ICES Journal of Marine Science

ICES Journal of Marine Science (2017), doi:10.1093/icesjms/fsx232

Temporal variability in offspring quality and individual reproductive output in a broadcast-spawning marine fish

Nancy E. Roney[1], Rebekah A. Oomen[1,2,3], Halvor Knutsen[3,4], Esben M. Olsen[3,4], and Jeffrey A. Hutchings[1,2,3]*

[1]*Department of Biology, Dalhousie University, Halifax, NS, Canada*
[2]*Department of Biosciences, Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo, Oslo, Norway*
[3]*Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His, Norway*
[4]*Centre for Coastal Research (CCR), University of Agder, N-4604 Kristiansand, Norway*

**Corresponding author: tel: +1 902 494 2687; e-mail: jhutch@dal.ca*

Roney, N. E., Oomen, R. A., Knutsen, H., Olsen, E. M., and Hutchings, J. A. Temporal variability in offspring quality and individual reproductive output in a broadcast-spawning marine fish. – ICES Journal of Marine Science, doi:10.1093/icesjms/fsx232.

Received 31 May 2017; revised 10 October 2017; accepted 3 November 2017.

The protracted spawning period of broadcast-spawning marine fishes has potential to generate considerable variability in metrics of individual reproductive output. We undertook a temporally detailed genetic study of larvae produced by Atlantic cod (*Gadus morhua*) from two spatially proximate populations spawning under controlled semi-natural conditions over 94 days. Based on daily samples of larvae ($n = 4489$ in total), we document fine-scaled temporal changes in, and correlates of, offspring phenotype and reproductive output (egg batches produced or fertilized). Larval length and standardized yolk-sac volume declined 11 and 49% over the spawning period, respectively. The adaptive significance of these trends is unclear. Longer, heavier females produced longer, better-provisioned larvae. Body size affected the number of egg batches to which an individual contributed genetically but not spawning duration. Males contributed gametes to a greater number of egg batches (19.5 vs. 9.2), and spawned over a longer period of time (48.9 vs. 30.8 days), than females. After accounting for body size and condition, egg batch number and spawning duration differed between adjacent populations separated by < 10 km. Our work highlights the need to understand the environmental and adaptive causes of temporal variability in offspring quality and its consequences to individual fitness and per capita population growth in batch-spawning fishes.

Keywords: Atlantic cod, larval length, offspring size-offspring number strategies, Skagerrak, spawning duration, yolk-sac volume.

Introduction

A rich literature has explored the conditions that favour selection for comparatively large or small offspring with correlated selection for relatively few or numerous offspring (Lack, 1947; Smith and Fretwell, 1974; Sinervo and McEdward, 1988; Einum and Fleming, 1999; Rollinson and Hutchings, 2013a, b). In fishes, the fitness implications of the trade-off between offspring size and number was first considered by Svärdson (1949) who argued, in effect, that fecundity is constrained by evolutionary pressures acting on offspring size, which in turn depends on how egg size—a presumed metric of offspring quality—affects offspring survival and parental reproductive success.

In some fishes (e.g. salmon, trout, char), the survival benefits accruing to offspring, and the fitness benefits to parents, resulting from the production of relatively large eggs appear to outweigh the fecundity costs of producing comparatively few of them (Hutchings, 2002; Rollinson and Hutchings, 2013a, b). In other species, however, particularly those for which parental care is absent and the fate of offspring is almost entirely dependent on a highly stochastic environment (e.g. broadcast-spawning marine fishes such as gadoids and flatfish), the marginal gains in offspring survival resulting from the production of larger offspring might not offset the fecundity costs of producing fewer offspring. Under these circumstances, the evolutionarily stable strategy

(ESS) of investment per offspring would be one of maximizing the number of eggs, each of which approaches the physiologically minimum size, within a brood (Hutchings, 2002). Such an ESS might be expected of species that have evolved life-history strategies that allow them to persist in highly unpredictable environments. The less predictable the environment, the greater the likelihood that selection will favour genotypes that spread the risk of reproductive failure across space or time (Childs *et al.*, 2010; Hutchings and Rangeley, 2011).

An interesting question emerges as to whether the offspring of such "bet-hedging" species should be relatively constant in size or quality, or whether they should differ, throughout a protracted spawning period. This might depend on the temporal scale of environmental stochasticity or unpredictability. If the stochasticity (and its influence on offspring survival) is primarily manifest on a short temporal scale (e.g. daily), one might predict that offspring quality would be relatively constant and near the physiological minimum. But if a breeding season occurs over a sufficiently long period of time, the offspring-survival consequences of short-term (daily) stochasticity might be superseded by longer-term trends in environmental variability that have a higher level of seasonal predictability, further altering the consequences of stochasticity on offspring size.

In marine fishes, offspring quality can be approximated by two readily measurable traits: larval size and yolk-sac volume. There is evidence that the former is positively related to larval survival (Pepin, 1991). Regarding nutritional provisions from maternal females, larvae can begin feeding exogenously before the yolk sac is exhausted, resulting in a period of overlap while transitioning from internal to external feeding sources (Neilson *et al.*, 1986, Morrison, 1992). Increases in energetic reserves (i.e. the size of the yolk sac) could lengthen this transitional period and contribute to lower mortality (Fiksen and Folkvord, 1999).

It is not clear then how offspring size and quality should vary during the spawning period of broadcast-spawning fishes. Our first objective is to address this uncertainty, using daily estimates of parentage and offspring-quality metrics for 4489 larvae, during a 94-day spawning period, from a free-spawning group of 73 wild adult Atlantic cod (*Gadus morhua*). Should variability in metrics of offspring quality be evident, a secondary question arises as to whether offspring phenotype is influenced by parental phenotype. In some marine species, for example, older individuals are reported to produce higher-quality offspring than younger individuals. A third objective is to examine relationships between parental phenotype and reproductive characteristics of the spawning period, such as the number of egg batches produced by females and fertilized by males; the latter has not previously been estimated in Atlantic cod.

