

REPRODUCTIVE SUCCESS IN ATLANTIC COD, *GADUS MORHUA*.

by

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For my parents.

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Abstract

This research highlights the complexity of the cod mating system while providing an in-depth empirical analysis of reproductive success in Atlantic cod. I performed a comprehensive examination of reproductive success in Atlantic cod, *Gadus morhua*, at unprecedented temporal resolution, spatial scale, and sample size. The parentage analysis using eight microsatellite markers was performed on 4489 individual larval samples, from 73 wild-caught adults, obtained daily over a 91-day period. Size had a positive influence on all three correlates of reproductive success: the number of offspring fertilized, the quality of offspring produced and the timing of reproduction (i.e. duration and the number of batches). The mating strategy of cod played a critical role in determining the number of offspring fertilized for both males and females. I hypothesized that male size was fundamental in determining its rank within a dominance hierarchy, and subsequently, top-ranked males were able to dominate spawning events resulting in disproportionately high reproductive success. The three large females had unexpectedly low reproductive success, a trend I attributed to there being a lack of suitably sized males in the spawning basin. This research highlights the complexity of the cod mating system while providing an in-depth empirical analysis into multiple metrics of reproductive success.

List of Abbreviations and Symbols Used

μm	micrometers
DNA	Deoxyribonucleic acid
GSI	Gonadosomatic Index
HSI	Hepatosomatic Index
mm	millimeters
OQ	Offspring Quality
RNA	Ribonucleic acid
R_s	The number of offspring fertilized
RT	Reproductive Timing
SD	Standard Deviation
SE	Standard Error

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Chapter 1: Introduction

Understanding variance in reproductive success is a fundamental issue in ecology and evolutionary biology (Howard 1979). Variation in individual reproductive success affects population-level processes such as gene flow, recruitment variability, and *per capita* population growth rate, making it of key importance to understanding a natural system (Møller and Legendre 2001, Freeland et al. 2011, Allendorf et al. 2013). Variance in reproductive success among individuals can result from processes varying from zygotic competition (e.g. Pacific oyster, *Crassostrea gigas* [Boudry et al. 2002]) to alternative mating strategies (e.g. lekking in Atlantic cod, *Gadus morhua* [Rowe et al. 2008]). Irrespective of the specific mechanism, reproductive success among individuals in a population can be so skewed that only a few individuals are responsible for the majority of offspring produced (Bekkevold et al. 2002, Rowe et al. 2008, Serbezov et al. 2010). In instances where large skews in reproductive success persist, it is particularly pertinent to understand the source of this variance and, furthermore, to identify what traits of individuals correlate with higher and lower levels of reproductive success.

Identifying sources of variance in reproductive success in a natural system is not a simple task (Howard 1979). In smaller, relatively closed systems such as lakes and streams, genetic markers have been successfully applied to obtain in situ estimates of reproductive success attributable to known individuals (e.g. brown trout, *Salmo trutta*, in a stream [Serbezov et al. 2010]). The results of such studies attain the most desirable estimations of reproductive success as they are representative of the production and survival of an individual's offspring under natural environmental conditions and selective

pressures. However, obtaining similar *in situ* estimates of reproductive success and further understanding the variance among individuals remains a challenge for populations in larger, open oceanic systems. Due to factors such as large population sizes, uninhibited dispersal, and migration, reliable and accurate estimates of *in situ* reproductive success are considerably more difficult to obtain, as they require extensive amounts of sampling and genetic power. As a result, many studies employ experimental methods to gain insight as to the extent to which individual traits correlate with reproductive success (Rowe et al. 2008, Ryder et al. 2009). By establishing experimental correlates of reproductive success, one can then estimate how these empirical relationships might translate to variance in a natural system (Scott et al. 2006).

Experimental studies in marine fishes have examined various metrics of reproductive success, including number of eggs produced (Buzeta and Waiwood 1982), number of offspring fertilized (Bekkevold et al. 2002), growth rate of larvae (Clemmesen et al. 2003) and larval time to starvation (Berkeley et al. 2004a). The variety of indicators used to experimentally represent reproductive success raises the question: what defines reproductive success? Lincoln et al. (1982) simply define it as "the number of offspring surviving at a given time". In this context, experimental representations of reproductive success have provided informative empirical tools for studying factors that affect the probability that a greater or lesser number of offspring survive at any given time.

