730 DNA Analyzer (Applied Biosystems), allele sizes were calculated against the internal LIZ size standard (GeneScan™ 500 LIZ™ dye Size Standard, Applied Biosystems) and, eletrophorograms were visualized using GeneMapper® v. 4.1 Software (Applied Biosystems). All genotyping was conducted twice, and the accuracy of all allele scorings generated by the software was visually confirmed.

The genotypes of the offspring were compared to that of the known mother and the 2 candidate fathers, and paternity was assigned manually based on exclusion.

Behavioural observations

Axis 210 Network Cameras (Axis Communications) were mounted above 4 of the 10 tanks (the 4 tanks of 1.8 m^3, tanks 4, 5, 7, and 8) (Table S1), such that the entirety of the tank was visible, and the cameras recorded continuously to a networked storage drive for the duration of the experiment. From the video recordings, 3 courting and 4 agonistic behaviours were assessed (Table 1).

Table 1. Behavioural interactions examined during spawning. Coerce is classified as an agonistic type in contrast to the 'paired swim' behaviour described by Brawn (1961) and Hutchings et al. (1999), because it appeared generally to be performed to restrict access to the female, or in some cases, a portion of the tank. The brush type was seen to initiate and accentuate the 'circling' behaviour described by Hutchings et al. (1999), which in turn was part of the 'flaunting display' described by Brawn (1961)

Interaction	Type	Description	Reference
Agonistic	Approach	One fish swimming directly to within one-half-body-length of another stationary fish	Hutchings et al. (1999)
	Chase	One fish swimming towards a swimming fish	Hutchings et al. (1999)
	Prod	Contact between the snout of one fish, and any part of another	Hutchings et al. (1999)
	Coerce	One fish swimming in a manner such that another fish was forced to swim in only a fraction of all potential directions	Brawn (1961), Hutchings et al. (1999)
Courting	Brush	One fish contacts another fish with its side	Hutchings et al. (1999)
	Lateral display	A fish maintains station in front of another stationary fish and extends its median fins	
	Ventral mount	One fish slips under another, grasps it with its pelvic fins and attempts to elicit spawning	Brawn (1961)

Fish were far less active and no spawns were observed during daylight, therefore only the behaviour of the fish during the night before eggs were collected was considered for analysis (i.e. the night in which spawning occurred). Although the whole video was screened, in the majority of cases, the actual release of gametes could not be unambiguously identified. The impacts of this were twofold: firstly, we were unable to examine how acting as the primary male (i.e. the male in the ventral mount with the female) influenced fertilization success, and secondly, we had to quantify the behaviour of the fish over the entire night of spawning. Thus, for each of the behaviour types listed in Table 1, we counted the number of behavioural actions each actor and recipient pair (Fig. 2) exhibited during one, randomly chosen, 5-min block per hour between 20:00 and 06:00 h (i.e. 'at night'). We then used the sum of the behavioural actions of each type of behaviour (all blocks, for all hours), for each actor-recipient pair in the analysis.

Each fish in a trio can act on, and in turn itself be acted upon, by the other 2 fish in the trio; thus, there are 6 potential actor/recipient dyads (Fig. 2). In light of this, the differences in the behaviour of fish of each origin were analyzed using 2 approaches. Firstly, for each of the behaviours listed in Table 1, the recipient of the behavioural events were not considered, and the total number of behavioural action events performed on both potential recipients were summed (Fig. 2). Next, for each of the behaviours listed in Table 1, the number of behavioural events directed at each of the potential recipients were considered separately (Fig. 2).

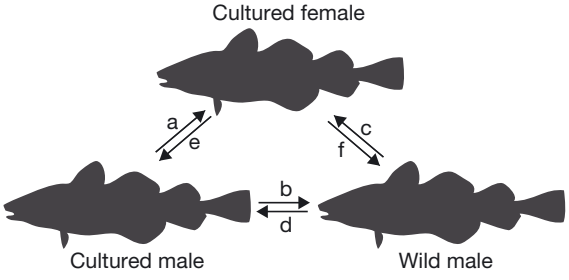

Fig. 2. Actor-recipient behavioural dyads with direction of action labelled a–e. Each fish is capable of acting on either, or both of the other 2 fish in the tank (e.g. for the female, arrows 'e' and 'f'). In turn, each fish can also be acted upon by either or both of the other fish (e.g. for the female, arrows 'a' and 'c'). Total behavioural actions are the sum of all behavioural actions an individual directs at both potential recipients (e.g. for the female, the sum of 'e' and 'f')

We also tested the following possibilities: (1) whether fish of different origins differed in their overall level of behaviour, (2) whether fish of different origins behaved in a qualitatively similar manner, and (3), if the behaviour of an individual in a trio influenced that of the others.

Statistical analysis

We tested for differences in weight, total length, and size-adjusted mean pelvic fin length between the wild males, the cultured males and the cultured females using ANOVA with permutation, using the aovp function from the lmPerm package (Wheeler 2010), and Tukey HSD tests (R Development Core Team 2011), where significant differences were

detected. The mean of the right and left pelvic fin lengths were calculated after they were first individually size-standardized using the formula

$$M_{std} = M_{obs}(51.65/TL_{obs})^{b}$$

where M is the trait measure, 51.65 is the mean total length of all fish, TL is the total length of a fish, b is the trait-specific common within-groups slope, and *obs* and *std* refer to the observed (raw), and the size-standardized measurements, respectively (Reist 1986). Despite heterogeneity of regression slopes between fish origins (wild or cultured), the common within-groups slope for each character was used because this is advised even when such heterogeneity exists (Reist 1986). We ensured the fish for which behavioural data were available were a representative subset of fish in the experiment by comparing their lengths and weights to those of all other fish of their origin in the experiment using paired *t*-tests (all $p > 0.05$) (R Development Core Team 2011).

Linear mixed-effects models (LLM; lme function from the package nlme) (Pinheiro et al. 2013), which can account for repeated and non-independent measures, were used because many trios spawned more than once; several fish were used in more than one round of experimentation and the behaviour of each member of a trio was not independent of that of the other members of the trio (Fig. 2). We assigned each fish a unique ID and these IDs were used in the mixed effects models as the random effects. Omnibus tests for LMM factor significance were performed using Type III ANOVA, and where significant differences were detected, post-hoc analysis using Tukey's HSD was conducted using the function glht in the multcomp package (Hothorn et al. 2008).

