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Male reproductive competition in spawning aggregations of cod (*Gadus morhua*, L.)

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Abstract

Reproductive competition may lead to a large skew in reproductive success among individuals. Very few studies have analysed the paternity contribution of individual males in spawning aggregations of fish species with huge census population sizes. We quantified the variance in male reproductive success in spawning aggregations of cod under experimental conditions over an entire spawning season. Male reproductive success was estimated by microsatellite-based parentage analysis of offspring produced in six separate groups of spawning cod. In total, 1340 offspring and 102 spawnings distributed across a spawning season were analysed. Our results show that multiple males contributed sperm to most spawnings but that paternity frequencies were highly skewed among males, with larger males on average siring higher proportions of offspring. It was further indicated that male reproductive success was dependent on the magnitude of the size difference between a female and a male. We discuss our results in relation to the cod mating system. Finally, we suggest that the highly skewed distribution of paternity success observed in cod may be a factor contributing to the low effective population size/census population size ratios observed in many marine organisms.

Keywords: effective population size, *Gadus morhua*, mating system, paternity assignment, reproductive skew, sperm competition

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Introduction

Patterns of mating behaviour and male reproductive success are poorly known for most species of marine fishes. Fish live in a medium in which it is normally difficult to observe reproductive behaviour and patterns of parentage. Further, most fishes have external fertilization, leading to an opportunity for high levels of sperm competition (Stockley *et al*. 1997), which is also reflected in the fact that a large number of species exhibit polymorphisms in male mating strategies (Taborsky 1998). The common scenario is that individual males either invest in attracting and monopolizing females, or in spermatogenesis and parasitizing pair spawnings, although more complex strategies are also implied (Taborsky 1998). Recently, DNA-based parentage inference has been successfully applied to investigate mating systems in a wide number of taxa and species (Birkhead & Møller 1998). In fishes, molecular tools have primarily been used to estimate the relative paternity contributions from males employing alternative reproductive strategies (e.g. Jordan & Youngson 1992; Thomaz *et al*. 1997; DeWoody *et al*. 2000; Fu *et al*. 2001), and few studies have addressed the relative contribution of groups of competing males over the length of a spawning season (for an exception see Taggart *et al*. 2001).

Cod, *Gadus morhua*, is an economically important fish species forming large populations in the North Atlantic. Cod are long-lived iteropars that reproduce during protracted annual spawning seasons. Males and females aggregate in spawning schools in which females spawn repeatedly over a period of 1–2 months, with an average of 8.3 (range 1–19) egg batches reported in a study (Kjesbu 1989). Female egg production and male sperm supplies are positively correlated with body size, which again is an increasing function of age (Oosthuizen & Daan 1974). Based on observations of captive cod, Brawn (1961) showed that reproductive behaviour includes males courting females and spawnings taking place when a male and a female release gametes in a ventral mount. In a recent

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study of 16 captive cod Hutchings *et al*. (1999) demonstrated male–male aggressive interactions resulting in a size-correlated dominance hierarchy for access to females. In addition, male sound production and complex courtship behaviour have been predicted to be sexually selected characters upon which female cod base an active choice of spawning partner (Engen & Folstad 1999; Hutchings *et al*. 1999). Based on these behavioural observations it has been suggested (Hutchings *et al*. 1999; Nordeide & Folstad 2000) that the cod mating system resembles that of lekking species. Lekking is characterized by males aggregating and establishing dominance hierarchies, followed by mating visits from females who choose males based on their dominance rank and sexually selected characters (Höglund & Alatalo 1995). A characteristic feature of leks is a high variance in reproductive success among males within an aggregation, with more attractive and dominant males gaining a disproportionate share of matings (Höglund & Alatalo 1995). In cod, this scenario would lead to the prediction that larger, more dominant males sire a larger proportion of offspring than less dominant males. Apart from intermale dominance relationships, individual male reproductive success may be affected by assortative mating. Because larger males have more sperm and larger females spawn more and larger eggs, discrimination among spawning partners in both sexes may lead to size-based positive assortative mating, a behaviour commonly reported from salmonids (Foote 1988).

In an evolutionary context, reproductive behaviour has important implications for population ecology and genetic structure. An example of this is when a limited number of males are able to monopolize a large number of females, which leads to a decrease of the genetically effective population size (N_e), a key parameter in evolution (Nunney 1993). In marine organisms large discrepancies are often found between estimates of N_e and census population sizes (e.g. Hedgecock *et al*. 1992; Ruzzante *et al*. 1996; Palumbi *et al*. 1997), and high variance in reproductive success among individuals has been suggested as a cause of this discrepancy (Hedgecock 1994; Frankham 1995).

Our study was conducted in order to estimate reproductive competition in cod males. The hypothesis that male dominance rank determines reproductive success was tested using enclosures with spawning cod, assuming that dominance rank is correlated with body size. Patterns of individual reproductive success were assessed using microsatellite-based parentage analyses of offspring from a large number of spawnings distributed across the entire spawning season. We show that a male's relative size (expressed by a size rank) had a highly significant effect on his reproductive success, that a large number of egg batches were sired by multiple males, and that the extent of male–female size difference had an effect on male reproductive success. Finally, we discuss how the cod mating system may affect the genetically effective population size/census population size ratio and thereby contributes to shaping the genetic structure of cod populations.