Material and methods

Field collection of parental fish

Risør Fjord on the Norwegian coastal Skagerrak (Figure 1), encompassing ~20 km^2, provides habitat for populations of Atlantic cod in the inner and outer fjord. Although cod are not physically restricted from moving between the inner and outer areas, gene flow is evidently quite limited, given their genetic differentiation from one another; dispersal between the populations has been estimated to be <2% per generation (Knutsen *et al.*, 2011). Additionally, significant differences in life history have been documented between the two populations, reflected in part by the slower growth of inner-fjord cod (Lekve *et al.*, 2002; Kuparinen *et al.*, 2015). In December 2014, 4–6 weeks prior to spawning, adult cod were collected by fyke net from two areas: Sørfjorden (hereafter, inner Risør fjord) and Østerfjorden (hereafter, outer Risør fjord).

Fish from each population were measured for length, tagged externally, using a T-Bar anchor tag labelled with a unique identification code, and subsequently placed in a 1-m deep, 9 × 5 m spawning basin (lined with natural rock) at the Institute for Marine Research, Flødevigen (~60 km south of Risør), where they spawned undisturbed. The sex ratio of spawning cod (determined by post-mortem inspection) was female-biased 1.6: 1.0 (45 females, 28 males). Within each population, the number of females: males was 24:12 and 21: 16 for outer- (length range: 45–63 cm) and inner-fjord cod (45–57 cm), respectively. Water in the spawning basin, pumped constantly from the coast at a depth of 75 m (a very stable environment with low variability in temperature and nutrients), averaged 7.4 °C, i.e. ambient temperature. Lights were adjusted to mimic the natural photoperiod and cod were fed ~2 kg of shrimp (*Pandalus borealis*) daily.

Laboratory sampling of larval and adult cod

The spawning period (94 days; 20 January to 24 April 2015) began when eggs were first present in the egg collector (positioned at the water surface at the spawning-basin outflow) and was considered to have ended when eggs were absent from the collector for 5 consecutive days. Eggs were present in the collector on all but 3 days. Egg batches were sampled on 90 of the days in the spawning period and then incubated separately in 1 of 15 tanks at 6.1 °C (*SD*: ±0.4) until the 50% hatch-development stage (15.0 ± 0.6 days, mean ± *SD*) (Supplementary Figure S1). When the offspring in a tank had achieved 50% hatch, a photograph and genetic sample were obtained for 50 individual larvae sampled at random. To obtain offspring measurements, larvae were placed in a solution of RNAlater and immediately individually photographed on a wet slide, using a Leica DFC425 C camera mounted to a Leica MZ16 A stereoscope at 20× magnification. A standardized length slide etched with a 1-mm scale bar was photographed at the beginning of each daily batch of photographs and later used to calibrate the image analysis software. After the photograph was taken, each whole larva was individually preserved in 1.5-ml micro tubes containing 250 µl of RNAlater for genetic analysis.

One month following completion of spawning, the following morphological data were recorded for each adult; total length (mm); pelvic fin lengths (mm); total weight (g); liver weight (g); gonad weight (g); and stomach weight with and without contents (g). The gonadosomatic index (GSI = gonad weight/somatic body weight) and the hepatosomatic index (HSI = liver weight/somatic body weight) were considered proxies for body condition (Lambert and Dutil, 1997). (We interpret condition/growth metrics measured 1 month after the termination of the spawning period to represent a defensible albeit imperfect measure of condition/growth prior to and during the spawning; nonetheless, we note that these data should be interpreted with caution.) Age determinations were made from examination of transversally sectioned otoliths, one per adult. Due to health concerns, two adults were sampled prior to the end of the spawning season, at which time their length, weight, and sex were recorded and an otolith removed for age determination.

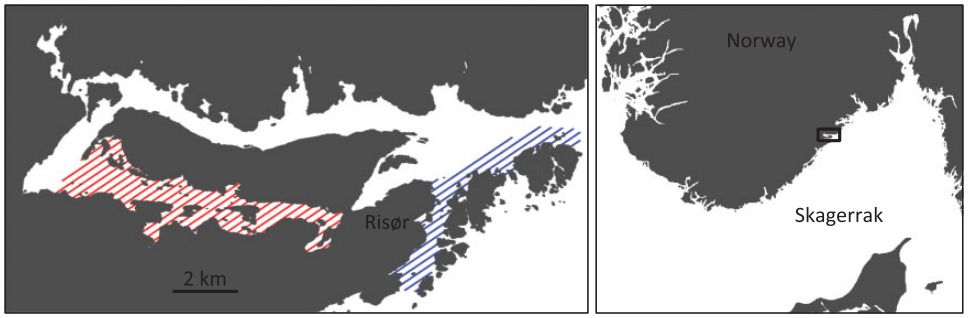

Figure 1. Immediately prior to the spawning period, Atlantic cod were collected from the inner (lined area on the left) and the outer (lined area on the right) Risør fjord on the Norwegian Skagerrak coast. Modified from Kuparinen *et al.* (2015).

Genetic analysis

DNA was extracted from parental fin clips, using an OMEGA Bio-tek tissue extraction kit, and from whole offspring, using the OMEGA Bio-tek 96-well plate DNAeasy extraction kit. All samples were amplified, using two multiplexes consisting of four loci each. Multiplex 1 was made up of three tetranucleotide repeat loci (*Gmo8, Gmo19*, and *Tch11*) and one trinucleotide repeat locus (*Gmo35*; Miller *et al.*, 2000; O'Reilly *et al.*, 2002). Multiplex 2 was made up of three dinucleotide repeat loci (*Gmo132*, *Gmo2*, and *Tch13*) and one tetranucleotide repeat locus (*Gmo34*; Brooker, 1994; Miller *et al.*, 2000; O'Reilly *et al.*, 2002). Both multiplexes were chosen based on the high levels of heterozygosity at each locus, genotyping reliability, and demonstrated efficiency for paternity studies in Atlantic cod (Dahle *et al.*, 2006; Wesmajervi *et al.*, 2006). Loci were amplified by polymerase chain reaction conditions, as specified by Wesmajervi *et al.* (2006) and Dahle *et al.* (2006), and then analysed using the capillary gel electrophoreses instrument, 3130*xl* Genetic Analyser (Applied Biosystems). Allelic sizes were scored with instrument-specific software and the programme GeneMapper (Applied Biosystems). To increase the accuracy of parental-genotype identifications, all adults were amplified three times per multiplex and scored independently by three different people. Disagreements on genotyping identification were referred to a fourth individual. The software MICRO-CHECKER (Van Oosterhout *et al.*, 2004) was used to test the microsatellite loci for evidence of stuttering or null alleles. There was limited evidence of potential null alleles for *Gmo19* and *Tch11* for inner- and outer-fjord cod, respectively. However, given this inconsistency between populations, and that the frequency of null alleles at these loci (<0.07) is considered low (Dakin and Avise, 2004), the loci were retained in the analysis given. None of the other markers exhibited evidence of scoring error, large allele dropout, or null alleles.