Before analyzing all detected spawning events together, we ensured that the spawning success of the wild and cultured males in the trios was unaffected by the different tank size (chi-squared = 5.55, df = 2, p = 0.06) or temporal round (3 experimental rounds) (chi-squared = 0.31, df = 2, p = 0.85) using Type III ANOVA on LMM. We then examined, in all detected spawning events, whether the wild and cultured males differed in their spawning success or in their behaviour. Differences in the spawning success of males of both types were also examined in terms of differences in the number of spawning event 'wins' and 'losses'. In this case, for each spawning event detected, a 'win' was awarded to the male who fertilized the greater proportion of eggs. If the 2 males within a trio fertilized an equal proportion of the eggs in a given spawning event, then neither a 'win' nor 'loss' can be awarded, and that event cannot be evaluated. Using the cultured males as the focal males, this was analyzed using a mixed-effects logistic regression with the IDs of the fish in the trio as the random effect.

The effects of relative size and behaviour on spawning success are reported for the spawning success of the cultured males only. This was done both for consistency and ease of interpretation, and because the spawning success data are proportions, therefore, if an effect is detected for one male, an inverse effect will be seen for the other male. The wild males were on average longer, heavier, and had longer pelvic fins (Tukey's HSD; all $p < 0.01$) (Table 2), than the cultured males and females, which did not differ significantly in these traits (Tukey's HSD; all $p > 0.88$) (Table 2). When examining how size influenced behaviour and spawning success, we considered both the overall size of the males compared to all other males of their origin, as well as differences in size between the 2 males in a trio. We looked at the within-origin effect of size since the purpose of this study was to examine differences between wild and cultured males, and because the significant interaction between size and origin made interpretation uncertain. Subsequently, because females were only able to evaluate and choose between the 2 spawning partners that she was presented, the effect of differences in the size of males within a tank (i.e. the 2 males in actual competition) were examined. Both the raw difference between the males and the $\log_{10}$ ratio of cultured male to wild

Table 2. Mean ± SD of the weight and length of wild and cultured males and cultured females used in the experiment, and ANOVA tests showing differences in weight and length between these groups. Different superscript letters denote significant differences ($\alpha = 0.05$) between groups in post-hoc tests (Tukey's HSD)

	Wild males (n = 16)	Cultured males (n = 22)	Cultured females (n = 19)	*F*	df	p
Weight (g)	2215.2 ± 183.5^{a}	1723.1 ± 91.9^{b}	1794.0 ± 60.4^{b}	9.88	2, 55	< 0.001
Total length (cm)	58.4 ± 1.4^{a}	49.1 ± 0.8^{b}	48.5 ± 0.6^{b}	33.91	2, 51	< 0.001
Mean pelvic fin length (mm)	75.2 ± 7.9^{a}	59.8 ± 6.0^{b}	55.4 ± 5.4^{b}	47.32	2, 49	< 0.001

male size was considered in order to assess the effects of raw, as well as proportional differences in size. We also tested the effect of differences in size between the males of both types and the female in the same manner. An effect of difference in wild and cultured male size on spawning success could indicate size-based dominance, whilst an effect of difference in size between either of the males and the female could indicate size-assortative mating.

We examined the influence of behaviour on the spawning success of the cultured males in the same way we examined the influence of size on their spawning success. That is, we first tested whether the number of behavioural action events performed during the night of spawning by fish of each origin for each behaviour, both when the recipient of the behavioural actions were considered, and when they were not, influenced the spawning success of the cultured male. We then tested the influence of differences in behaviour between the fish in the trio on the spawning success of the cultured male. Again, we first looked at the influence of the raw difference in the number of each type of behavioural action performed between each fish, then at the $\log_{10}$ ratios of cultured male to wild male behavioural actions. We also tested for evidence of female behavioural preference for either male type, and if so, whether this preference was reflective of spawning success.

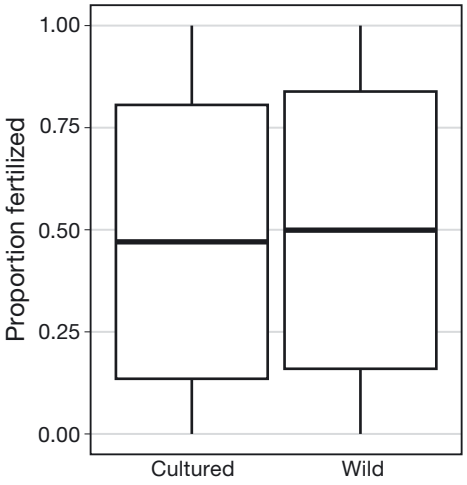

Fig. 3. Proportion of the 25 eggs (genotyped per batch) fertilized by either the wild or cultured male. Data comprise all spawns for each trio that was successful in spawning for all tanks and all rounds of experimentation. Boxes denote the interquartile range, with mid-line representing the median, and whiskers extending to 1.5 times the inter-quartile range

RESULTS

Spawning success

Of the 30 trios, 23 spawned a total of 61 times (mean = 2.65, range = 1–6) (see Table S1 in the Supplement). Across all spawning events there was no significant difference (ANOVA on LMM; chi-squared = 0.22, df = 1, p = 0.64; note that all subsequently mentioned ANOVAs are ANOVAs on LMM) in the proportion of eggs fertilized by the wild (mean 50%, range 0–100) and cultured (mean = 47%, range = 0–100) males (Fig. 3). The paternity of 3% of all eggs could not be resolved because shared alleles in the males precluded the exclusion of either male as the candidate father. The wild male fertilized all eggs in a given batch for 6 spawns across 5 unique trios, while the cultured male sired all eggs within a given batch during 3 spawns across 3 unique trios. There was no significant difference in the number of spawning 'wins' (i.e. when a male fertilized the greater proportion of eggs) between the wild and cultured males (ANOVA; chi-squared = 0.04, df = 1, p > 0.86). Of the 61 detected spawning events, the cultured male won 29, the wild male won 30, and they both fertilized an equal proportion (i.e. 50%) in 2 spawning events. Qualitatively similar results were found in the subset of spawnings for which behavioural data were available.