Methods

Experimental set-up

The spawning experiments were performed at the Parisvatnet Field Station, Bergen, Norway. Cod used for the experiment were coastal North Sea cod derived from two sources in order to maximize the size range of spawning cod. One group (24 fish of mean total length = 66.5 cm, C.V. = 20.4%) was caught from a local (60°35′ N, 04°51′ E) population 11 months prior to the experiment. The group had been kept with other cod in a 125-m^3 enclosure rigged at sea, and fed according to standards for captive cod. The other group (26 fish of mean total length = 44.2 cm, C.V. = 6.9%) consisted of 2-year-old offspring of ≈ 100 wild-caught cod (from the same population as the first group) that had spawned as one large captive group. The eggs had been collected, hatched and larvae reared to maturity under conditions similar to those in which the mature wild-caught cod were kept. All fish were tagged for identification. Prior to the experiment, the fish were anaesthetized, and their sex and spawning condition determined using catheterization (based on the presence of ripe eggs in ovaries or mature sperm in testes). All fish were measured (total length to nearest 0.5 cm), and tissue samples (dorsal fin clips) were taken and stored in 96% EtOH.

Six $2.5 \times 1.7 \times 2$ m (length × width × depth) enclosures were rigged at sea. At the beginning of the spawning season in February 2000, on average four males and four females were placed in each of the six enclosures (Table 1). The density of cod within enclosures was ≈ 0.9 fish/m^3, which is within reported natural densities of cod in spawning aggregations (Rose 1993). The specific composition of cod was aimed at creating among-enclosure variance in body size. However, practical availability of individuals resulted in a slight variance in male and female numbers. Cod are known to spawn readily in captivity and the size of the enclosures was presumed to allow natural mating behaviour. Ambient seawater (salinity 34‰, temperature range 4–7 °C) was continuously pumped into the enclosures and outflowing surface water was sieved through a 0.08 m^3 mesh bag collecting all spawned eggs, which are buoyant in seawater. The cod were allowed to spawn in the enclosures until most females had ceased spawning 35 days later.

Sampling of eggs

Eggs spawned over a 24-h period were collected daily between 08.00 and 10.00 hours. For each enclosure each

Table 1 Total body length (cm) of parental cod ranked from largest to smallest for each of the six enclosures

	1	2	3	4	5	6
Males	86	96	79	67.5	46.5	46
	72.5	57	75	66	45	43.5
	72*	47	68	—	44	40.5
	46.5	40	48	—	44	—
	42†	—	—	—	42	—
	—	—	—	—	39	—
Mean length (SD)	63.8 (18.78)	60.00 (24.99)	67.5 (13.77)	66.75 (1.06)	43.42 (2.62)	43.33 (2.75)
Females	77	68	80	76	47	48.5
	68	50	68	71	46.5	47
	65	45	44.5	52	44	46.5
	44	40	40	50.5	40	46.5
	—	—	—	44.5	—	42
Mean length (SD)	63.5 (13.96)	55.4 (14.83)	58.13 (19.06)	58.8 (13.82)	44.38 (3.20)	46.1 (2.43)

*Male died on day 16 of the experiment and was replaced same day by male ‡.

day (= each batch), the total volume of eggs spawned was estimated by measuring egg volume to the nearest 50 mL. From each batch the fertilization rate was estimated based on a random sample of 100 eggs, and the development stage of the eggs was examined to ascertain that all collected eggs were freshly spawned (within the last 24 h). Fertilization rates were high overall (average = 91.5%, SE = 1.6, $N = 66$), and in nearly all cases the collected eggs had been fertilized within the past 8 h. Fewer than 1 of 1000 eggs were fertilized more than 24 h prior to egg collection. This indicates that water currents rapidly transported most eggs from the spawning enclosures into the collecting bags, and hence that temporally separated spawning batches from the same female could be distinguished based on their collecting date. From each batch a random sample of 50 mL eggs ($\approx 3 \times 10^4$ eggs) was incubated in seawater in 10-L containers. Seawater was replaced daily and when eggs hatched (or were < 24 h from hatching) a random sample of ≈ 500 eggs/larvae from each batch was stored in 96% EtOH for subsequent genetic analyses.

DNA extraction and microsatellite analysis

DNA was extracted from fin tissue of parental fish and from whole larvae or eggs using the Chelex extraction method of Estoup *et al*. (1996). From a range of available cod microsatellite DNA loci, three tetranucleotide repeat loci, *Gmo19*, *Gmo37* (Miller & Beacham 2000), *Gmo8* (Brooker *et al*. 1994), and one dinucleotide repeat locus which did not exhibit stutter-bands, *Gmo2* (Brooker *et al*. 1994) were chosen based on their high levels of heterozygosity and genotyping reliability. Parental fish and offspring were genotyped for the four microsatellite loci using PCR conditions specified in Brooker *et al*. (1994) and Miller & Beacham (2000), and subsequently analysed on a Pharmacia ALF express automated sequencer according to the recommendations of the manufacturer. All loci in parental fish were amplified and run three times to ensure correct genotyping. Standard individuals were run on all gels to check consistency in fragment lengths.

Level and distribution of genetic diversity

Observed and expected heterozygosity of the loci were estimated for the pool of parental fish. Deviations from Hardy–Weinberg genotype frequencies were assessed using the exact probability test implemented in the program GENEPOP Version 3.1d (Raymond & Rousset 1995a). The same program was used to test for nonrandom association of pairs of loci, and to test for differences in allele frequencies between the two groups of mature cod used as parental fish (wild-caught vs. 2-year sea-ranched), and differences in allele frequencies between males and females [using an exact test (Raymond & Rousset 1995b) and combining probabilities over loci using Fisher's method].