Family reconstruction of the allelic data from both offspring and parents was performed with the programme COLONY v2.0.6.1 (Jones and Wang, 2010). Larvae were run in batches of 10 sampling days (~500 larvae per batch). All runs used the full-likelihood method with high precision and a random seed number. Genotyping error was set to 0.02 per locus. Each analysis was repeated, using medium, long and very long runs, to assess whether maximum likelihood configuration had been reached.

Offspring quality indicators

The offspring quality indicators were the length of the larvae and the volume of the yolk sac standardized to the length of each larva [This standardization was necessitated by a highly significant positive association between lengths of larvae and yolk-sac volumes; Pearson correlation coefficient = 0.312 ($t = 21.634$, df = 4339, $p < 0.001$); Spearman rank correlation coefficient = 0.377 ($S =$ 8 491 900 000, $p < 0.001$).]. Length was measured as the distance from the snout to the end of the notochord. Yolk-sac volume (V) was calculated as $V = \pi\ (6LH^2)^{-1}$ (Uusi-Heikkilä *et al.*, 2010), where L is the length (horizontal measurement; μm) and H is the height (vertical measurement; μm) of the yolk sac. All measurements were taken using the image analysis software Fiji (Schindelin *et al.*, 2012).

Batch verification

Before analysing trends in offspring phenotype and reproductive output, it was necessary that eggs could be confidently ascribed to single egg batches, i.e. the group of eggs produced by a single female following a single mating episode (A mating episode is defined as the release of eggs by a single female that are then fertilized by one or more males; see Rowe *et al.* [2008] for more details.) We assumed that all of the eggs collected on a single day that had been genetically assigned to a particular female:male combination, were the result of a single mating episode by that female. There was, however, a low probability that not all eggs produced on a given day had been retained by the egg collector within 24 h of the occurrence of the mating event(s). That is, some eggs resulting from a given batch might have been retained in the spawning basin for >24 h, resulting in their collection one or more days later.

To ascertain the correct egg batch corresponding to each larva, we examined the temporal variation in the number of offspring produced, and the ratio of yolk-sac volume standardized to the length of the larvae for each female. The volume of the yolk sac divided by larval length averaged 1330 mm^2 at hatch, steeply declining to zero after 5–9 days when the larvae had exhausted their yolk sacs and increased in length. For a given female, if the offspring she had produced (verified genetically) were present in the egg collector for 2 consecutive days, the offspring collected on the second day were considered to be offspring that had been retained from a previous batch. Thus, eggs produced by a single female that had been collected over 2 consecutive days were considered to comprise two separate egg batches for that female if: (i) the number of larvae collected on the second day exceeded the number collected the previous day, and (ii) the mean standardized yolk-sac volume on the second day had not declined relative to that estimated for the previous day. As reported below,

this batch-verification protocol resulted in the removal of a small percentage (3%) of the genetically identified larvae from the analysis.

Timing of reproduction

The number of egg batches and the duration of spawning were used to examine variation in the timing of reproduction among individuals. The number of batches ascribed to each individual equaled the number of days on which offspring genetically linked to that individual were observed (subject to the identification protocol articulated in the previous section earlier). The duration of spawning for each individual was the difference between the first and last day on which offspring were observed.

Statistical analyses

The cumulative seasonal change in offspring quality was examined by applying a sequence of first- through third-order polynomial regressions for offspring quality as a function of the day of the spawning period. This was done for both metrics of offspring quality: length-at-hatch and standardized yolk-sac volume.

To determine whether a female consistently produced offspring of presumed higher or lower quality throughout the spawning season, we first removed the temporal trend that was evident in the data. To do so, residual analyses were conducted on the final models from the pooled offspring quality analyses. Given the constancy in developmental time (Supplementary Figure S1), a positive residual was considered indicative of a larva of above-average quality whereas a negative residual was considered indicative of a larva of comparatively poorer quality. Finally, to obtain mother-specific estimates, the mean of each individual's residual offspring quality values across the spawning season was calculated. This was done for both the length of offspring and their standardized yolk-sac volume.

Generalized linear models were used to examine a female's overall relative offspring quality (OQ). Mean residual offspring quality, either for length or standardized yolk-sac volume, was a function of population identity (inner/outer fjord), length (prior to spawning), the individual difference in length before and after spawning (Length.Diff – a proxy for growth) and several variables measured at the termination of the spawning period: weight, HSI, GSI, age, and mean pelvic fin length (i.e. calculated from the residuals of linear regressions between pelvic fin length and body length *sensu* Skjæraasen *et al.*, 2006):

$$\mathrm{OQ} \sim \mathrm{population} + \mathrm{length} + \mathrm{lengthdiff} + \mathrm{weight} + \mathrm{HSI} + \mathrm{GSI} + \mathrm{age} + \mathrm{mean\ pelvic\ fin\ length} \quad (1)$$

Variation in reproductive timing (RT) (number of batches and duration of spawning) was examined using generalized linear models run separately for each sex, such that each of the two reproductive timing metrics was a function of population, length, weight, HSI, GSI, age, and the residual mean pelvic fin length:

$$\mathrm{RT} \sim \mathrm{population} + \mathrm{length} + \mathrm{weight} + \mathrm{HSI} + \mathrm{GSI} + \mathrm{age} + \mathrm{mean\ pelvic\ fin\ length} \quad (2)$$

Model selection was performed by stepwise reduction, following Zuur *et al.* (2009). To examine the robustness of the model selection and final models, stepwise forward model selection was also performed. All analyses were conducted with R version 3.1.0 (R Core Team, 2014).