Relationship between fish size and spawning success

When all spawns were examined, neither the size of the fish nor differences in their size were found to effect spawning success. The weight, total length, and size-standardized mean pelvic fin length of the wild and cultured males were not found to be related to the fertilization success of the cultured male (ANOVAs; df = 1, all p > 0.12). Nor was the weight or length of the female found to affect the proportion spawned by the cultured male (ANOVAs; df = 1, all p > 0.37). There was no evidence to suggest that size-based dominance influenced spawning success because neither raw differences nor $\log_{10}$ ratios in weight, total length, or pelvic fin size between the wild and cultured male had an effect on the spawning success of the cultured male (ANOVAs; df = 1, all p > 0.05). Differences in length, weight and pelvic fin size between the female and either of the males were not found to have a significant effect on cultured male fertilization success (ANOVAs; df = 1, all p > 0.05), thus size-assortative mating was not apparent.

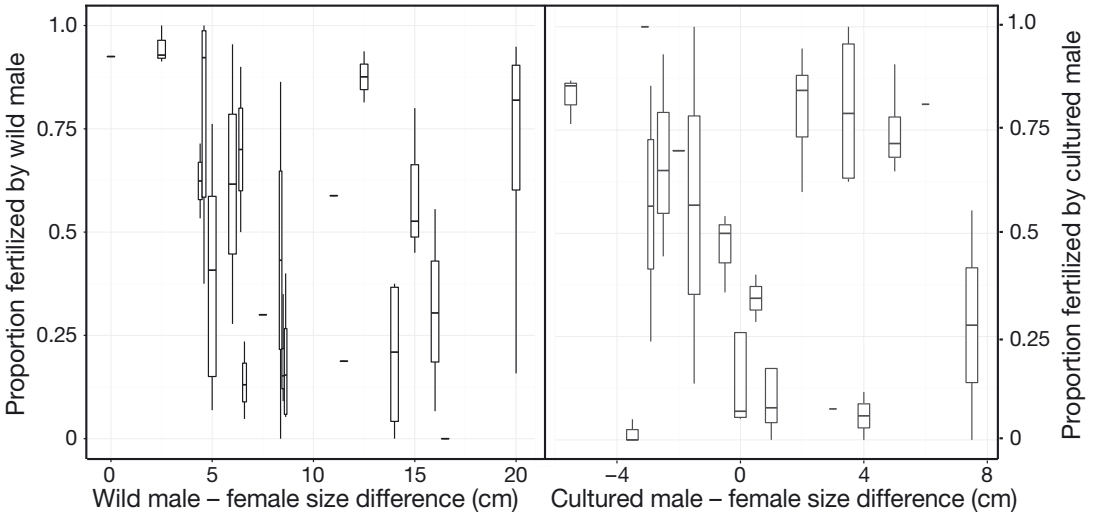

Fig. 4. Relationship between the proportion of eggs fertilized by each male and the difference in male-female size (length of the male minus the length of the female with which he spawned). Negative numbers indicate the female was longer than the male. Boxes denote the interquartile range, with the mid-line representing the median, and whiskers extending to 1.5 times the inter-quartile range. Dashes indicate that a group spawned once (thus no variance calculated)

There was also no evidence (Fig. 4) of a dome-shaped response characteristic of size-assortative mating (i.e. proportional fertilization peaking when the male-female size difference is minimal, and decreasing as the difference in size increases).

Behaviour

Behavioural data were available for 23 spawning events, representing 9 trios; 4 trios were filmed in each of 3 rounds, but some did not spawn (Table S1). Note that the behaviour of one of the females during the night of one of the spawning events was dramatically different from both her behaviour during the other night in which she spawned, as well as from the behaviour of every other female. During the night in question, this female was found to direct an inordinate number of approach and brush behavioural events towards the cultured male in the trio, which in turn had an undue influence on her aggregated behaviours (see Table 1 for description of behaviours). To address this, the data were first analyzed with this aberrant spawning event included, and then with it removed, because this single event was found to drive the majority of the relationships found with female behaviour.

When the recipient of the behavioural action events was not considered, there were significant differences among wild and cultured males and females in every type of behavioural action, apart from ventral mounts (Table 3). Post-hoc analysis revealed that, with the exception of ventral mounts, the cultured males performed significantly more agonistic and courting behavioural events than the females (Table 3). Furthermore, while cultured males tended to also perform more behavioural events than wild males, the only significant difference between the 2 was in the number of brushes (Table 3). The wild males tended to perform more behavioural events than cultured females, but only the difference in the number of approaches was significant (Table 3). Note that the variability of the behavioural data is large in relation to the sample size (see Table 3), which likely accounts for the lack of statistical significance despite relatively large differences in means. These results were not altered by the exclusion of the aberrant spawning event.

Taking the recipient of each behavioural action into account revealed that the cultured males directed more lateral displays, chases, brushes and approaches towards the female than the female directed towards either of the males (Table 4). Additionally, the cultured males directed more coerce behaviour events towards wild males than did the females towards wild or cultured males, and more than the wild males directed towards the females (Table 4). The cultured males also performed more brush behavioural events on the females than the wild males performed on either the females or the cultured males (Table 4). The cultured males approached the wild males more than the females did (Table 4). Overall,

Table 3. Mean ± SD of total number of behavioural actions (defined in Table 1) performed during the night of spawning. Numbers are the sum of the actions an individual directed at both possible recipients. ANOVA on LMM results show differences in behavioural actions between groups. Different superscript letters denote significant differences (α = 0.05) between groups