Parentage analysis

Assessment of parentage of the tested offspring was performed using the program CERVUS Version 1.0 (Marshall *et al*. 1998). CERVUS performs parentage inference based on co-dominant markers such as microsatellites by generating locus-by-locus likelihood scores for each parental candidate of each offspring (Marshall *et al*. 1998). The program incorporates potential mis-scoring and mutation when assigning parentage to an offspring by allowing for a specified level of errors in the genotype data. This way, both otherwise genetically excluded and multiple nonexcluded parental types can be statistically distinguished. Parentage of an offspring is assigned based

on finding the parental candidate with the highest allele frequency based logarithm of the odds (LOD) score. A delta statistical confidence is determined for assigned paternities through a simulation program, yielding an approach which has been shown to perform with high statistical confidence (Slate *et al*. 2000). CERVUS also generates estimates of null-alleles, and an estimated null-allele frequency > 0.05 was considered significant (Summers & Amos 1997). Because all possible parental genotypes were known it was implied that a genetic mismatch between offspring genotypes and either all males or all females in an enclosure was due to either mis-scoring or mutation.

Parentage assignment was performed for each offspring in the following hierarchical order: first, LOD score tests were performed for all enclosure-specific maternal genotypes. Overall, information from the four loci combined yielded high exclusion probabilities, and most offspring were genetically compatible with only one female, who was then assigned maternity of the offspring. However, for 226 of the 1340 offspring, two females simultaneously had high LOD scores with one or no genetic mismatches. In these cases, two separate paternity analyses were performed with each of the two females designated as mother. Second, LOD scores were produced for each paternal genotype for all offspring assigned a maternal genotype. In all cases in which two females simultaneously had a high probability of maternity of an offspring, only one of the subsequent two paternity analyses revealed paternity assignment with a high certainty, and the offspring was subsequently assigned to this pair. In order to test the robustness of this approach, parentage assignment was also performed in the reverse order, with paternity assigned before maternity, and this yielded corresponding results. Based on these procedures, offspring were assigned parentage when: (i) they were compatible with no more than one parental pair with both high LOD scores and no genetic incompatibility; and (ii) a given parental combination with high LOD scores yielded genetic incompatibility between offspring/putative mother/putative father at no more than one locus (allowing for mis-scoring and mutation). The rate of typing error was set at 3% (based on CERVUS-generated estimates of rates of genetic mismatches between assigned mothers and offspring) and strict and relaxed confidence levels were set at 95 and 80%, respectively.

Offspring analysed

Female cod have been reported to spawn at 2–6-day intervals (Kjesbu 1989), and it was therefore assumed that all offspring assigned to a specific female on a specific date were the result of a single spawning. From each of the enclosures an average of 11 (SD = 3.0) batches were chosen distributed over the spawning season. From each of the batches (66 in total), between 20 and 30 (in a single case 70) offspring were genotyped and included in the parentage analyses.

Correlates of male reproductive success

Male reproductive success was calculated for each male as the proportion of offspring from a single spawning (= maternal sib group from one egg batch) that was sired by that male. Only spawnings from which at least five offspring were genotyped were included in these and the subsequent analyses. To be able to compare the effect of male size across enclosures with varying mean male size, males were ranked according to size within enclosures. As an estimator of male sperm competition the genetically effective paternity frequency was calculated for each spawning as $m = 1/\Sigma p_i^2$, where p_i is the proportion of offspring sired by male i. The effects of male rank, body size of the spawning female, date, and enclosure on male reproductive success were investigated by a general linear model (GLM) using the software package GLMSTAT Version 3.0. A GLM with binomial errors and a logit link function was used, as male reproductive success was calculated as the proportion of offspring sired within a single spawning. To analyse whether size-based assortative mating affected male reproductive success, a separate GLM analysis was carried out. The difference in female and male body length was calculated for all combinations of individuals within an enclosure and grouped into 10 cm increments. Female-minus-male size difference increments were then included as a continuous predictor variable and as a quadratic term, thus evaluating if the response variables showed a curvilinear relationship with differences in body length (Crawley 1993; p. 192). In all cases of multiple tests, table-wide significance levels were applied using the sequential Bonferroni technique (Rice 1989).

Results

Measures of genetic diversity

All 50 parental cod and a total of 1340 offspring were genotyped for the four microsatellite loci. The parental cod showed high levels of genetic variation for the four loci, with an average of 8.0 (SD = 1.2) alleles per locus, and an average expected heterozygosity (H_E) of 0.860 (SD = 0.071) taken over enclosures (see Table 2 for enclosure-specific levels of variation). All alleles found in the offspring could also be found in the enclosure-specific pool of candidate parents. No significant differences were found between allele frequencies (estimated by exact tests combined over loci) between the two source groups of parental fish [wild-caught: mean no. of alleles (± SD) = 14.00 ± 6.00, H_E = 0.873 ± 0.055; sea-ranched: mean no. of alleles = 13.75

Table 2 Estimates of genetic parameters based on four microsatellite loci in parental cod and shown for each of the six spawning enclosures. H_E denotes expected heterozygosity under Hardy–Weinberg equilibrium. Null-allele estimates were generated using CERVUS. Asterisks denote estimates of null-alleles in significant proportions