Results

Parentage analysis

Microsatellite genotypes were obtained for 4489 of 4500 larvae. Parental assignment was based on maximum likelihood inference, using the results of the long runs (the long runs yielded nearly identical results to those produced by the very long runs). All unknown parentage genotypes were referenced against known parents. If an unknown parentage genotype matched five of eight loci of a known parent, the unknown parent was re-assigned as the known parent. Given that all parental genotypes are known, any mismatches in genotype are a result of either mutation or scoring error. The final parentage analysis resulted in successful paternal assignment of 94.0% (4221 of 4489) of the larvae and successful maternal assignment of 93.5% (4198 of 4489) of the larvae.

Metrics of offspring quality

Using the batch-verification protocol described above, only 3% ($n = 135$) of larvae were estimated to have been retained in the spawning basin for >24 h after they had been spawned; these were excluded from further analysis. As one example, in the sample plot for individual F16, it is clear which larvae are representative of true unique batches and which are likely residual larvae that had been retained (Supplementary Figure S2).

The total length of larvae at hatch declined relatively consistently during the spawning period (Figure 2). Between the first and final days of the spawning period, the model-estimated length of cod larvae declined 11% (4430–3940 μm). Female body weight was positively ($p = 0.006$) associated with larval length (Table 2).

Standardized yolk-sac volume appeared to increase during the initial third of the spawning period but to decline thereafter (Figure 3, Table 1). Between the first and final days of the spawning period, model-estimated yolk-sac volume (after accounting for the observed seasonal trend in larval length) declined 49% (1222–624 $\mu m^3 \cdot \mu m^{-1}$); yolk-sac volume on the final day of the spawning period is estimated to be 62% less than the peak standardized volume (1635 $\mu m^3 \cdot \mu m^{-1}$, attained near the end of the first third of the spawning period; Figure 3). Following model selection for the mean residual standardized volume of the yolk sac, the best model included positive effects of female length and the length difference of a female between the beginning and end of the spawning period (Table 2).

Timing of reproduction

Males contributed gametes to a greater number of egg batches, and spawned over a longer period of time, than females. Regarding the former, the average number of egg batches to which each male contributed sperm (19.5 ± 14.4 *SD*) was more than double that produced by females (9.2 ± 4.6 *SD*) ($p = 0.0002$). Spawning duration of males (48.9 ± 20.9 *SD* days) was 58% longer than that of females (30.8 ± 17.9 *SD* days) ($p = 0.0009$). Comparing the two populations, inner-fjord males contributed to significantly more egg batches (22.2 ± 14.9) than outer-fjord males (14.1 ± 12.4) ($p = 0.0237$ (Table 3) although their spawning-period durations did not differ significantly (inner fjord: 53.1 ± 18.8 days; outer fjord: 40.6 ± 23.6 days;

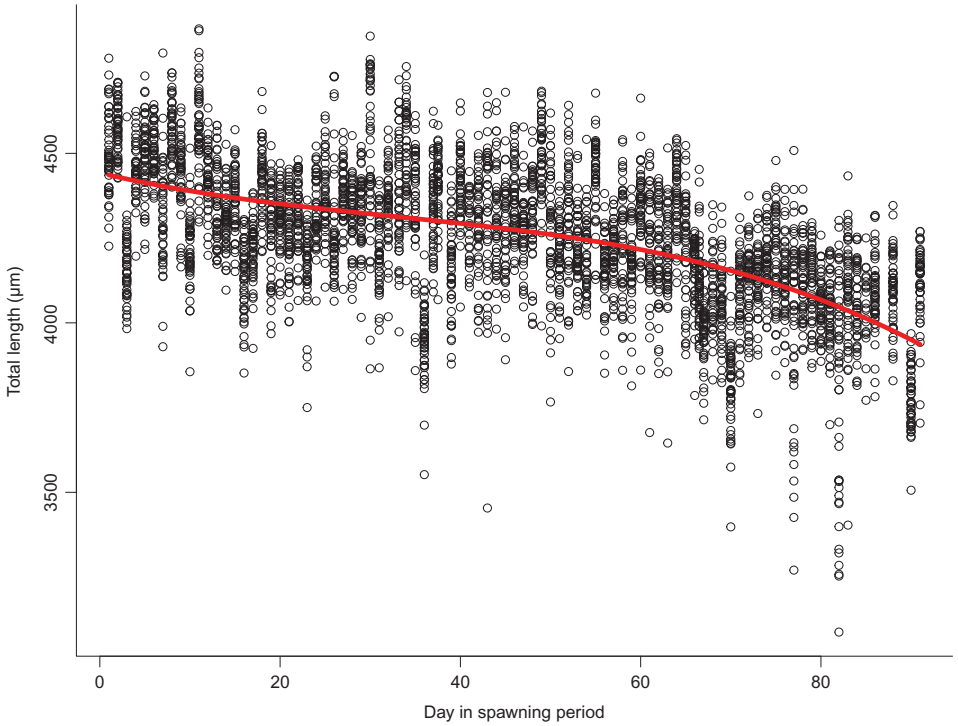

Figure 2. Seasonal trend in the total length-at-hatch of larvae ($n = 4489$) produced by 73 wild-caught Atlantic cod, *Gadus morhua*, during the course of an entire spawning season. The model fit for the polynomial (red line): total length = ß0 + ß1(batch-day #)^2 + ß2(batch-day #)^3, computed using the total length vs. batch-day number.

Table 1. Parameter estimates for the pooled seasonal trend in metrics of offspring quality measured for 4489 larvae produced by a free-spawning population of 73 wild-caught Atlantic cod.