The number of offspring fertilized is the one of the most commonly used experimental metrics of reproductive success (Bekkevold et al. 2002, Rowe et al. 2008, Uusi-Heikkilä et al. 2010). This metric constitutes the most basic representation of an individual's reproductive success and provides a fundamental baseline to any future

estimates of the number of offspring surviving at a given time. Assuming that selection pressures affect all offspring equally, the relative variance of initial fertilization success among individuals should be relatively representative of future relative values of reproductive success within a system.

However, from a parental perspective, while the number of offspring fertilized is fundamental to an individual's reproductive success, other intrinsic factors, such as the quality of the offspring produced and the timing at which they are produced, will influence the continued success of offspring, and hence parental reproductive success. This variance in offspring quality and reproductive timing is of particular relevance for long-lived, batch-spawning species for which there is expected to be a greater variability in individual reproductive success, often with older spawners producing better quality offspring (Berkeley et al. 2004a, Fitzhugh et al. 2012, Hixon et al. 2013). For batch-spawning species, the increased temporal window over which offspring are produced allows for greater variation in environmental conditions experienced by different batches of offspring (Wright and Trippel 2009). Thus, in a long-lived, batch-spawning species such as cod, while the absolute level of fecundity of a fish is important, likely affecting the number of offspring fertilized, the variation in the quality of offspring and reproductive timing can further influence the reproductive success of an individual.

Using an experimental approach, I examined reproductive success in Atlantic cod, a species whose life history attributes and alternative mating strategies make it intriguing to study within the context of behavioural ecology, but a species that has provided one of the most compelling and dramatic examples of how over-exploitation can contribute to population collapse (Rowe and Hutchings 2003, Hutchings and Rangeley 2011).

Originally thought to have had a simple mating strategy, cod have been found to exhibit a complex mating system that includes male competition, dominance hierarchies, courtship displays, agonistic interactions, and (potentially) mate choice (Brawn 1961, Hutchings et al. 1999). This long-lived, bet-hedging species releases multiple batches of eggs over the course of a 1-3 month spawning period (Rowe and Hutchings 2003, Hutchings and Rangeley 2011) and their lekking mating strategy results in large variance in reproductive success among individuals (Bekkevold et al. 2002, Rowe et al. 2007, 2008). Thus, their life-history attributes and alternative mating strategy make them an interesting candidate species in which to examine reproductive success in a marine fish.

The thesis is a comprehensive experimental examination of reproductive success in a free-spawning aggregation of Atlantic cod. Chapter 1 introduces both Chapter 2 and Chapter 3. Both Chapter 2 and Chapter 3 utilize data from the same reproductive success experiment conducted in the winter of 2014. The spawning group size (73 adults), temporal resolution of samples (daily over a 93-day spawning period), and number of larvae sampled (4500 larvae) combine to exceed both the resolution and breadth at which a reproductive success experiment of a broadcast-spawning marine fish has previously been examined.

Chapter 2 examines the number of offspring fertilized from males and females throughout the spawning period. The primary objectives of this chapter were: 1) to improve understanding of individual variance in reproductive success, and 2) to examine temporal variation among reproductively successful individuals. I test the null hypotheses that reproductive success is independent of individual traits such as body size, and that there is no temporal trend in the mean size of reproductively successful individuals.

Chapter 3 then focuses on key factors related to individual allocation of reproductive effort: offspring quality and the timing of reproduction. The main objectives of this chapter were: 1) to examine how offspring quality varies over the course of the spawning period, 2) to improve understanding of the causes and consequences of individual variation in offspring quality, and 3) to investigate how the timing of reproduction differs among individuals. I test the null hypotheses that offspring quality is independent of time in the spawning period and maternal traits. Furthermore, for both males and females, I tested the null hypothesis that reproductive timing was independent of individual traits.

The general implications of the findings of the thesis research are discussed in Chapter 4.