Enclosure	Locus	No. of alleles	H_E	Null alleles
1	*Gmo2*	7	0.863	−0.065
	Gmo8	11	0.941	−0.056
	Gmo19	12	0.948	−0.034
	Gmo37	8	0.882	−0.039
2	*Gmo2*	5	0.745	0.050
	Gmo8	11	0.941	−0.070
	Gmo19	6	0.745	−0.071
	Gmo37	6	0.797	−0.012
3	*Gmo2*	5	0.825	0.151*
	Gmo8	11	0.950	−0.064
	Gmo19	7	0.883	−0.056
	Gmo37	5	0.792	−0.035
4	*Gmo2*	4	0.758	0.074*
	Gmo8	9	0.879	−0.037
	Gmo19	7	0.857	0.034
	Gmo37	7	0.802	−0.093
5	*Gmo2*	5	0.774	0.030
	Gmo8	15	0.968	−0.066
	Gmo19	10	0.916	−0.061
	Gmo37	7	0.811	−0.061
6	*Gmo2*	9	0.883	−0.011
	Gmo8	11	0.950	−0.025
	Gmo19	8	0.900	−0.045
	Gmo37	6	0.825	−0.005

± 6.65, $H_E = 0.851 \pm 0.080$, $P = 0.510$], and neither were there significant differences between allele frequencies in males and females [males: mean no. of alleles (± SD) = 14.25 ± 6.70, $H_E = 0.881 \pm 0.059$; females: mean no. of alleles = 12.00 ± 4.24, $H_E = 0.846 \pm 0.064$, $P = 0.454$). After sequential Bonferroni correction for multiple tests no significant linkage disequilibria or significant deviations from Hardy–Weinberg proportions were observed.

Analyses of parentage

Using the four microsatellite loci, varying detection probabilities were observed in the six enclosures, but given a correctly assigned mother, the exclusion power of paternal types was very high in all enclosures (Table 3). A total of 1321 offspring (98.6%) was assigned parentage according the criteria stated above, with 92% of the assigned offspring being assigned paternity at a 95% confidence level, and 8% at an 80% confidence level. Of the assigned offspring, 14% showed one genetic mismatch with their assigned parents. The locus *Gmo2* apparently had significant levels of null-alleles for two of the enclosures (Table 2). Difficulties with typing this locus were reflected by the fact that 46% of the genetic mismatches in the putative parent/offspring analyses occurred at this locus. Re-amplification of the locus for these offspring sometimes, albeit inconsistently, revealed second alleles in putatively homozygous offspring, a recognized problem when only small amounts of template DNA are available for polymerase chain reaction (Goossens *et al*. 1998). Thus, the problem appeared to be allelic 'drop-out' rather than null alleles *per se*. Because re-extraction of DNA was not an option as whole eggs or larvae had been used, we were not able to estimate the magnitude of this allele 'drop-out' problem. Nonetheless, including the locus *Gmo2* in the parentage analysis increased detection probability and did not lower the overall confidence level at which offspring were assigned.

Table 3 Exclusion power estimates combined over loci, given for first and second assigned parent for each of the enclosures (see text for explanation)

Enclosure	No. of offspring analysed	Exclusion power of first parent (mother)	Exclusion power of second parent (father)
1	370	0.965	0.994
2	190	0.887	0.970
3	180	0.922	0.983
4	220	0.867	0.968
5	160	0.953	0.991
6	220	0.947	0.990

A total of 102 spawnings, for which an average of 12.22 (SE = 0.61, range 5–42, $N_{total} = 1247$) offspring were identified, was included in the paternity analyses. Multiple paternal genotypes were identified in the majority of spawnings (77 of 102 spawnings), with the overall average number of fathers contributing to a spawning being 2.12 (SE = 0.08). The mean genetically effective paternity frequency (± SE) over all enclosures and spawnings was 1.69 ± 0.06. There was no significant difference in effective paternity frequency among the six enclosures (analysis of variance: $F_{5,96} = 1.873$, $P = 0.106$), and neither did the enclosure specific effective paternity frequencies co-vary with the number of males present (regressing effective paternity frequencies on no. of males present: $r^2 = 0.03$, $F_{1,100} = 2.830$, $P = 0.100$). The average proportions of offspring sired, and the genetically effective paternity frequencies in spawnings with various numbers of males siring offspring are shown in Table 4. The distribution of paternal contributions to spawnings showed a U-shaped distribution, i.e. most males participating in spawnings acquired paternity of either most offspring (> 80%) or of a relatively small proportion of the eggs in a single spawning (Fig. 1).

Table 4 The average (± SE) paternity proportions for spawnings in which from one to four males were detected to contribute sperm. The distribution of paternity proportions is given for each male ranked from most successful to least successful male, respectively. The average genetically effective number of fathers to each of the four spawning scenaria is also given (± SE)

No. of males participating in spawning	No. of spawnings (% of total)	Paternity proportion of most successful male	Paternity proportion of second-most successful male	Paternity proportion of third-most successful male	Paternity proportion of fourth-most successful male	Genetically effective no. of fathers to a spawning
1	25 (25)	1	—	—	—	1
2	42 (41)	0.768 (± 0.017)	0.232 (± 0.017)	—	—	1.553 (± 0.039)
3	33 (32)	0.609 (± 0.036)	0.247 (± 0.028)	0.144 (± 0.018)	—	2.209 (± 0.081)
4	2 (2)	0.414 (± 0.014)	0.268 (± 0.018)	0.171 (± 0.029)	0.146 (± 0.004)	3.399 (± 0.121)

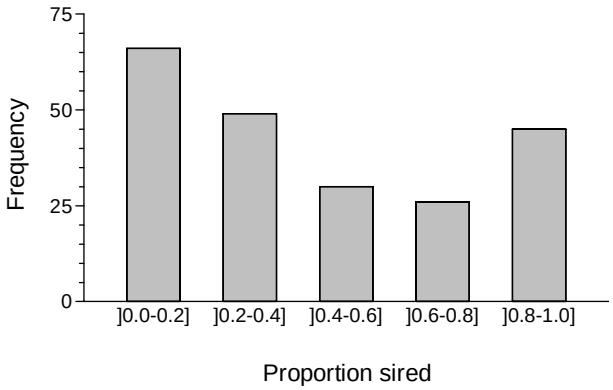

Fig. 1 The distribution of siring frequencies (the proportion of offspring sired by individual male ejaculates) in 102 spawnings.

