

Shifting reproductive success in a shoal of Atlantic Cod, *Gadus morhua* L.

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Abstract Individual and temporal variability in the spawning behaviour of Atlantic Cod (*Gadus morhua* L.) was examined by studying wild and reared individuals from the same putative population in a common spawning arena. The number of eggs fertilized per male was best explained by the number of aggressive interactions initiated by each male. A strong skew in male reproductive success and a temporal shift in male fertilization success were also observed. The latter co-occurred with an apparent similar temporal shift in the reproductive behaviour of the individual males. We hypothesize that energetic costs associated with reproduction, potentially mediated by sperm depletion in dominant individuals, may be responsible for temporal shifts in mating behaviour and reproductive success of male Atlantic Cod. We suggest that this hypothesis merits further study.

Keywords Mating system · Aggression · Fertilization success · Dominance · Spawning behaviour

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Introduction

Broadcast-spawning fish, such as gadoids, aggregate in large shoals during reproduction (Brander 1994) wherein considerable amounts of small, positively buoyant pelagic eggs are released by females (Kjesbu 1989; Blanchard et al. 2003). Traditionally, such systems have been thought to provide little opportunity for mate choice (e.g., Berglund 1997; Stockley et al. 1997). However, at least one broadcast spawner, the Atlantic Cod (*Gadus morhua* L.), appears to have a much more complex mating system than previously thought. During spawning, male cod exhibit distinctive behaviours that appear to be associated with both visual and auditory courtship displays (Brawn 1961; Hutchings et al. 1999; Rowe and Hutchings 2004) and frequent antagonistic intra-sexual interactions (Brawn 1961; Hutchings et al. 1999). It has been argued that this behavioural repertoire, coupled with acoustic communications (Rowe and Hutchings 2008), may provide a basis for female choice, resulting in large mating skew and high variance in reproductive success among males (Hutchings et al. 1999; Bekkevold et al. 2002; Rowe et al. 2008). Coupled with field studies of migration patterns (Morgan and Trippel 1996; Nordeide 1998) and spatio-temporal stability of spawning shoals (Robichaud and Rose 2001, 2003; Windle and Rose 2007), this body of research has lent support to the hypothesis that cod mate in leks (Morgan and Trippel 1996; Hutchings et al.

1999; Nordeide and Folstad 2000), a mating system in which sexual selection is typically strong and individual variability in fertilization success high (Bradbury et al. 1985; Alatalo et al. 1992).

In the present study, wild and domesticated cod stemming from the same putative population were introduced into a common spawning arena to quantify individual variability in the phenotypic and behavioural characteristics most closely linked with male reproductive success.

Methods

Study population

The reared cod were first-generation offspring of wild-caught cod from the NAFO (Northwest Atlantic Fishery Organization) subdivision 4X (41° N, 64° W) off southwestern Nova Scotia, Canada. Eggs were collected from the parental stock which was allowed to breed undisturbed in the extremely large (684 m³) Pool Tank in the Aquatron Facility at Dalhousie University in February–March 2001. After collection, eggs were kept in incubators until hatching in mid-April 2001. Larvae were fed a diet of natural zooplankton until mid-June when they were fed dry pellets. Larvae and juveniles experienced a simulated natural photoperiod for the entire duration of the pre-experimental period (Halifax; 44° 40' N, 63° 36' W). In February 2002, the reared cod were transferred to the 10-m deep, circular Tower Tank (117 m³) at the Aquatron. At the beginning of the experimental period in 2003, individuals were selected at random from a pool of approximately 150 reared cod. Wild cod were caught from NAFO subdivision 4X by a commercial vessel, transported to Dalhousie University, and placed in the Pool Tank in January 2003.

The experiment

On February 21, all cod were sedated with Metakain (0.5 g L⁻¹) and measured for total length and pelvic fin length (fin base to the tip of the longest fin ray), a sexually dimorphic characteristic (Skjæraasen et al. 2006), using vernier callipers. Each fish was also weighed and sexed by ultrasound (Karlsen and Holm 1994). Based on the ultrasound examination, 10 reared and 10 wild-origin cod were chosen for the

study (sex ratio was 1:1). All males extruded sperm following the application of mild pressure on their ventral sides. The ultrasound images were used to select females thought to have a high probability of spawning in the near future. These experimental fish were then marked individually with a two-tag system, using the dorsal fins as landmarks (following Hutchings et al. 1999), that permitted unambiguous visual identification.