Model [ß$_0$ + ß$_1$(batch-day #) + ß$_2$(batch-day #)^2+ ß$_3$(batch-day #)^3]		**Estimate**	***SE***	***p*-value**
Length ~ Batch-Day #	ß$_0$	4.43×10^{2}	1.08	<0.001
	ß$_1$	-5.12×10^{-1}	1.01×10^{-1}	<0.001
	ß$_2$	8.02×10^{-3}	2.56×10^{-3}	0.002
	ß$_3$	-9.01×10^{-5}	1.85×10^{-5}	<0.001
Yolk-sac volume per total length ~ Batch-Day #	ß$_0$	1.19×10^{3}	3.18×10^{1}	<0.001
	ß$_1$	3.26×10^{1}	2.98	<0.001
	ß$_2$	-6.74×10^{-1}	7.55×10^{-2}	<0.001
	ß$_3$	2.72×10^{-3}	5.45×10^{-4}	<0.001

Length, or yolk-sac volume per total length, is considered to be a function of a sequence of first- through third-order polynomial regressions for batch-day number (*SE* represents standard error).

$p = 0.0879$). The same population dichotomy in reproductive timing was evident among females, inner-fjord females producing significantly more batches (10.7 ± 4.0) than outer-fjord females (7.0 ± 4.7) ($p = 0.0245$); inner-fjord females spawned over significantly longer time periods (inner fjord: 37.5 ± 18.0 days; outer fjord: 20.5 ± 12.4 days; $p = 0.0044$) (Table 4).

The two metrics of reproductive timing were correlated with body size and differed between the inner- and outer-fjord populations, depending on sex. Among males, larger individuals fertilized greater numbers of egg batches, and inner-fjord males fertilized more batches than outer-fjord males (Table 3). Among females, number of egg batches was positively and negatively associated with prespawning length and post-spawning weight, respectively. There was a significant population effect on the timing of reproduction for females, those originating from the outer fjord producing fewer egg batches over shorter periods of time (Table 4). There were no significant differences between populations in the date on which spawning was initiated either for males or females.

Discussion

The present study documented fine-scaled temporal changes in, and phenotypic correlates of, offspring quality and key metrics of reproductive timing in a batch-spawning marine fish. Based on analyses of almost 4500 Atlantic cod larvae spawned throughout a 3-month period, the mean length of larvae and the mean length-standardized yolk-sac volume declined 11 and 49%, respectively. Longer, heavier females tended to produce longer larvae and to provision their offspring with relatively larger yolk sacs. Body size affected the number of egg batches to which an individual contributed genetically but not the duration of the spawning period. Males spawned for a longer period of time (49 days) than females (31 days); neither sex spawned throughout the entire spawning period of 94 days. After accounting for body size and condition, there was evidence of significant differences in number of egg batches and spawning duration between two spatially proximate populations separated by <10 km.

Trends in offspring phenotype

Our work suggests that offspring quality, as reflected by larval length and yolk-sac volume, can vary throughout the spawning period of broadcast-spawning fishes for which offspring survival is subject to high levels of environmental and demographic stochasticity. It is unclear, however, whether this finding is consistent with

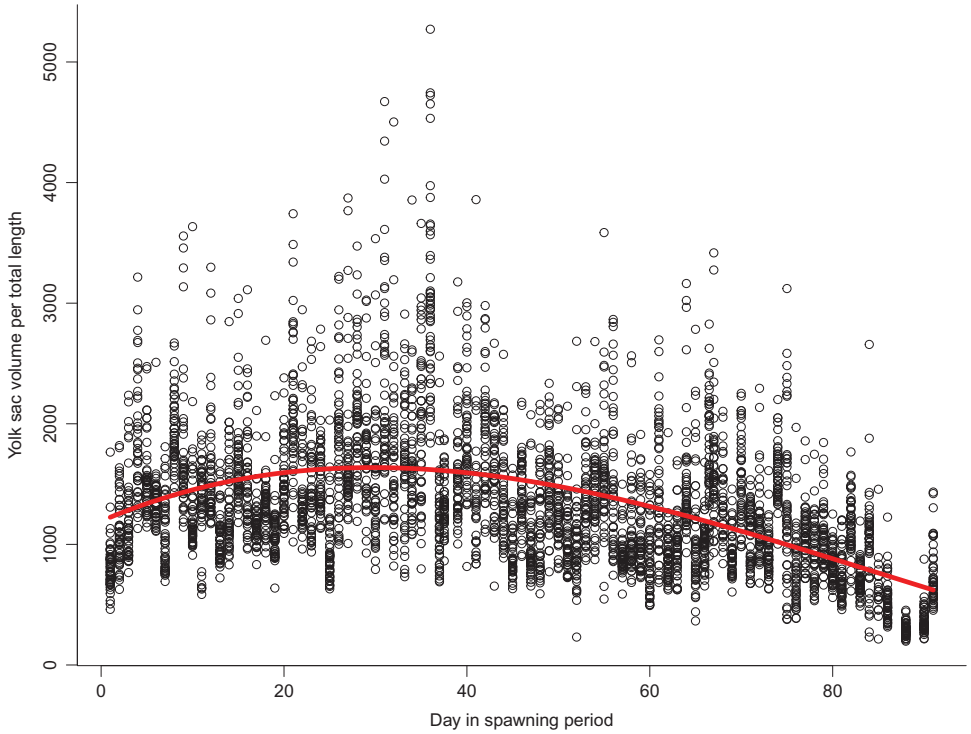

Figure 3. Seasonal trend in the yolk-sac volume standardized to length at hatch ($\mu m^3 \cdot \mu m^{-1}$) of larvae ($n = 4489$) produced during the spawning period by 73 wild-caught Atlantic cod. The model fit for the polynomial: yolk-sac volume per total length $= ß_0 + ß_1$(batch-day #) $+ ß_2$(batch-day #)$^2 + ß_3$(batch-day #)^3 was computed, using the yolk-sac volume per total length vs. batch-day number.

Table 2. Generalized linear models for offspring quality in females.

	Effect	Estimate	*SE*	*t*-value	*p*-value
Residual larval length	Weight	0.011	0.004	2.966	0.006
Residual volume/length	PreLength	2.005	0.875	2.291	0.030
	Length.Diff	4.167	1.838	2.267	0.031

The response variable, the mean residual offspring quality (length or volume/length), was considered to be a function of population identity, length prior to spawning, weight, HSI, GSI, length difference between the beginning and the end of the experiment, and residual mean pelvic fin length. The best model (fixed effects for which $p < 0.05$ are presented) for residual length included the variable "Weight" (body weight). The best model for residual volume of the yolk sac standardized by length included the variables "PreLength" (length prior to spawning) and "Length.Diff" (the length difference between the beginning and the end of the experiment).