Chapter 2: Individual and temporal variation of reproductive success

2.1 Methods

2.1.1 Study Populations

A comprehensive reproductive success experiment was conducted using cod in spawning condition obtained from Risør fjord (~ 20km²), located on the southern coast of Norway and opening eastward to the Skagerrak Sea via a small channel (Fig. 1). More specifically, cod were sampled from Sørfjorden (hereafter, inner Risør fjord) and Østerfjorden (hereafter, outer Risør fjord). While cod are not physically restricted from moving between the inner and outer areas, dispersal between the putative populations has been estimated to be as low as 0.7%, and statistically significant levels of genetic differentiation between the inner and outer fjord groups has been documented (Knutsen et al. 2011). In addition, there are significant differences in life history between the two populations, reflected in part by slower growth rates experienced by inner cod (Dannevig 1949, Lekve et al. 2002, Kuparinen et al. 2015).

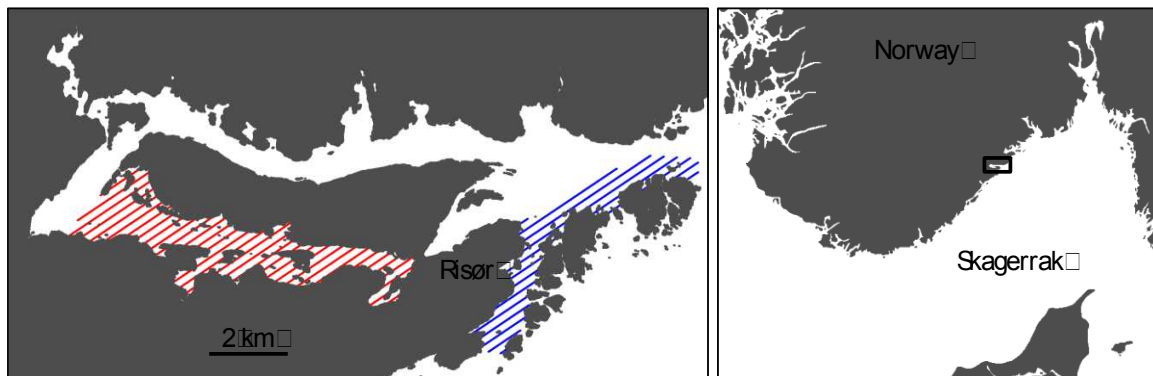


Figure 1: Adult collection areas. Atlantic cod were collected from the inner (red lined area) and the outer (blue lined area) Risør fjord on the Norwegian Skagerrak coast. Modified from Kuparinen et al. (2015).

2.1.2 Adult Collection and Experimental Spawning Conditions

In December, one month prior to the 2014 winter spawning season, adult cod were collected from fyke nets set within the inner (n=36) and outer (n=37) Risør fjord areas. After transport to the Institute for Marine Research Flødevigen research station, cod were placed in a single spawning basin where they remained uninterrupted for the entire experiment (Fig. 2). Prior to placement in the spawning basin, fish were measured for length and tagged externally, using a T-Bar anchor tag labeled with a unique identification code (Fig. 3).