Prior to the introduction of the experimental fish into the Tower Tank, a metal plate having the same cross-sectional area as the tank (10.5 m², radius = 1.83 m) was positioned 3 m below the surface to restrict cod vertical movements to a depth at which there was sufficient light for the video recordings. The volume of the experimental arena was 33 m³. The Tower Tank was illuminated by six overhead lamps, including four 1,000-watt phosphor-coated metal halide lamps and two 400-watt mercury vapour lamps. The photoperiod in the tank was controlled by two separate timers, set to approximately 08:00–16:00, during the entire experimental period. An egg collector (100 L) that regularly skimmed the water surface of the tank was mounted prior to the start of the experiment. Eggs were collected for the first time on February 26 and for the last time on April 16. On April 20, the experiment was terminated and the cod were sacrificed by an overdose of anaesthetic.

Behavioural recordings

Three cameras were used to record spawning behaviour. One was mounted directly above the centre of the tank, 2.5 m above the surface. Additional cameras were mounted on side viewing windows at depths 1 m and 2 m below the surface. Each camera was connected to a separate video-cassette recorder (VCR). From February 26 to April 20, recordings were made daily from 08:00–16:00 with the exceptions of March 12 and 17, when the VCRs malfunctioned.

The behavioural interactions examined here (Table 1) represent a compilation of behaviours described by Brawn (1961) and Hutchings et al. (1999). Given the absence of paternity analyses after March 23 (see below), the analyses were restricted to the behaviours recorded from February 26 to March 23. Each recording was first examined at 8X natural speed. If any courtship or aggressive behaviour was observed, the individuals involved were manually

Table 1 The behavioural interactions considered during spawning

Interaction	Name	Description	Reference
Courtship	Paired swim	Two fish are in motion following each other's movements tightly, resulting in a circling pattern either close to the bottom or in the water column.	Brawn ^a (1961)
	Mount	The male mounts another cod ventrally, starting on top of the fish and 'grabbing' it with the pelvic fins. Thereafter the male slides down along the side, ending up underneath the other fish, stomach-to-stomach, and with genital apertures close together.	Brawn (1961)
	Circling	One or more males circle around a female that lies motionless on the bottom.	Hutchings et al. (1999)
Aggression	Chase	Swimming towards a swimming fish	Hutchings et al. (1999)
	Approach	Swimming towards a stationary fish	Hutchings et al. (1999)
	Prod	Contact between one fish's snout and another fish's body	Hutchings et al. (1999)
	Nip/ Bite	One fish biting or attempting to bite another	Hutchings et al. (1999)

^aThe circling pattern was a part of the "flaunting display" described by Brawn, but other elements in the display she described were not observed

scored, using Observer 3.0 (Noldus 1991). Behaviours were primarily scored from recordings taken from the top, centre-mounted camera. The side-mounted cameras were used primarily to assist in fish identification. In total, 192 h of recordings were analysed. All behavioural analyses were done independently and "blind" of the paternity analysis.

Egg collection

The egg collector was checked daily at approximately 16:00. When eggs were found, they were placed in a 20 L aquarium supplied with circulating water and aeration and left undisturbed until the larvae were about to hatch. This time frame allowed for sufficient larval tissue to develop to permit isolation of DNA with relative ease. After hatching, larvae were preserved in ethanol for determination of paternity. Unfortunately, a problem with the water supply in these aquaria led to a substantial loss of samples, reducing the number of sampling days to 13 between February 26 and March 23 (the dates for which we have samples are February 26–28 and March 2,3,5,6,7,11,14,15,17 and 23).