Behaviour	Cultured male	Wild male	Cultured female	χ^2	p
Total actions	99.0 ± 88.1[a]	53.8 ± 55.3[ab]	15.3 ± 27.0[b]	38.23	<0.001
Agonistic behaviours					
Total agonistic	63.8 ± 50.7[a]	38.8 ± 36.6[ab]	10.4 ± 15.5[b]	43.04	<0.001
Approach	41.0 ± 30.4[a]	28.0 ± 25.7[a]	6.9 ± 11.8[b]	45.18	<0.001
Chase	3.9 ± 5.8[a]	1.5 ± 2.9[ab]	0.4 ± 1.1[b]	20.18	<0.001
Prod	8.2 ± 9.4[a]	4.7 ± 6.1[ab]	1.5 ± 2.6[b]	21.02	<0.001
Coerce	10.75 ± 12.4[a]	4.6 ± 7.0[ab]	1.7 ± 2.4[b]	25.12	<0.001
Courting behaviours					
Total courting	35.2 ± 40.8[a]	15.0 ± 22.5[ab]	4.9 ± 12.4[b]	24.26	<0.001
Brush	19.0 ± 19.4[a]	8.2 ± 10.0[b]	4.2 ± 12.1[b]	22.84	<0.001
Lateral display	15.9 ± 23.2[a]	6.4 ± 15.0[ab]	0.7 ± 2.1[b]	18.61	<0.001
Ventral mount	0.3 ± 1.1	0.5 ± 1.3	0.02 ± 0.2	2.43	0.296

the cultured males were observed to direct significantly more agonistic behaviour towards females than the females did to either male type (Table 4). The cultured males also directed more overall courting towards females than did the wild males (Table 4), while females showed no significant preference for either male type. Exclusion of the aberrant spawning event did not affect these results.

The ratio of total agonistic to total courting behavioural events revealed no significant differences in the manner in which individuals of different origins interacted (including and excluding aberrant spawning events) (ANOVAs; all $p > 0.05$). Interestingly, within trios, there was a significant relationship between the total number of behavioural events (t = 2.9, df = 12, $p < 0.05$), and the total agonistic behavioural events (t = 3.6, df = 12. $p < 0.01$) performed by one male and the number performed by the other male, but there was no relationship between the number of total courting behavioural events they performed (t = 2.0, df = 12, $p > 0.063$). Furthermore, there was no relationship, between the total number of behavioural events, the total agonistic behavioural events, and the total courting behavioural events performed by either male in a trio and the female in that trio (all $p > 0.094$).

Relationship of behaviour to body and pelvic fin size

Neither male length, weight, nor standardized mean pelvic fin size of the wild male had a statistically significant effect on the total number of behavioural events, the total number of agonistic events,

Table 4. Differences in the behavioural interactions (defined in Table 1) among the fish in the trios. WM, CM and Fem denote wild male, cultured male, and cultured female, respectively. Arrows represent the direction of behavioural interaction, with actor on the left, and recipient on the right. > indicates that the number of behavioural actions performed by the first actor/recipient pair was greater than the number performed by the second pair. All entries are significant at α = 0.05. * denotes entries significant only after the aberrant spawning was excluded from analysis

Behaviour	Significant differences
Total actions	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM
Agonistic behaviours	
Total agonistic	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM CM ⇒ WM > Fem ⇒ CM*
Approach	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ WM > Fem ⇒ WM
Chase	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM
Prod	–
Coerce	CM ⇒ WM > WM ⇒ Fem CM ⇒ WM > Fem ⇒ CM CM ⇒ WM > Fem ⇒ WM
Courting behaviours	
Total courting	CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM CM ⇒ Fem > WM ⇒ Fem
Brush	CM ⇒ Fem > WM ⇒ Fem CM ⇒ Fem > WM ⇒ CM CM ⇒ Fem > CM ⇒ WM* CM ⇒ Fem > Fem ⇒ CM CM ⇒ Fem > Fem ⇒ WM
Lateral display	CM ⇒ Fem > Fem ⇒ WM
Ventral mount	–

the total number of courting events or the number of each of the individual types of behavioural events performed when the recipient of the interaction was not considered (ANOVAs; df = 1, all $p > 0.33$). Likewise, wild male size had no effect on either the raw differences in the number of each type of behavioural events performed between the cultured and wild male in a trio, or on the ratio of the number of behavioural events between the cultured and wild male in a trio (ANOVAs; df = 1, all $p > 0.54$).

This pattern was similar for cultured males, with the exception of a negative relationship between their total length, and the number of chases observed when the recipients were not considered (ANOVA; chi-squared = 6.82, df = 1, $p < 0.01$). However, this relationship appeared to be driven by the smallest male studied having performed the greatest number of chases of all cultured males, and when the one spawning in which he participated was removed the relationship became non-significant (ANOVA; chi-squared = 1.79, df = 1, $p > 0.18$).

For females, there was some evidence of positive relationships between their size and the number of total courting, brush and chase behavioural events (ANOVAs; df = 1, all $p < 0.05$). When the aberrant spawning event was removed from the analysis none of the significant relationships remained.

Neither raw or $\log_{10}$ ratios of differences in weight and length between the wild and cultured male, or between the female and either of the wild or cultured males, had a significant effect on the absolute number of, or the difference in the number of individual or aggregated behavioural events performed (ANOVAs; df = 1, all $p > 0.11$).

Relationship between behaviour and spawning success

No relationship between female behaviour and the spawning success of either cultured or wild males was found after the removal of the aberrant spawning event (ANOVAs; df = 1, all $p > 0.46$). Cultured male spawning success however, was positively related to the total number of brush behaviours they exhibited (to ANOVA; chi-squared = 6.64, df = 1, $p < 0.01$), as well as to the total number of agonistic (ANOVA; chi-squared = 5.71, df = 1, $p < 0.05$) and approach (ANOVA; chi-squared = 9.09, df = 1, $p < 0.01$) behavioural actions performed by the wild male. When the direction of interaction was considered, it was the brushes (ANOVA; chi-squared = 7.75, df = 1, adjusted $p < 0.05$) and approaches (ANOVA; chi-squared = 7.10, df = 1, adjusted $p < 0.01$) that the wild male performed on the female that had the positive effect on cultured male spawning success. There were no other significant relationships between male behaviour and spawning success.