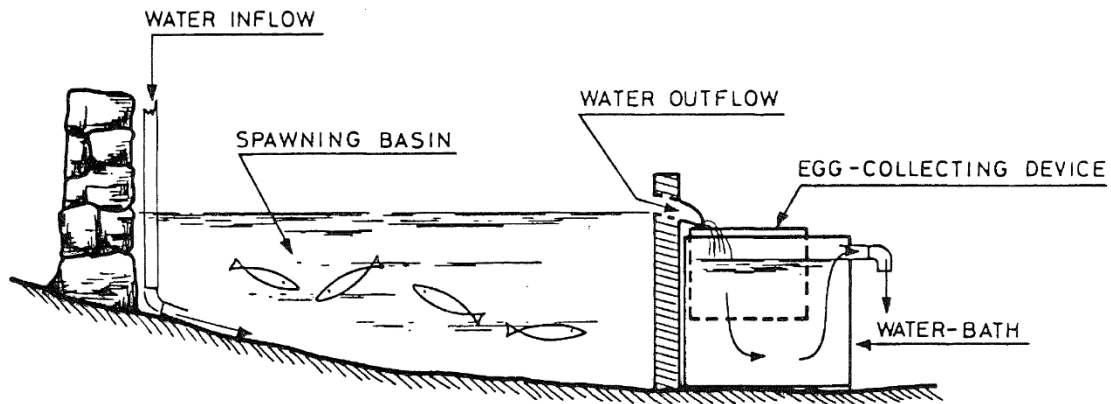


Figure 2: Flødevigen spawning basin. Taken from Moksness and Riis-Vestergaard (1982).

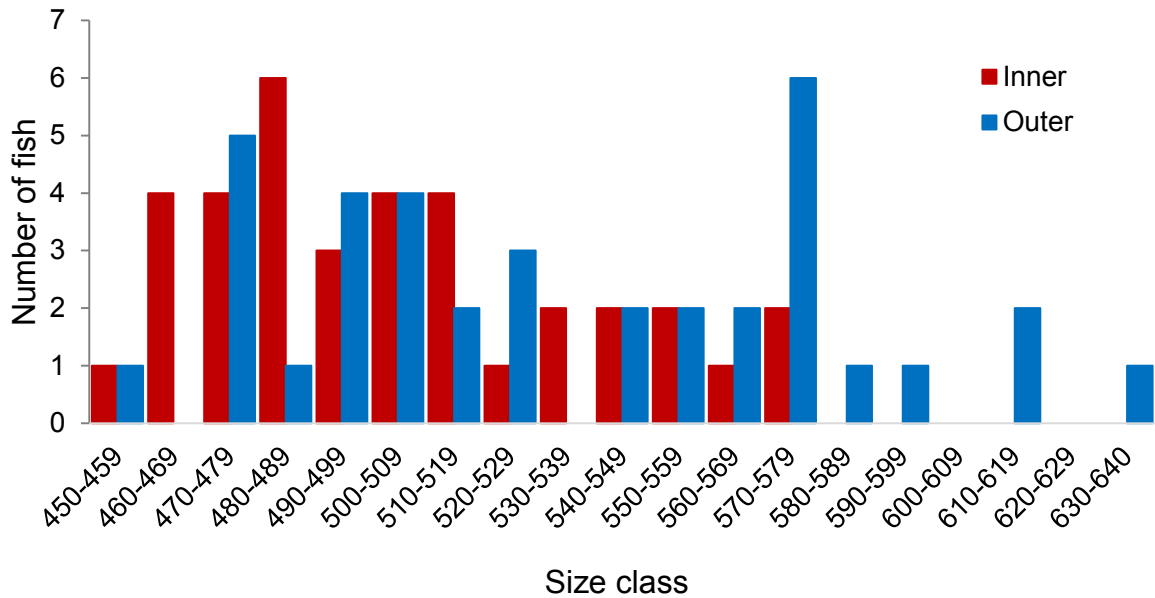


Figure 3: Size frequency distribution of all fish put into the spawning basin at the beginning of the experiment. Blue bars are fish from the outer fjord population and red bars are from the inner fjord population.

The Flødevigen spawning basin (45m³), originally built in 1880 for the specific purpose of collecting eggs for a coastal cod restocking program, is a semi-natural basin built into the rock-bed foundation, allowing for a balance between natural spawning environment and laboratory control. The flow rate of 0.5-6.0 litres sec⁻¹ results in 0.8-12.0 complete exchanges of water daily (Moksness and Riis-Vestergaard 1982). Lighting was adjusted weekly to mimic the natural light rhythm throughout the experiment. The temperature of the spawning basin was the natural incoming temperature of coastal water pumped into the basin from a depth of 75 meters (Fig. 4). Salinity ranged from 33.5‰ to 34.8‰ during the experiment.