Table 3. Generalized linear model for the reproductive timing metric of number of egg batches in male Atlantic cod.

Response	Effect	Estimate	*SE*	*t*-value	*p*-value
Number of batches	PopOuter	−14.731	6.015	−2.449	0.024
	Weight	0.018	0.008	2.209	0.039

The response variable was considered to be a function of population identity, length, weight, HSI, GSI, and residual mean pelvic fin length. The best model (fixed effects for which $p < 0.05$ are presented) for number of batches included "PopOuter" (population) and "Weight" (body weight). Degrees of freedom: (2, 20).

the hypothesis that females are favoured evolutionarily to maximize the number of offspring each of which is at or near the physiologically minimum size. All else being equal, declining larval length is associated with increased larval mortality (Pepin, 1991), and one would think that a halving of yolk-sac volume might have similar consequences. Based on Pepin's (1991) empirical model for marine fish ($M = 0.25e^{\circ.067T}L^{-0.68}$, where $T = 6.1$ mm and $L = 4.43$ or 3.94 mm), the 11% reduction in larval length throughout the spawning period reported here is predicted to be associated with an 7% increase in daily larval mortality. Thus, the observed decrease in metrics of offspring quality observed here might not be adaptive but reflect a form of energetic "fatigue", a reduction in the ability of females to maintain the production of higher-quality offspring throughout the spawning period because of energetically related costs of reproduction.

Alternatively, the observed trends might be consistent with the ESS of maximizing the number of minimally sized offspring (Hutchings, 2002). To be adaptive, the presumed fitness cost (reduced offspring survival) associated with producing smaller, less well-provisioned larvae as the spawning period progresses would need to be offset by environmental conditions that are increasingly favourable to offspring, such as increased food supply. There is compelling evidence that these conditions of increasing productivity exist. Based on a 28-year dataset, Tiselius *et al.* (2016) reported order-of-magnitude seasonal increases in both primary and secondary productivity in Skagerrak waters during the January-through-April spawning period for cod. This temporal shift in food abundance could be interpreted as meaning that the physiologically minimum offspring size (and quality) of cod declines during the spawning period because of steadily improving food-supply conditions. This adaptation-based hypothesis might reduce the presumed fitness costs associated with reductions in larval length and yolk-sac volume during the lengthy

Table 4. Generalized linear models for reproductive timing metrics in females: number of batches and spawning duration.

Response	Effect	Estimate	*SE*	*t*-value	*p*-value
Number of batches	Weight	−0.006	0.002	−2.523	0.017
	PopOuter	−3.639	1.534	−2.372	0.024
	PreLength	0.047	0.021	2.223	0.034
Spawning duration	PopOuter	−18.561	5.994	−3.097	0.004

The response variable, the spawning metric, was considered to be a function of population identity, length, weight, HSI, GSI, and residual mean pelvic fin length. The best model (fixed effects for which $p < 0.05$ are presented) for number of batches included "Weight" (body weight), "PopOuter" (population) and "PreLength" (length prior to spawning). The best model for the length of spawning included "PopOuter" (population) only. Degrees of freedom for both analyses: (3, 28).

spawning period under natural conditions. Temperature also increase during the spawning period for these coastal Norwegian cod and might be hypothesized to create increasingly favourable conditions for developing larvae (Steinarsson and Björnsson, 1999), although the extent to which these conditions might be countered by the increasing stress of heightened metabolic demands (Chauton *et al.*, 2015) is not known. Predation is another likely source of larval mortality (Pepin, 1993), although it is not known if predation risk to larval cod increases from January through April. It is also not known if the presumed reductions in offspring quality documented here would have a meaningful impact on predation risk relative to the maternal fitness cost of producing fewer, albeit larger (and potentially less vulnerable) offspring.

To our knowledge, the present work is the first to report seasonal declines in the length of larvae and yolk-sac volume during the spawning period in a batch-spawning marine fish. Previous studies (e.g. Chambers and Waiwood, 1996; Kjesbu *et al.*, 1996; Trippel, 1998; Vallin and Nissling, 2000) have documented reductions in egg diameter during the spawning period. The temporal decline in egg size documented by Trippel (1998) was relatively constant with time, whereas Chambers and Waiwood (1996) reported a peak in egg size halfway through the spawning period, corresponding roughly to the timing of the model-estimated peak in yolk-sac volume reported here.

Parental correlates of offspring quality

The positive influence of female body size on larval length is consistent with previously documented associations between female size and both larval length-at-hatch and egg size (Kjesbu, 1989; Kjesbu *et al.*, 1996; Vallin and Nissling, 2000; Marteinsdottir and Begg, 2002). Our finding that female size correlates with relative yolk-sac volume accords with Solemdal *et al.*'s (1993) correlation between female size and the amino acid content of eggs, and Ouellet *et al.*'s (2001) report of a positive link between prespawning condition and egg energy content.

We also found evidence that larval length is positively correlated with the increase in body size experienced by females during and (or) after the spawning period; Chambers and Waiwood (1996) reported a similar association between growth and egg size. Given the existence of a growth cost of reproduction (energy allocated to reproduction is not available for somatic growth; Hutchings, 1999; Jørgensen and Holt, 2013), one might not have expected faster growth to be associated with greater production of energetically costly, higher quality offspring. However, this trade-off is a *within-individual* cost, meaning that a negative correlation need not be expected between growth rate and offspring quality among different individuals. The data reported here suggest that females who experienced superior growth were higher-quality individuals that were also able to produce better quality larvae.

Correlates of reproductive timing

Larger individuals contributed to a greater number of egg batches than smaller individuals. This was true for both males and females, although the significantly positive correlate of body size was weight in males and length in females. Oddly, the correlation coefficient for body weight was negative for females. Upon closer examination, however, we find that this can be attributed to the fact that the two heaviest females experienced very low reproductive success. Excluding these females from the analysis, there is no relationship between maternal weight and number of egg batches (although population identity and length retain their significant effects). The low number of fertilized batches produced by the largest females might be attributable to a lack of suitably sized males for paired spawning (Brawn, 1961; Rakitin *et al.*, 2001; Bekkevold *et al.*, 2002). Rowe *et al.* (2007), for example, reported that cod achieve their highest reproductive success when breeding with mates larger than themselves. In our spawning group, there were no males larger than the two heaviest females.