DISCUSSION

Relation of findings to Atlantic cod mating system

Contrary to our hypothesis, larger or more aggressive males did not enjoy greater spawning success. Finding equality in the spawning success of male wild and cultured cod is unique to this experiment, and could be the result of the interplay between the cod mating system and our experimental setup. Male cod typically form dominance hierarchies several weeks prior to the first spawning event (Brawn 1961, Hutchings et al. 1999) and previous researchers have assigned male rank within a dominance hierarchy based on spawning success or on relative levels of agonistic behaviour (Brawn 1961, Hutchings et al. 1999, Bekkevold et al. 2002). Both spawning success and male rank seem to be highly positively correlated to one another, and to body size (Brawn 1961, Hutchings et al. 1999, Bekkevold et al. 2002). While dominance hierarchies may have formed prior to spawning in our experiment, they were not detected in the behavioural analysis. In contrast to other studies, we found a lack of relationship between agonistic behaviour observed during the night of spawning, spawning success and body size. This is suggestive evidence that behavioural dominance during the night of spawning did not influence the outcome of spawnings in this experiment, and also that dominance dyads may not have formed within the trios. Given that Skjæraasen et al. (2010) and Skjæraasen & Hutchings (2010) also found no relationship between male size and dominance rank, it may be that competition between cultured and wild male cod leads to a breakdown of size-stratified dominance ranks, and thus lack of relationship between male size and dominance rank is the norm in cultured/wild interactions. This has some support in the results of Skjæraasen & Hutchings (2010), who found a significant relationship between wild male length (but not weight), condition or pelvic fin length and dominance rank when the much smaller cultured males were excluded from their analysis. This suggests that something particular to the cultured fish was causing the breakdown of the typically size-stratified dominance hierarchy.

Abnormal cultured male behaviour has been observed during mating competition with wild males in salmonids. The cultured males do not follow the usual agonistic exchange typical among wild males, and while cultured and wild male salmonids show similar levels of aggression, the cultured individuals do not appropriately cede victory (Fleming & Gross 1993, Fleming et al. 1996, 1997). A breakdown of the size-stratified dominance hierarchy typically observed in male cod could occur if wild and cultured male cod also have similar differences in their response thresholds when evaluating competitors, the point at which they switch from display to overt, physical aggression, or the point at which they cease physically contesting interactions or cede victory. Under such a scenario, wild males may trade current for future reproductive success, and/or they may choose to adopt alternate mating strategies and act as a satellite spawner. This is supported by Skjæraasen & Hutchings (2010) and Skjæraasen et al. (2010) who found that across all males in their studies, male agonistic behaviour, but not body size, is positively related to reproductive success; dominance hierarchies existed, but were stratified based on behaviour. Skjaeraasen et al. (2010) and Skjaeraasen & Hutchings (2010)'s findings of dominance hierarchies based on behaviour are not consistent with our results. We found that neither levels of agonistic behaviour performed during the night of spawning, nor body size, had an effect on the spawning success of males of either origin.

An alternative explanation for our findings is that lack of dominance and effect of size on spawning success may be a feature of competitive interaction in cod trios. Using an experimental set up similar to ours, Rakitin et al. (2001) explicitly tested for and found no effect of size on spawning success in wild male cod. They found that the male in the trio that fertilized the greater proportion of eggs alternated randomly between batches of eggs, and also that there was no association between activity level and fertilization success, which could indicate lack of female mate choice. Similarly, Skjæraasen (2003) also found no relationship between male size and fertilization success for trios of both wild and cultured males tested separately. However, Skjæraasen (2003) did find a relationship between male behaviour and spawning success. Whilst this may explain why we saw no evidence of positive size-assortative mating, which has been reported elsewhere (e.g. Bekkevold et al. 2002), these results differ intrinsically from ours. Skjæraasen (2003) found evidence of inter-batch consistency in spawning success for both wild and cultured males, which we also observed to some degree (e.g. wild male no. 13 with female no. 1, wild male no. 9 with female no. 15, and wild male no. 6 with female no. 20) (Fig. 5). Taken together with the fact that, unlike Skjæraasen (2003), we saw no evidence that dominance played a role in determining the outcome of mating competition, this intra- and inter-trio consistency indicates that female mate choice could have played a role in shaping the outcome of our experiment. However, the characteristics on which the females were basing their choices are not obvious.

Courtship in cod is behaviourally complex, involving visual and acoustic displays, and female mate choice may be based on cues from any or all of these

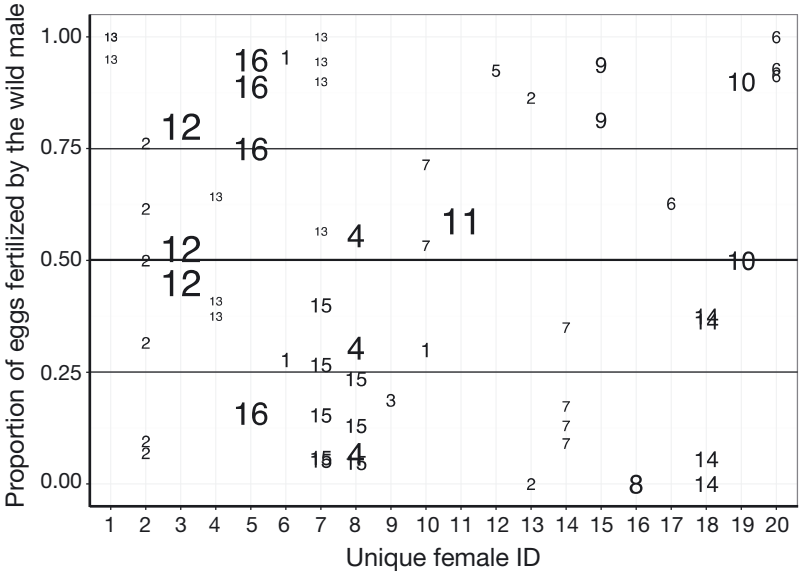

Fig. 5. Intra- and inter-trio spawning success of wild males. The spawning success of the cultured males is not shown, because it was not used in more than one trial, and thus does not show inter-trio variability. However, within each trio, the spawning success of the cultured males is the inverse of that of the wild male. The *y*-axis is the proportion of eggs fertilized by the wild male, while the *x*-axis is the unique identity of the female with which a male spawned. Individual wild males are plotted using unique numbers, and the font size is proportional to the weight of that male. Each point is reflective of the proportion of eggs fertilized by a wild male in one spawning event with the female indicated on the *x*-axis. More than one unique number above a female indicates she was used in more than one trial; the unique ID of a wild male occurring above more than one unique female indicates that he was used in more than one trial