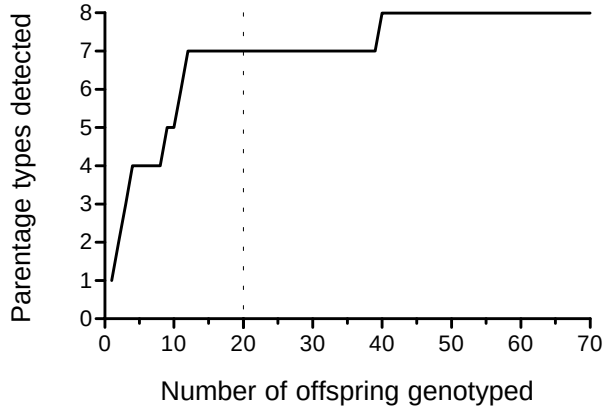

Fig. 2 The number of parental combinations detected in a batch as a function of a randomised number of genotyped offspring. The dotted line at $x = 20$ indicates the minimum number of offspring analysed per batch.

To assess the reliability of basing estimates of reproductive success on a minimum of 20 offspring analysed per batch, the number of parental combinations being identified as a function of the number of offspring genotyped was estimated, based on 70 offspring from a single batch from enclosure 1. The distribution of observed parental combinations as a function of a randomised number of genotyped offspring is shown in Fig. 2. When 20 offspring were genotyped, seven of a total of eight observed parental combinations were identified (offspring from three females and four males were identified). The offspring from rare parental combinations that were not observed in 20 offspring made up < 1.5% of the total number of offspring.

The effective paternity frequencies estimated per spawning based on 20 offspring from this batch were relatively high (average = 2.63) in comparison with the average estimated across all spawnings (1.69). Consequently, in most batches 20 offspring should provide an even more robust estimate of paternity frequencies, simply because fewer males would be involved per spawning. Also, there was no significant relationship between the number of offspring analysed per spawning and the number of fathers detected ($r = 0.168$, $P = 0.09$, $N = 102$ spawnings).

In the 66 batches analysed, on average 1.6 (SE = 0.08, range 1–4) females were identified as spawners per day, and there was no significant difference among enclosures in the number of females that spawned per day (Kruskal–Wallis test: $H = 9.9$, $N = 6$, $P = 0.08$). The distribution of the number of females spawning per day, per enclosure was not significantly different from a random (Poisson) distribution, showing that females did not exhibit synchronous spawning (chi-square tests for deviance from Poisson distribution, all tests nonsignificant). This indicates that the magnitude of resources that males competed for (number of females in spawning condition) remained equal over enclosures and throughout the observed spawning period.

Male reproductive variance

The GLM analysis of factors affecting individual male reproductive success showed that male size rank within enclosures had a highly significant effect on reproductive success, but also that the individual enclosures differed with respect to patterns of male reproductive success (Table 5). There was a further significant interaction between male rank and enclosure, showing that the effect