The present study provides experimental verification of a model-based prediction that male Atlantic cod spawn for longer periods of time than females (Hutchings and Myers, 1993). This longer spawning period would allow the average male to contribute gametes to a greater number of egg batches than the number of batches produced by the average female (here, the average number of batches to which each male contributed sperm was more than double that produced by females). Hutchings and Myers (1993) postulated that large females would have a longer spawning duration than small females. Although we found no correlation between these two variables, the statistical power of the analysis might have been low given comparatively small ranges in body size; 80% of males and females were between 50 and 60 cm in length.

To our knowledge, sexual differences in batch number have not previously been described in cod or for any other batch-spawning marine fish. Our ranges in egg batches per female (1–18) and spawning duration (1–76 days) are not dissimilar to those documented in other experimental studies (Kjesbu, 1989; Chambers and Waiwood, 1996; Kjesbu *et al.*, 1996; Trippel, 1998).

Conclusion

Our findings highlight patterns of fine-scaled temporal variability in metrics of offspring quality, parental correlates of these metrics, and reproductive timing in Atlantic cod. We found that larval size and yolk-sac volume generally declined throughout the spawning period, although it is not clear whether this was due to the physiological demands of batch spawning or an adaptive response attributable to increasingly favourable environmental conditions during the spawning period. Offspring size and yolk-sac volume are positively influenced by female body size and by growth. With respect to reproductive timing, parental size affected the number of egg batches to which an individual contributed genetically but not the duration of the spawning period.

After accounting for multiple phenotypic differences, number of egg batches and spawning duration differed between adjacent cod populations, contributing to a growing body of literature indicating that biologically meaningful variability can exist within broadcast-spawning marine fishes at surprisingly small spatial scales (Knutsen *et al.*, 2011; Kuparinen *et al.*, 2015; Roney *et al.*, 2016). There is a need for research that quantifies the fitness consequences of individual and population differences in temporal variability in offspring quality and reproductive timing and, by extension, per capita population growth and productivity.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the article.

Acknowledgements

We would like to acknowledge the contributions of the staff, technicians, students (especially E. Juliussen and D. Rivas-Sánchez), and volunteers at the Institute of Marine Research Flødevigen that assisted with experimental maintenance and data collection. We are grateful for the extensive, detailed comments provided by two referees. This study was funded by Canada's Natural Sciences and Engineering Research Council (Discovery Grant to J.A.H. and Canada Graduate Scholarships to N.E.R. and R.A.O.), the Norwegian Research Council (HAVKYST Grant to J.A.H. and Leiv Eiriksson Mobility Grant to R.A.O., the Prediction and Observation of the Marine Environment network (mobility grants to N.E.R. and R.A.O.), and the European Regional Development Fund (Interreg IVa. "MarGen" project).

References

Bekkevold, D., Hansen, M. M., and Loeschcke, V. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua* L.). Molecular Ecology, 11: 91–102.

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

Brooker, A. L. 1994. Polymorphic microsatellites: tools for measuringgenetic diversity in subpopulations of Atlantic cod *(Gadus morhua*). MSc thesis, Department of Biology, Dalhousie University, Halifax, N.S., Canada.

Chambers, R. C., and Waiwood, K. G. 1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Canadian Journal of Fisheries and Aquatic Sciences, 53: 1986–2003.

Chauton, M. S., Galloway, T. F., Kjørsvik, E., Størseth, T. R., Puvanendran, V., van deer Meeren, T., Karlsen, Ø., Rønnestad, I., and Hamre, K. 2015. H NMR metabolic profiling of cod (*Gadus morhua*) larvae: potential effects of temperature and diet composition during early developmental stages. Biology Open, 4: 1671–1678.

Childs, D. Z., Metcalf, C. J. E., and Rees, M. 2010. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. Proceedings of the Royal Society B, 277: 3055–3064.

Dahle, G., Jørstad, K., Rusaas, H., and Otterå, H. 2006. Genetic characteristics of broodstock collected from four Norwegian coastal cod (*Gadus morhua*) populations. ICES Journal of Marine Science, 63: 209–215.

Dakin, E. E., and Avise, J. C. 2004. Microsatellite null alleles in parentage analysis. Heredity, 93: 504–509.

Einum, S., and Fleming, I. A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): Norms of reaction to environmental quality. Proceedings of the Royal Society of London B, 266: 2095–2100.

Fiksen, Ø., and Folkvord, A. 1999. Maternal effects and the benefit of yolk supply in cod larvae in different environments - a simulation model. ICES Council Meeting 1999/Y: 21, 1–6.

Hutchings, J. A. 1999. The influence of growth and survival costs of reproduction on Atlantic cod, *Gadus morhua*, population growth rate. Canadian Journal of Fisheries and Aquatic Sciences, 56: 1612–1623.

Hutchings, J. A. 2002. Life histories of fish. *In* Handbook of Fish Biology and Fisheries Vol. 1. Fish Biology, pp. 149–174. Ed. by P. J. B. Hart and J. D. Reynolds. Blackwell Science, Oxford.

Hutchings, J. A., and Myers, R. A. 1993. Effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua*, in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2468–2474.

Hutchings, J. A., and Rangeley, R. W. 2011. Correlates of recovery for Canadian Atlantic cod (*Gadus morhua*). Canadian Journal of Zoology, 89: 386–400.

Jones, O. R., and Wang, J. 2010. COLONY: A program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10: 551–555.

Jørgensen, C., and Holt, R. E. 2013. Natural mortality: its ecology, how it shapes fish life histories, and why it may be increased by fishing. Journal of Sea Research, 75: 8–18.

Kjesbu, O. S. 1989. The spawning activity of cod, *Gadus morhua* L. Journal of Fish Biology, 34: 195–206.

Kjesbu, O. S., Solemdal, P., Bratland, P., and Fonn, M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 53: 610–620.