Paternity analyses

Tissue was extracted using Qiagen's DNeasy 96 Well Extraction Kits. Polymerase Chain Reactions (PCRs) were carried out for 5 loci: GMO 8, GMO 19, GMO 34, GMO 35, and TCH 5, for which primers were hex labelled. Samples were run on Acrylamide Gels and

images scanned, using FMBio II software. Samples were scored and parentage determined by the PAPA programme (Duchesne et al. 2002). Larvae were extracted in 96-well plates, using Eyeball Buffer and Protease. The same PCR technology and sample imaging protocol used for adult samples was used for larvae samples. Of 1,248 offspring analysed, 95% could be assigned unambiguously to a single male and female.

Data analyses

Observations were divided into 2-hour intervals to determine whether behavioural activity varied diurnally. We then applied a general linear mixed-effects model to the data (e.g., Pinheiro and Bates 2000). The time intervals were coded as 1, 2, 3 and 4 in the model. Observation day was used as a random effect and aggressions and courtships as fixed effects. The model was run with both homo- and heteroschedastic variance and with or without a random component in the slope as well as the intercept. The *Aikaike* (AIC) and *Bayesian* (BIC) information criteria (Pinheiro and Bates 2000) were used for model selection.

To examine if there was a difference in the numbers of courtships directed at wild and reared females, we calculated the proportions of courtships directed at wild females and tested if this differed from an expected 50:50 ratio. All proportions were normalised by the arcsine transformation and weighted by the total number of individual displays performed by each male.

To examine potential associations between male behaviour and reproductive success, the relative values of courtship and aggression initiated by each male were first calculated (Table 2). These fractions were then arc-sine square-root transformed and regressed separately against the arc-sine square-root transformed fractions of eggs sired by the different males (Table 2).

Simple regressions between weight, total length, condition (i.e. the residual of total weight after controlling for total length), pelvic fin ray length and the residual of pelvic fin ray length (after controlling for total length), and their associations with male reproductive success were undertaken to identify potential morphological correlates of male reproductive success.

Results

Comparing the wild cod with their reared counterparts, the former were significantly longer and heavier

(Table 2; two-tailed *t*-test, d.f.=18, $p<0.0001$), although reared cod were in better condition (separate-slopes ANCOVA, $F_{2,18}=6.84$, $p<0.05$). After controlling for total length, pelvic fin length did not differ between cod types (ANCOVA, $F_{1,15}=0.06$, $p=0.81$). At the end of the experiment, all males were either spent or still in spawning condition. In contrast, the gonads of all wild females were either spent or in a resting state; only one reared female had initiated spawning. All females that had contributed eggs to the DNA-fingerprinted batches were of wild origin (Table 2).

Both the number of courtship and aggressive interactions exhibited a diurnal pattern. Displays were at a minimum in the morning and increased linearly during the course of the day (Fig. 1; d. f.=165, $p<0.0001$). While there was no difference in the number of courtship and aggressive interactions observed from 08.00 to 10.00 (Fig. 1; d. f.=165, $p=0.47$), there was a greater increase in courtship interactions during the day (Fig. 1; d. f.=165, $p<0.0001$). Based on both the *AIC* and *BIC*, the preferred model was one with a random component

Table 2 Data on morphology, behavioural interactions initiated and number of eggs contributed genetically for individual cod

Papa number	Weight (g)	Length (cm)	Pelvic Fin (cm)	Courtships initiated	Aggressions initiated	Eggs contributed
FR1	929	44	3.9	–	–	0
FR13	713	43	4.3	–	–	0
FR14	811	43	3.2	–	–	0
FR20	842	42	2.5	–	–	0
FR7	588	38	2.9	–	–	0
MR10	1,048	47	4.4	176	54	1
MR11	621	40	3.7	3	4	0
MR15	605	38	2.9	1	1	4
MR19	839	41	3.6	0	1	1
MR4	668	37	3.5	0	1	15
FW1	3,614	70	8.3	–	–	335
FW12	4,200	75	7.1	–	–	107
FW16	3,336	70	3.2	–	–	174
FW17	3,070	69	7.8	–	–	128
FW9	4,800	78	7.9	–	–	376
MW18	2,960	68	7.1	65	99	184
MW2	3,216	69	7.8	227	115	724
MW5	2,670	67	8.8	176	67	260
MW6	2,708	66	8.2	24	4	0
MW8	2,286	66	6.9	2	0	0