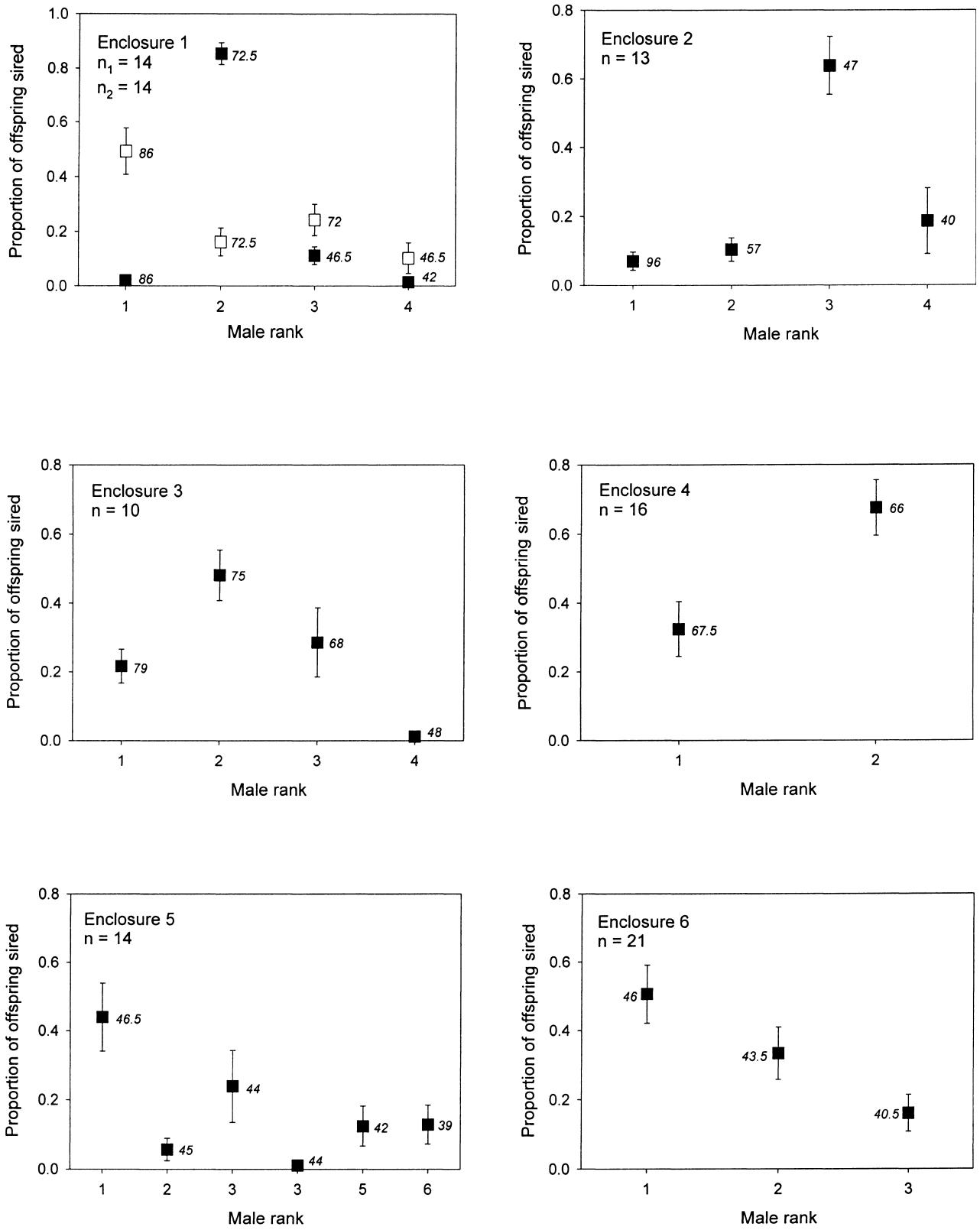

Fig. 3 Average male reproductive success (proportion of offspring sired in a spawning ± SE) in relation to male size rank for each of the six enclosures. The size of each male is given with the rank. The number of spawnings analysed is given for each enclosure. For enclosure 1, two sets of values are given: □, the initial period before male 2 died; ■, values following the replacement of male 2 with a smaller male (see Table 1).

Table 5 General linear model analysis on the effect of male size rank within enclosures, female length, enclosure and date on male paternity success. Only significant interactions are shown

Effects	Scaled deviance	df	Chi-square	*P*
Male rank	48.21	5	193.5	< 0.0001
Female length	0.00006	1	0.000244	0.9938
Enclosure	19.85	5	79.64	0.0013
Date	0.00207	1	0.00830	0.9637
Male rank × female length	15.40	5	61.80	0.0088
Male rank × enclosure	118.8	11	476.9	< 0.0001
Male rank × date	29.13	5	116.9	< 0.0001
Male rank × date × enclosure	78.12	11	313.5	< 0.0001

Table 6 General linear model analysis with the effects of female-minus-male size difference, enclosure and date on male paternity success. Only significant interactions are shown

Effects	Scaled deviance	df	Chi-square	*P*
Female–male difference	49.16	1	284.0	< 0.0001
Enclosure	32.98	5	190.5	< 0.0001
Date	0.776	1	4.479	0.379
(Female–male difference)^2	57.24	1	330.6	< 0.0001
Female–male diff. × date × enclosure	10.51	3	60.75	0.0147

of male size rank differed between enclosures (Fig. 3). Female body size had no significant effect on paternity patterns, but there was a significant interaction between female length and male rank, showing that the effect of male rank was not uniform over females of different sizes. There was no effect of date, but significant interactions among male rank and day, and male rank, day and enclosures. This was an effect of a shift in reproductive dominance over the course of the spawning season, as a number of high-ranking males seemingly experienced sperm depletion, and reproductive dominance was subsequently acquired by the second ranking male (see for example Fig. 3, enclosure 1).

When the effect of female–male size difference was included in a GLM analysis, highly significant effects of both size difference and its quadratic term were found (Table 6, Fig. 4). However, a significant effect of enclosure was also found, indicating that the significance of female–male size differences may be affected by the actual composition of males competing for fertilization within an enclosure. The result was further hampered by the significant three-way interaction among enclosure, date and female–male size difference (Table 6), which was probably a result of the shift in reproductive success for some males over the spawning season, as mentioned above.

There was no significant effect of male origin (wild-caught or sea-ranched) on the average proportion of offspring sired (average proportion sired ± SE, wild-caught: 0.51 ± 0.03, n = 120, sea-ranched: 0.42 ± 0.03, n = 96, t-test on arcsine transformed paternity proportions over all spawnings: t = 1.933, df = 214, P = 0.055), and neither did male origin significantly affect whether males were able to dominate the spawnings they participated in, as wild-caught and sea-ranched males sired > 80% of the offspring in, respectively, 31 and 21 spawnings of a total of 216 male ejaculates siring offspring (Fisher's exact test: P = 0.240).