Knutsen, H., Olsen, E. M., Jorde, P. E., Espeland, S. H., André, C., and Stenseth, N. C. 2011. Are low but statistically significant levels of genetic differentiation in marine fishes "biologically meaningful"? A case study of coastal Atlantic cod. Molecular Ecology, 20: 768–783.

Kuparinen, A., Roney, N. E., Oomen, R. A., Hutchings, J. A., and Olsen, E. M. 2015. Small-scale life history variability suggests potential for spatial mismatches in Atlantic cod management units. ICES Journal of Marine Science, 71: 1088–1099.

Lack, D. C. 2008. The significance of clutch-size. Ibis, 89: 302–352.

Lambert, Y., and Dutil, J.-D. 1997. Condition and energy reserves of Atlantic cod (Gadus morhua) during the collapse of the northern Gulf of St. Lawrence stock. Canadian Journal of Fisheries and Aquatic Sciences, 54: 2388–2400.

Lekve, K., Ottersen, G., Stenseth, N. C., and Gjøsæter, J. 2002. Length dynamics in juvenile coastal Skagerrak cod: Effects of biotic and abiotic processes. Ecology, 83: 1676–1688.

Marteinsdottir, G., and Begg, G. A. 2002. Essential relationships incorporating the influence of age, size and condition on vari- ables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. Marine Ecology Progress Series, 235: 235–256.

Miller, K. M., Le, K. D., and Beacham, T. D. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). Molecular Ecology, 9: 238–239.

Morrison, C. 1992. The digestive tract of the cod eleutheroembryo ("yolk-sac larva") and larva. NAFO Scientific Council Studies, 18: 23–24.

Neilson, J., Perry, R., Valerio, P., and Waiwood, K. 1986. Condition of Atlantic cod *Gadus morhua* larvae after the transition to exogenous feeding: morphometries, buoyancy and predator avoidance. Marine Ecology Progress Series, 32: 229–235.

O'Reilly, P. T., McPherson, A. A., Kenchington, E., Taggart, C., Jones, M. W., and Bentzen, P. 2002. Isolation and characterization of tetranucleotide microsatellites from Atlantic haddock (*Melanogrammus aeglefinus*). Marine Biotechnology, 4: 418–422.

Ouellet, P., Lambert, Y., and Berube, I. 2001. Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. ICES Juornal of Marine Science, 58: 672–686.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Canadian Journal of Fisheries and Aquatic Sciences, 48: 503–518.

Pepin, P. 1993. An appraisal of the size-dependent mortality hypothesis for larval fish: comparison of a multispecies study with an empirical review. Canadian Journal of Fisheries and Aquatic Sciences, 50: 2166–2175.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation Statistical Computing, Vienna, Austria. http://ww.r-project.org.

Rakitin, A., Ferguson, M. M., and Trippel, E. A. 2001. Male reproductive success and body size in Atlantic cod *Gadus morhua* L. Marine Biology, 138: 1077–1085.

Rollinson, N., and Hutchings, J. A. 2013a. Environmental quality predicts optimal egg size in the wild. American Naturalist, 182: 76–90.

Rollinson, N., and Hutchings, J. A. 2013b. The relationship between offspring size and fitness: integrating theory and empiricism. Ecology, 94: 315–324.

Roney, N. E., Hutchings, J. A., Olsen, E. M., Knutsen, H., Albretsen, J., and Kuparinen, A. 2016. Fine-scale life-history structure in a highly mobile marine fish. Evolutionary Ecology Research, 17: 95–109.

Rowe, S., Hutchings, J. A., and Skjæraasen, J. E. 2007. Non-random mating in a broadcast spawner: mate size influences reproductive success in Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 64: 219–226.

Rowe, S., Hutchings, J. A., Skjæraasen, J. E., and Bezanson, L. 2008. Morphological and behavioural correlates of reproductive success in Atlantic cod *Gadus morhua*. Marine Ecology Progress Series, 354: 257–265.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. 2012. Fiji: an open-source platform for biological-image analysis. Nature Methods, 9: 676–682.

Sinervo, B., and McEdward, L. R. 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. Evolution, 42: 885–899.

Skjæraasen, J. E., Rowe, S., and Hutchings, J. A. 2006. Sexual dimorphism in pelvic fin length of Atlantic cod. Canadian Journal of Fisheries and Aquatic Sciences, 20: 347–353.

Smith, C. C., and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. American Naturalist, 108: 499–506.

Solemdal, P., Bergh, Ø., Dahle, G., Falk-Petersen, I.-B., Fyhn, H., Grahl-Nielsen, O., Haaland, J. et al. 1993. Size of spawning arcto-Norwegian cod (*Gadus morhua* L.) and the effects of their eggs on early larvae. ICES Statutory Meeting G, 41: 1–22.

Steinarsson, A., and Björnsson, B. 1999. The effects of temperature and size on growth and mortality of cod larvae. Journal of Fish Biology, 55: 100–109.

Svärdson, G. 1949. Natural selection and egg number in fish. Report of the Institute of Freshwater Research Drottningholm, 29: 115–122.

Tiselius, P., Belgrano, A., Andersson, L., and Lindahl, O. 2016. Primary productivity in a coastal ecosystem: a trophic perspective on a long-term time series. Journal of Plankton Research, 38: 1092–1102.

Trippel, E. A. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Transactions of the American Fisheries Society, 127: 339–359.

Uusi-Heikkilä, S., Wolter, C., Meinelt, T., and Arlinghaus, R. 2010. Size-dependent reproductive success of wild zebrafish Danio rerio in the laboratory. Journal of Fish Biology, 77: 552–569.

Vallin, L., and Nissling, A. 2000. Maternal effects on egg size and egg buoyancy of Baltic cod, Gadus morhua Gadus. Implications for stock structure effects on recruitment. Fisheries Research, 49: 21–37.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535–538.

Wesmajervi, M. S., Westgaard, J.-I., and Delghandi, M. 2006. Evaluation of a novel pentaplex microsatellite marker system for paternity studies in Atlantic cod (*Gadus morhua* L.). Aquaculture Research, 37: 1195–1201.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.

Handling editor: Francis Juanes