(Brawn 1961). In our experiment, in addition to having no effect on agonistic interaction, body size and pelvic fin length had no effect on courting behaviour or on spawning success. Only courting behaviours were found to influence male reproductive success. The cultured males performed significantly more courting behavioural events than the wild males. In particular, the cultured males directed a significantly greater proportion of their courting behavioural actions towards the females than they did towards the wild males, while the wild males directed a statistically equal number of courting behavioural events towards both the cultured males and females. This finding that wild and cultured males differed in the number of courting displays they exhibited as well as to whom they directed them, is in contrast to the results of Skjæraasen et al. (2010), who found that wild and cultured males both directed more courting events towards other males, than they did towards females, which the authors attributed to males courting fish in their vicinity. While male cod are capable of sex determination, male-male courting appears common, and sexual recognition often does not occur until after a behaviour or physical contact has been initiated (Skjæraasen et al. 2010). Our findings suggest that the cultured males were able to visually distinguish between the female and the wild male, while the wild males were unable to visually determine the sex of the cultured fish. This may have been because the wild and cultured males had different search images for ripe females based on the condition of the females with which they have previous experience. The mean condition of the cultured males in our study (mean Fulton's K = 1.41), was greater than the mean condition of the wild females in Skjæraasen & Hutchings (2010) (mean K = 1.10), and Skjæraasen et al. (2010) (mean K = 1.06), and this may have led the wild males to confuse the cultured males and females and to behave inappropriately towards both. We found the number of brush and approach behavioural events the wild males directed towards the female had a negative effect on the wild males' spawning success, suggesting that the behaviour of the wild males towards the females may not have been appropriate and that the females were selecting against them based on this. It was impossible to sex the cod prior to maturation, and thus male and female cod of each type were housed communally, which may have led to the cultured males having an inherent advantage through prior exposure.

We found no evidence for our second hypothesis either. Despite differences in male behaviour towards females, the females did not differ in the number of agonistic or courting behaviours directed towards either male type indicating they had no behavioural preference for males of either origin. However, female mate choice may be mediated by behaviours not quantified, such as tendency to break away from ventral mounts, and decisions of whether or not to release eggs.

In addition to prior exposure to ripe females, prior spawning experience may have also influenced the spawning success of the fish in our study. Growth rate, while highly variable, is generally slower and because age at maturity is directly related to growth rate (Thorpe 2004), age at maturity is consequently higher in wild (Knickle & Rose 2013) than in cultured cod (Svåsand et al. 1996). Thus, wild cod mature at a greater age, and at a slightly larger body size than do cultured cod. If past spawning experience improves male reproductive success, a smaller cultured male cod with more seasons of spawning experience may have higher reproductive success than a larger, less experienced, wild fish. Such an effect has been documented in the Pecos pupfish *Cyprinodon pecosensis* (Echelle & Echelle, 1978), wherein spawning success increased with experience, independent of male body size (Kodric-Brown 1995). Skjæraasen et al. (2008), found that repeat-spawning cultured cod males invest more in their drumming muscle mass and less in the length of their pelvic fins than do recruit spawners, while the opposite is seen in wild males. This could indicate that, in an effort to increase their spawning success, experienced males are able to tailor their displays and/or secondary sexual characteristics to either the environment they experience or to the preference of females. While we do not know the exact age or spawning history of the wild fish in this study, based on their size they are likely a mixture of naive and repeat spawners (Knickle & Rose 2013). It is likely that the cultured females are naive spawners, but a proportion of the cultured males may have matured the previous year.

The importance of multiple paternity in determining the outcome of this study, and in the mating system of cod cannot be overstated. Multiple paternity in batches of eggs appears to be the norm in cod under tank-based experimental conditions, and likely also in the wild (e.g. Hutchings et al. 1999, Rakitin et al. 2001, Bekkevold et al. 2002). In the current study, the success of both the wild and cultured males when acting as the satellite male could be quite high (at least 50%); in the absence of visual observation of all spawnings, it cannot be concluded if the fertilization success of the satellite male exceeded that of the primary male.

Rowe et al. (2008) found that while mating success of males within spawning groups is highly skewed, and males that are larger and more aggressive generally sire a greater proportion of eggs, some males are able to sire offspring without courting females or aggressively competing with fellow males. These authors suggest that not only is this possible evidence for alternate mating tactics in cod, but also that this is the cause of the statistical breakdown of a relationship between morphological and behavioural correlates, and spawning success. In our experiment, this hypothesis can be taken a step further. In experiments with more than 4 males in competition, one or more males are generally fully excluded from spawning by the agonistic behaviour of the dominant males (Bekkevold et al. 2002, Skjæraasen & Hutchings 2010). In our experiment, in which there were only 2 males, once either of the males paired with the female in a ventral mount, there was nothing to prevent the other from satellite spawning. This illustrates a very important assumption within this, and some other studies: that the male that was genetically detected to fertilize the greater proportion of eggs was presumed to be the primary spawner (i.e. the male ventrally mounted to the female). While this is generally found to be true in other studies, it cannot be positively concluded that the satellite spawner could not have obtained greater fertilization success than the primary spawner through sperm competition, genetic incompatibilities, or mis-timing of gamete release by the primary male (Fleming et al. 1996, Weir et al. 2004, Berejikian et al. 2009). Genetic incompatibilities cannot be ruled out either as having influenced fertilization success, however such evidence is weak. Rudolfsen et al. (2005) assert that finding no optimal male for all females is indicative of genetic incompatibility, and we found that fertilization success of wild male 13 with female 4 was generally lower than his success with either females 1 or 7 (Fig. 5), which supports this assertion. However it must be noted that his fertilization success in the spawning event with the highest fertilization success with female 4 was actually higher than that observed in the spawning event with the lowest fertilization success with female 7. While this finding could be suggestive of genetic incompatibility, alternative explanations such as female choice or timing of gamete release cannot be excluded.

Potential for introgression

This study is the first to show that in the absence of multi-male dominance hierarchies, the spawning success of cultured male cod is equal to that of wild males, despite these first-generation cultured cod differing both behaviourally (this study) and morphologically (Wringe 2015) from wild fish of the same source population. These results also provide further evidence that interbreeding between wild and escaped cultured cod is likely. It is also probable that through both intentional and unintentional selection within the culture environment, these differences will become magnified in future generations.