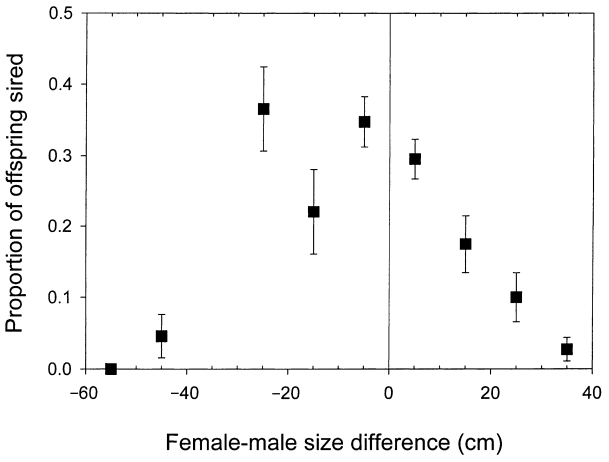

Fig. 4 Average male reproductive success (the proportion of offspring from one spawning sired by that male) plotted as a function of the difference between the female size minus the male size (with values grouped into 10 cm increments), i.e. negative values indicate that the male is larger than the female.

Discussion

Before discussing the biological implications of our results we first briefly address issues related to the reliability of parentage assignment and whether the spawning behaviour detected in the enclosures is representative of spawning in the wild. First, the applicability of paternity studies relies on correct parentage assignment of offspring. In our study, the DNA markers applied were chosen to maximize genetic resolution and minimize typing errors, and although allele drop-out seemed to be a problem at the dinucleotide locus *Gmo2*, the overall result was that 98.6% of all offspring analysed were assigned paternity with high confidence.

Second, Fleming *et al*. (1997) found that sea-ranched salmon, *Salmo salar*, males had significantly lower success with monopolizing spawnings, and participated in more multiple male spawnings than naturally produced males. Because the group of sea-ranched cod males used in our experiment was smaller on average, and relatively small males had a lower reproductive success, the difference in male reproductive success may have been attributed to the differences in male origin irrespective of rank. However, the overall variance in male reproductive success did not co-vary with male origin, and sea-ranched males were able to dominate spawnings at frequencies equal with those of wild-caught males.

Finally, the level of stress may affect the establishment of stable male–male hierarchies and hence patterns of paternity in mating aggregations (Tuyttens & Macdonald 2000). In a comparison of a group of experimentally stressed captive cod with a group of unstressed cod, stressed cod performed altered courtship sequences, although spawning and fertilization rates remained comparable with those of the unstressed cod (Morgan *et al*. 1999). However, the cod in our experiment were not subject to direct human disturbance and were not visibly stressed. Courtship behaviour (cf. Brawn 1961) was observed on several occasions, and the density of spawning fish in the enclosures was within natural limits. We therefore do not expect stress to have had a severe effect on the observed paternity patterns. We found no correlation between the number of males present in the enclosures and estimated paternity frequencies. However, the absolute numbers of fish present in spawning groups is expected to affect individual spawning strategies. Our study set-up represented a closed system, whereas spawning groups under natural conditions are open systems which individuals are able to freely join or leave. Individual males may move around in response to cues such as local individual density and substructure in dominance hierarchies, which again may change over the course of a spawning season as some individuals cease spawning. In our set-up male reproductive strategies may thus have been altered in reaction to confinement and the actual availability of females, although we have no direct indication of this being the case. Very little is known about individual behaviour in large spawning aggregations, and in the following we cautiously assume that the patterns observed in our experimental set-up resemble those under natural spawning conditions.

Male reproductive success

Male reproductive competition by ejaculates from multiple males competing for fertilization has been documented in a rapidly increasing number of genetic paternity studies of a wide range of fish species (see DeWoody & Avise 2001 for a recent review). However, relatively little is still known about relationships between the reproductive success of individual males and patterns of male morphology and/or intersocial relationships. The mating system of cod has previously been termed promiscuous (Breder & Rosen 1966), although no estimates of the numbers of males and females participating in spawnings have been reported. Based on ratios of gonad weight to body weight, Stockley *et al*. (1997) predicted a high level of sperm competition in cod. However, Hutchings *et al*. (1999) argued that the observed cod spawning behaviour implied a high skew in individual male reproductive success. In our study, paternity analyses showed that male size rank had a highly significant effect on paternity success with larger males acquiring a larger proportion of offspring than smaller males. This pattern was consistent when the relative reproductive success of individual males was compared over a large number of spawnings, and over varied compositions of male aggregations (Fig. 3). Male–male dominance relationships and spawnings could not be observed directly in our study. Nonetheless, coupling the observations of cod reproductive behaviour by Brawn (1961) and Hutchings *et al*. (1999) with our study showing a correlation between male size rank and reproductive success, leads to a corroboration of the hypothesis that cod males experience reproductive hierarchies, and that larger cod males are expected to sire the largest proportion of offspring recruited into a population. However, our paternity analyses also revealed that that sperm from multiple males contributed to offspring in 75% of the examined spawnings, and hence that cod males experience high sperm competition intensity, as predicted by Stockley *et al*. (1997). Even though there were several cases of all or most offspring being sired by one male only, there was a proportion of spawnings in which paternity was more equally distributed among participating males (Fig. 1). This was also illustrated by the average genetically effective paternity frequency per spawning being close to 1.7, and thus significantly > 1. Male dominance was hence rarely complete during spawnings, and individual males may perform varied mating strategies, sometimes investing

in courtship, and sometimes ejaculating with other spawning couples.