F female, *M* Male, *R* Reared, *W* wild cod

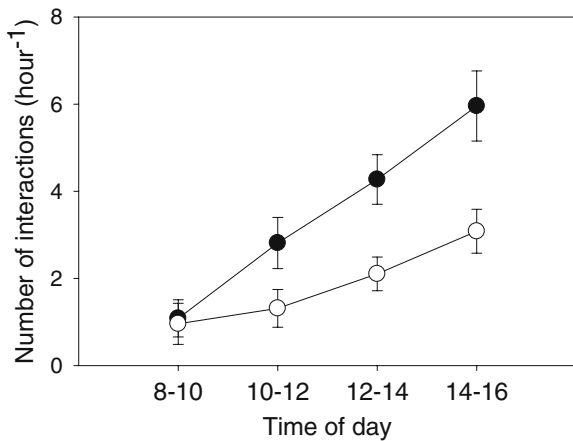


Fig. 1 Periodicity of courtship (black circles) and aggressive interactions (white circles). Error bars are the standard error of each measurement. Results are pooled from 24 observation days

in both the slope and intercept and having heteroschedastic variance.

The most common courtship interaction was a paired swim, followed by ventral mounts and circling. Only wild males performed the circling behaviour. Chase was by far the most common form of aggression. Of 674 courtship interactions observed, 96% were initiated by the wild males MW2, MW5, MW18 and the reared male MR10 (Table 2). The same quartet performed 335 of 346 aggressions (Table 2). The wild, spawning females received significantly more courtships than the reared, non-spawning females (Fig. 2; d.f.=7, $t=14.05$, $p<0.01$).

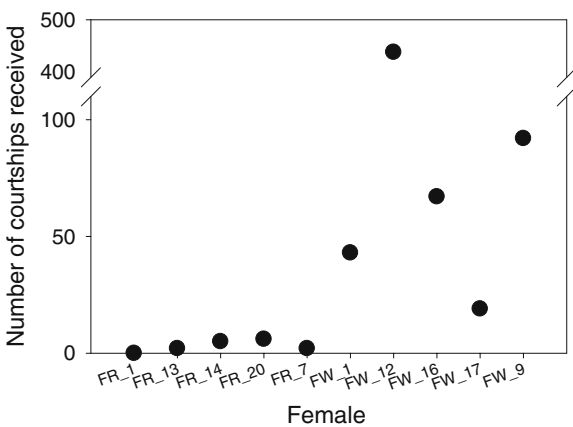


Fig. 2 Number of courtships received by the different females. Notice the break in scale. FR denotes a reared female and FW a wild female

Overall, both courtship ($F_{1,8}=8.9$, $p<0.05$) and aggressive behaviours ($F_{1,8}=17.4$, $p<0.01$) initiated were significantly correlated with male reproductive success (see Table 2 for individual data), and there was a large skew in male reproductive success (Fig. 3). However, there was also a clear temporal pattern in the number of displays performed by the three most successful males (Fig. 4), a pattern that was also reflected in their reproductive success. From February 26 to March 7, one male (MW2) performed the most aggressions on each sampling day and the most courtship interactions on all days but one (Fig. 4). This male also sired 82% of all the eggs during this period (Fig. 4). From March 8 to March 23, the male performing the most interactions varied among egg-sampling days. During this period, the three wild males who were active in courtship and aggressive behaviour sired 96% of the eggs with no significant difference in reproductive success between them (Fig. 4). This shift in the dominance hierarchy, and the coherence between the behavioural observations and the paternity results, was further supported by the results of two-way ANOVAs, using male and the two time periods as grouping factors and using courtship, aggression (observation day⁻¹) or reproductive success (eggs sired sampling day⁻¹) as test variables. For all ANOVAs, the interaction between male and sampling period was significant (Fig. 4; $p<0.05$).