The use of only cultured females in this experiment may cause an overestimation of cultured male success, given that the spawning success of cultured male cod in competition with wild males has been found to be higher when they mate with cultured rather than with wild females (Skjæraasen et al. 2010). However, when considering risk of introgression, even low cultured male fertilization success, presumably such as may be attained through satellite spawning, cannot be discounted, and our results show that both the cultured and wild males took part in the majority of spawning events. Nonetheless, there is evidence to suggest that cultured males may be excluded even from satellite spawning in the wild. Tagging studies have shown that after simulated escape, the habitat use of cultured male and female cod generally overlaps with that of wild cod (Uglem et al. 2008, Meager et al. 2009, 2010, Zimmermann et al. 2013). However, within spawning aggregations, the distribution of the cultured males was physically separated from that of the wild males and the cultured males appeared to be excluded from the spawning arenas (Meager et al. 2009, Meager et al. 2010). These studies did find that female cultured cod were associated with the wild males in the spawning aggregations, and the results of our study, along with those of Skjæraasen et al. (2010) demonstrate that wild males will readily spawn with cultured females suggesting that escaped cultured females may act as the primary vector of introgression as previously observed in Atlantic salmon (Fleming et al. 1996, 2000).

Caveats aside, the lack of clear dominance, either behaviourally or through monopolization of spawning events by either the wild or cultured males, while still finding some consistency in intra- and inter-trio fertilization success, suggests that the competitive ability of individual males is quite varied. Thus, in the case of a large-scale escape event, the likelihood exists that some fraction of the male escapees may be competitively superior to their wild conspecifics and hybridization between them and wild females may occur. In fact, given that cod will spawn within cages

and the resultant eggs will 'escape' and develop in the wild (Jørstad et al. 2008, 2014), exposure to the wild environment may result in 'farmed' offspring possessing a wild-type phenotype which may be inherently as fit as their wild counterparts. This may occur through some combination of a plastic phenotypic response to the wild environmental conditions or through a different selection regime in the wild which may result in the survival of a portion of the 'farmed' offspring most akin to their wild counterparts (phenotypically and genetically). Furthermore, for cod that escape from culture, their potential to hybridize may also increase in subsequent spawning seasons if experience plays a role in determining success, and as the escapees become larger and as their external morphology converges on that of the wild-type phenotype.

Acknowledgements. The authors acknowledge the contributions of Ed Trippel to the planning and design of the experiment. Corinne Conway was indispensible throughout the project, and was a great asset. John Brattey and Dennis Ivany helped in the collection of the wild cod. The assistance of Beth Perry and the staff at the Genomics and Proteomics Laboratory at Memorial University was invaluable in performing the genetic analyses. We also thank the members of the Fleming and Purchase labs for their assistance and friendship. This paper was much improved by the comments of several anonymous reviewers. Natural Sciences and Engineering Research Council funded this research through a strategic grant to I.A.F. and C.F.P., and the Research and Development Corporation of Newfoundland provided additional support to B.F.W.

LITERATURE CITED

Beacham TD, Brattey J, Miller KM, Le KD, Withler RE (2002) Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation. ICES J Mar Sci 59:650–665

Bekkevold D (2006) Male size composition affects male reproductive variance in Atlantic cod *Gadus morhua* L. Spawning aggregations. J Fish Biol 69:945–950

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

Berejikian BA, Van Doornik DM, Scheurer JA, Bush R (2009) Reproductive behavior and relative reproductive success of natural- and hatchery-origin Hood Canal summer chum salmon (*Oncorhynchus keta*). Can J Fish Aquat Sci 66:781–789

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

COSEWIC (2010) COSEWIC assessment and status report on the Atlantic cod *Gadus morhua* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa

Dauer MB, Seamons TR, Hauser L, Quinn TP, Naish KA (2009) Estimating the ratio of hatchery-produced to wild adult steelhead on the spawning grounds using scale pattern analyses. Trans Am Fish Soc 138:15–22

Fleming IA, Einum S (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES J Mar Sci 54:1051–1063

Fleming IA, Gross MR (1993) Breeding success of hatchery and wild coho salmon (*Oncorhynchus kisutch*) in competition. Ecol Appl 3:230–245

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 33:893–905

Fleming IA, Lamberg A, Jonsson B (1997) Effects of early experience on the reproductive performance of Atlantic salmon. Behav Ecol 8:470–480

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

Glover KA, Pertoldi C, Besnier F, Wennevik V, Kent M, Skaala O (2013) Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. BMC Genet 14:74–93

Gross MR (1998) One species with two biologies: Atlantic salmon (*Salmo salar*) in the wild and in aquaculture. Can J Fish Aquat Sci 55:131–144

Hall TE, Smith P, Johnston IA (2004) Stages of embryonic development in the Atlantic cod *Gadus morhua.* J Morphol 259:255–270

Hansen LA, Dale T, Damsgård B, Uglem I, Aas K, Bjørn PA (2008) Escape-related behaviour of Atlantic cod, *Gadus morhua* L., in a simulated farm situation. Aquacult Res 40:26–34

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

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

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

Jensen Ø, Dempster T, Thorstad EB, Uglem I, Fredheim A (2010) Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. Aquacult Environ Interact 1:71–83

Jørstad KE, Van Der Meeren T, Paulsen OI, Thomsen T, Thorsen A, Svåsand T (2008) 'Escapes' of eggs from farmed cod spawning in net pens: recruitment to wild stocks. Rev Fish Sci 16:285–295

Jørstad KE, Otterå H, van der Meeren T, Dahle G, Paulsen OI, Bakke G, Svåsand T (2014) Genetic marking of farmed Atlantic cod (Gadus morhua L.) and detection of escapes from a commercial cod farm. ICES J Mar Sci 71: 574–584

Knickle DC, Rose GA (2013) Comparing growth and maturity of sympatric Atlantic (*Gadus morhua*) and Greenland (*Gadus ogac*) cod in coastal Newfoundland. Can J Zool 91:672–677

Kodric-Brown A (1995) Does past reproductive history predict competitive interactions and male mating success in pupfish? Anim Behav 50:1433–1440