Assortative mating was detected in the analysis with males that were either much larger, or much smaller than the spawning females both acquiring relatively low paternity frequency (Fig. 4). Males that were much smaller than a spawning female did, however, often participate in spawnings, albeit with a low paternity success on average. In a total of 16 spawnings, males that were smaller than the spawning females by > 20 cm in body length were observed to sire an average of 23% (SD = 22%) of the offspring (data not shown). However, based on these experiments it was not possible to distinguish fundamentally different male mating strategies (sneaking vs. courting). Unfertilized cod eggs and sperm remain viable in seawater for a long time (> 60 min) enabling sperm to compete for fertilization during a relatively long period following ejaculation (Trippel & Morgan 1994), and ejaculating even when far removed from a spawning female may enable subordinate males to acquire some fraction of reproductive success during spawning peaks. The degree of assortative mating is also expected to be affected by the actual variance in female and male sizes within groups of spawning cod. A more detailed analysis of individual female spawning patterns over the mating season and with differential levels of male competition is expected to add information to this component of cod reproduction (D. Bekkevold, manuscript in preparation).

An assumption for explaining the observed male reproductive skew as an effect of dominance hierarchies and/or female mate choice is that the male siring the dominant proportion of offspring in a spawning was the male eliciting egg release in the female. As we did not directly observe spawnings, we cannot exclude that larger males sired more offspring simply by having larger ejaculates, which swamped those of smaller males, rather than because they acquired more matings with females. However, the ventral mount may be expected to facilitate first male precedence to a larger degree than in other external fertilizers, which do not show a direct female–male physical pairing during spawning with multiple ejaculating males (e.g. salmonids; Jones 1959). Another factor influencing male reproductive variance is that sperm from different males may vary in competitive ability. Rakitin *et al*. (1999) reported a significant skew in paternity frequencies in cod eggs when equal numbers of spermatozoa from two males were added in *in vitro* fertilization. This, combined with the observation that the fertilization success of sperm from individual males varied depending on the specific female egg donor, suggests additional cod mate 'choice' taking place at the level of gametes (Rakitin *et al*. 1999). In addition, variance in individual parentage patterns may be associated with assortative mating and mate choice reflecting major histocompatibility complex variation in individuals (Landry *et al*. 2001). Such factors may explain some of the variance in individual reproductive success, but based on this study they seem to be of less importance than male size-correlated effects.

Variation in mating success is fundamental to sexual selection. The overall variance in male mating success found in our study lies within the range given in a comparison of male reproductive variance in 20 species of birds and mammals known to mate on leks (Mackenzie *et al*. 1995). This supports an hypothesis of cod being lekking. However, the extent to which the reproductive skew is a result of stable male–male dominance hierarchies and/or female mate choice based on sexually selected traits, as has been shown for other lekking species, remains to be explored further. Male mating success on leks is expected to be dependent on both proximate factors of male–male dominance hierarchies, but also on female choice tactics and error rates (Johnstone & Earn 1999). The magnitude of importance of these factors is at present not known for cod, and investigations into the basis for female choice of spawning partners merit further consideration.

Relevance to genetic population structure

Cod has been shown to be subdivided into genetically distinct and temporally stable populations (Mork *et al*. 1985; Ruzzante *et al*. 1996, 1999, 2001; Nielsen *et al*. 2001; Pogson *et al*. 2001). Given the huge census population sizes in cod and many other marine fish species, genetic drift is expected to be negligible and it may in fact be considered surprising that detectable genetic differentiation is at all present at presumably neutral loci. It has been suggested that currently observed genetic differentiation reflects reminiscences of initial founder events followed by restricted gene flow (e.g. Pogson *et al*. 2001). However, other studies of marine organisms, including fishes, suggest low ratios of effective to census population sizes (N_e/N) (Hedgecock 1994). Low N_e/N ratios could be the result of high variance in reproductive success, which decreases the genetically effective size of a population without affecting census population size. This factor is expected to be especially important in highly fecund species, such as cod, in which most of the mortality occurs during the egg and larval stages (Hedgecock 1994). If eggs and larvae from temporally and spatially separated spawnings experience large variations in physical and biological conditions, the result may be that only relatively few individuals reproduce under optimal conditions. Thus, entire cohorts of recruits could be the product of relatively few spawnings ('sweepstakes selection').

Our results point to an additional factor, i.e. skewed reproductive success of individual males in a spawning aggregation, which could lead to high variance in reproductive success and thereby contribute to a low N_e/N ratio.

Given that cod census population sizes (N) should be counted in millions, it is unlikely that N_e is sufficiently small to lead to detectable genetic drift in cod populations on a short (ecological) timescale. However, over thousands of years, i.e. on an evolutionary timescale, male reproductive skew could contribute to a detectable low N_e/N ratio.

A further evaluation of the importance of this factor relative to other factors such as 'sweepstakes selection' would, however, require information both on the magnitude of male reproductive skew under natural conditions in larger spawning assemblages and on average lifetime reproductive success of individual males.

Conclusions

In conclusion, our results illustrate that assumptions about 'random mating' are often not valid when the mating system of a species is subject to closer investigations, even in marine species with huge census population sizes. Cod is not a promiscuous mass spawner, but exhibits a complicated mating structure. Reproductive success is highly skewed among males with larger males siring larger proportions of offspring, although an effect of assortative mating was also indicated. Even though one male often dominates, multiple males contribute sperm to most spawnings, leading to high sperm competition intensity. The observed size-correlated male mating skew supports previous suggestions of cod being lekking, although the direct criteria for male reproductive success under natural conditions remain to be elucidated. Finally, skewed male reproductive success may be a factor contributing to the low N_e/N ratios often observed in marine organisms.

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