Male reproductive success was not correlated with male weight, length, condition, or pelvic fin length (all p 's>0.05). Our metric of male condition and

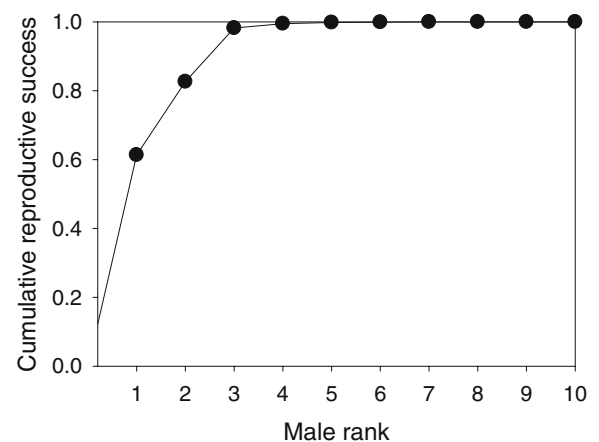
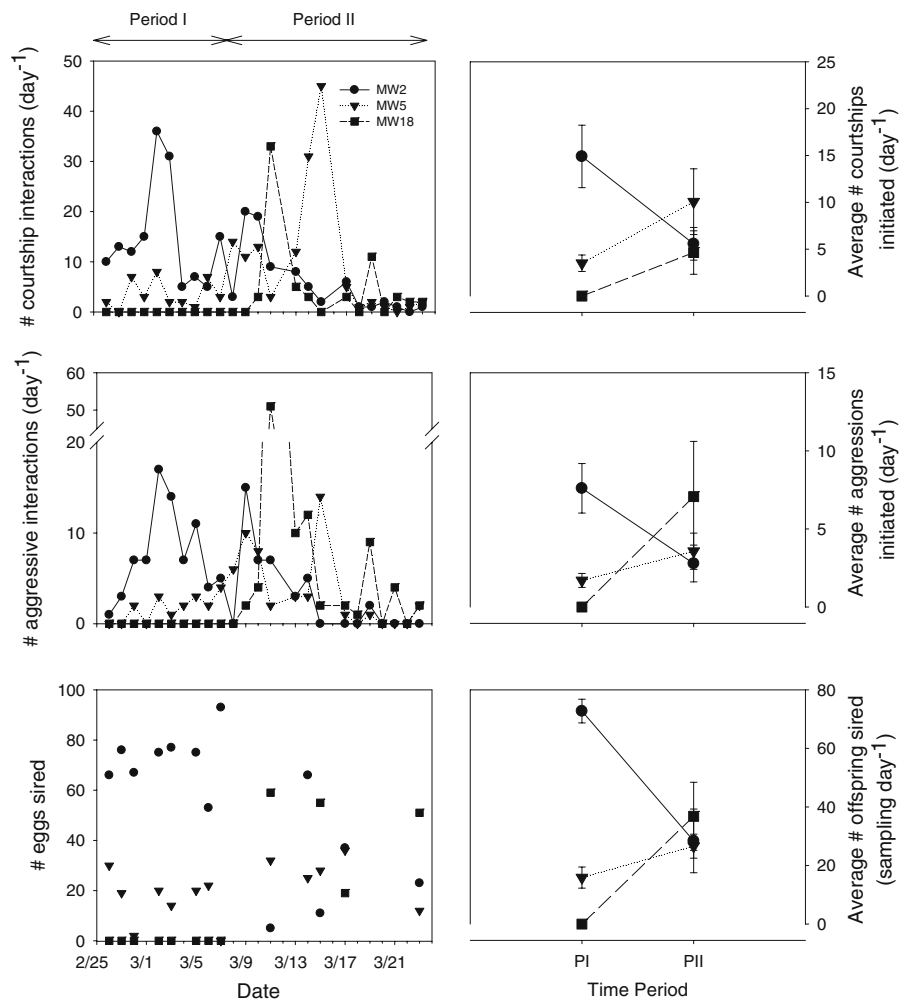


Fig. 3 Cumulative reproductive success of males

Fig. 4 The number of interactions initiated daily by the three males that sired more than 95% of the eggs—MW2 (circles), MW5 (triangles), MW18 (squares)—and their reproductive success on the different sampling days. Right side panels are the same data combined into two separate periods, February 26 to March 7 (Period I (PI)) and March 8 to March 23 (PII)



residual pelvic fin length were also included with total length in a multiple regression analysis, but the resulting correlation coefficient was not significant. Overall, behavioural interactions initiated remained the only significant correlate of male reproductive success. Considering wild males only, male length (R^2 -adj.=0.73, d. f.=4, $p<0.05$) was positively correlated with reproductive success, whereas male weight, condition, pelvic fin length or residual fin length were not (all p 's>0.05).