Lura H, Sægrov H (1991) Documentation of successful spawning of escaped farmed female Atlantic salmon, *Salmo salar*, in Norwegian rivers. Aquaculture 98:151–159

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 B 270: 2443–2450

Meager JJ, Skæraasen JE, Fernö A, Karlsen O, Løkkeborg S, Michalsen K, Utskot SO (2009) Vertical dynamics and reproductive behaviour of farmed and wild Atlantic cod *Gadus morhua.* Mar Ecol Prog Ser 389:233–243

Meager JJ, Skjæraasen JE, Fernö A, Løkkeborg S (2010) Reproductive interactions between fugitive farmed and wild Atlantic cod (*Gadus morhua*) in the field. Can J Fish Aquat Sci 67:1221–1231

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

Naylor R, Burke M (2005) Aquaculture and ocean resources: Raising tigers of the sea. Annu Rev Environ Resour 30: 185–218

O'Reilly PT, Canino MF, Bailey KM, Bentzen P (2000) Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. J Fish Biol 56:1074–1086

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2013) Nlme: linear and nonlinear mixed effects models. R package version 3.1-111. http://CRAN.R-project.org/package=nlme

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

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

Reist JD (1986) An empirical-evaluation of coefficients used in residual and allometric adjustment of size covariation. Can J Zool 64:1363–1368

Rose GA, Bradbury IR, deYoung B, Fudge SB and others (2008) Rebuilding Atlantic cod: lessons from a spawning ground in coastal Newfoundland. In: Kruse GH, Drinkwater K, Ianelli JN, Link JS, Stram DL, Wespestad V, Woodby D (eds) Resiliency of gadid stocks to fishing and climate change: 24th Lowell Wakefield Fisheries Symposium. Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks, AK

Rowe S, Hutchings JA, Skjæraasen JE, Bezanson L (2008) Morphological and behavioural correlates of reproductive success in Atlantic cod *Gadus morhua.* Mar Ecol Prog Ser 354:257–265

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

Skaala O, Glover KA, Barlaup BT, Svåsand T, Besnier F, Hansen MM, Borgstrøm R (2012) Performance of farmed, hybrid, and wild Atlantic salmon (*Salmo salar*) families in a natural river environment. Can J Fish Aquat Sci 69: 1994–2006

Skjæraasen JE (2003) Implications of behaviour for maturation and spawning in cod. PhD thesis, University of Bergen

Skjæraasen JE, Hutchings JA (2010) Shifting reproductive success in a shoal of Atlantic cod, *Gadus morhua* L. Environ Biol Fishes 88:311–318

Skjæraasen JE, Meager JJ, Karlsen O (2008) The expression of secondary sexual characteristics in recruit- and repeat-spawning farmed and wild Atlantic cod (*Gadus morhua*). ICES J Mar Sci 65:1710–1716

Skjæraasen JE, Meager JJ, Karlsen O, Mayer I, Dahle G, Rudolfsen G, Fernö A (2010) Mating competition between farmed and wild cod *Gadus morhua.* Mar Ecol Prog Ser 412:247–258

Somarakis S, Pavlidis M, Saapoglou C, Tsigenopoulos CS, Dempster T (2013) Evidence for 'escape through spawning' in large gilthead sea bream *Sparus aurata* reared in commercial sea-cages. Aquacult Environ Interact 3: 135–152

Svåsand T, Jørstad KE, Otterå H, Kjesbu OS (1996) Differences in growth performance between Arcto-Norwegian and Norwegian coastal cod reared under identical conditions. J Fish Biol 49:108–119

Svåsand T, Kristiansen TS, Pedersen T, Salvanes AGV, Engelsen R, Nævdal G, Nødtvedt M (2000) The enhancement of cod stocks. Fish Fish 1:173–205

Thorpe JE (2004) Life history responses of fishes to culture. J Fish Biol 65:263–285

Thorstad EB, Fleming IA, McGinnity P, Soto D, Wennevik V, Whoriskey F (2008) Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. NINA Special Report 36, Norwegian Institute for Nature Research, Trondheim

Uglem I, Bjørn PA, Dale T, Kerwath S and others (2008) Movements and spatiotemporal distribution of escaped farmed and local wild Atlantic cod (*Gadus morhua* L.). Aquacult Res 39:158–170

Uglem I, Knutsen Ø, Kjesbu OS, Hansen ØJ and others (2012) Extent and ecological importance of escape through spawning in sea-cages for Atlantic cod. Aquacult Environ Interact 3:33–49

Webb JH, Youngson AF, Thompson CE, Hay DW, Donaghy MJ, McLaren IS (1993) Spawning of escaped farmed Atlantic salmon, *Salmo salar* L., in western and northern Scottish rivers: egg deposition by females. Aquacult Res 24:663–670

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

Wesmajervi MS, Westgaard JI, Delghandi M (2006) Evaluation of a novel pentaplex microsatellite marker system for paternity studies in Atlantic cod (*Gadus morhua* L.). Aquacult Res 37:1195–1201

Wheeler B (2010) Lmperm: Permutation tests for linear models. R package version 1.1-2. h ttp://cran. r-project.org/web/packages/lmPerm/vignettes/lmPerm.pdf

Wringe B, Fleming I, Purchase C (2015) Rapid morphological divergence of cultured Atlantic cod of the northwest Atlantic from their source population. Aquacult Environ Interact 7: in press

Wroblewski JS, Smedbol RK, Taggart CT, Goddard SV (1996) Movements of farmed and wild Atlantic cod (*Gadus morhua*) released in Trinity Bay, Newfoundland. Mar Biol 124:619–627

Zimmermann EW, Purchase CF, Fleming IA (2012) Reducing the incidence of net cage biting and the expression of escape-related behaviors in Atlantic cod (*Gadus morhua*) with feeding and cage enrichment. Appl Anim Behav Sci 141:71–78

Zimmermann EW, Purchase CF, Fleming IA, Brattey J (2013) Dispersal of wild and escapee farmed Atlantic cod (*Gadus morhua*) in Newfoundland. Can J Fish Aquat Sci 70:747–755

Editorial responsibility: Jana Davis, Annapolis, Maryland, USA

Submitted: October 29, 2014; Accepted: June 27, 2015
Proofs received from author(s): August 24, 2015