Discussion

The reproductive success of reared cod was essentially nil (Table 2), a finding consistent with the reduced mating success reported for domestic salmonids in

spawning competition with their wild counterparts (e.g. Fleming et al. 1996; Weir et al. 2004). However, the considerable size difference between wild and reared cod in our experiment is a likely explanation for this outcome (Rowe et al. 2008) and future studies with size-matched reared and wild cod are needed to determine whether there is any difference in the competitive ability of wild and domestically reared cod.

Overall, the results showed a clear association between mating behaviour and reproductive success (Table 2, Fig. 4). The importance of behaviour is further emphasized by the fact that males almost exclusively directed their courtships towards spawning females (Fig. 2). The spawnings observed in our study all occurred during the later part of the day (13:50–16:05), i.e. the time when males were the

most active (Fig. 1). Similarly, Rowe and Hutchings (2006) found that sound production of spawning cod varied diurnally with peak intensity at dusk.

The link we observed between aggression and reproductive success (Table 2), and the overall reproductive skew among different males (Fig. 3), is consistent with previous work on this topic (Hutchings et al. 1999; Rowe et al. 2007, 2008). In addition to potential indirect benefits, such as good genes, a direct benefit of mating with dominant males may be a decreased likelihood of disrupted mating. This may have particular relevance to cod reproduction as other males will commonly try to interrupt mating (e.g. Rowe and Hutchings 2006). Although not often associated with courting pairs in the present study, it was not unusual for as many as three sub-dominant males to follow mating couples, to try to displace the ventrally-mounted male, and to release sperm during egg release. During one spawning event, for example, one male (MW2) maintained his ventral mount for more than 60 s, presumably until the female had shed all her eggs, despite the efforts of two other males to displace him during this period. Female choice of dominant males might serve to minimize such disruptions (Hutchings et al. 1999). Brawn (1961) reported that the dominant male cod chased away other males from his and the female's immediate vicinity and was observed to mate undisturbed on four occasions. Noticeably, in our study, MW2 only mounted females on four different occasions, which resulted in spawning on three of these occasions with three different females. Numerous mounts by other males did not once elicit egg release by the female recipient. MW2 was overall by far the most successful male siring more than 60% of the total amount of eggs.

The only non-behavioural characteristic that influenced male mating success was male length, when considering only wild males. Male weight, condition and pelvic fin length were not correlated with male reproductive success in the present study. In accordance with these results, Rowe et al. (2008) documented a consistent positive effect of male length on reproductive success across cod populations, and a small positive effect of pelvic fin length in one of four populations.

One interesting feature of the results presented here is the temporal variation in reproductive success among males, a pattern seemingly closely reciprocated by the behavioural observations. One explanation for

this observation is sperm depletion in the most successful males. However, the initially dominant male (MW2) continued to fertilise eggs throughout the experiment, and it would seem unlikely that this explanation can fully account for our results. Another possibility is that the energetic demand of display behaviour may mediate temporal changes in dominance hierarchies and thereby reproductive success among individual males. This has been documented in several lek-breeding species (e.g., Höglund and Alatalo 1995). Consistent with our hypothesis that the temporal shift may be attributable to an energetic cost, reproductive behaviour seems to be associated with increased weight loss of male cod during spawning (Skjæraasen et al. in review). However, given that we have documented this phenomenon in only one mixed shoal, and in light of the limited number of males participating in behavioural interactions observed here, further research on the temporal dynamics of cod mating behaviour in other populations is warranted.

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