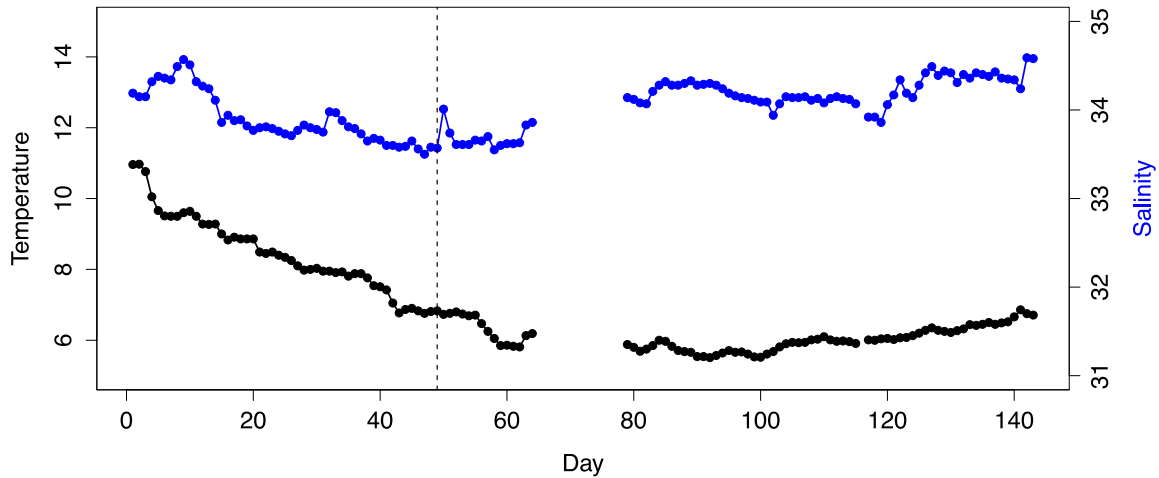


Figure 4: Spawning basin temperature and salinity during the experimental period (2 December 2013 to 24 April 2014). Dashed line represents the start date of spawning on 20 January 2014.

2.1.3 Larval Sampling

Sampling of larvae occurred throughout the spawning period, beginning on the date when eggs were first present in the egg collector and terminating when no eggs had been collected for five consecutive days. Eggs were collected daily between 8:00 and 10:00 hours. Upon collection, the volume of the eggs was measured using a 4-*l* graduated cylinder and then placed into one of 14 incubation tanks. The spawning period lasted 94 days, from 20 January to 24 April; eggs were collected on 91 days (Fig. 5).

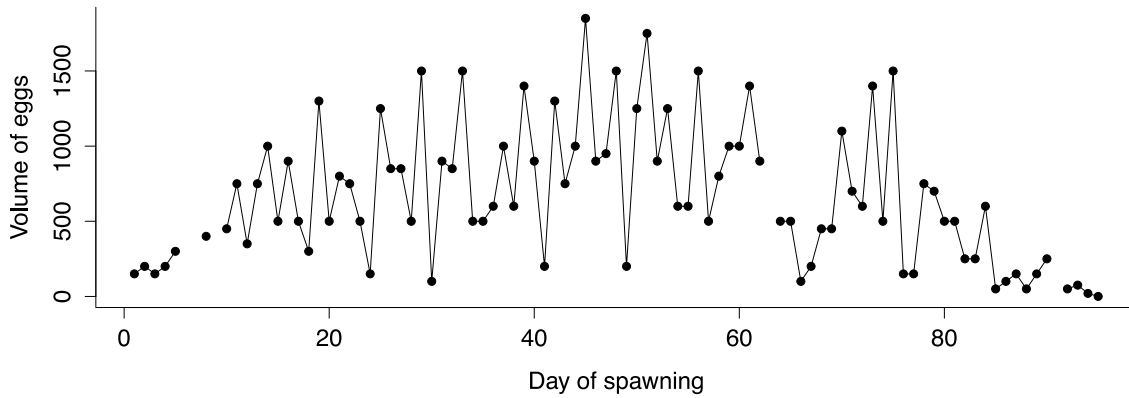


Figure 5: Daily volume (ml) of eggs collected throughout the spawning period. Absent estimates of egg volume are due either to the absence of eggs or an overflow of the egg collector, thus resulting in an incorrect volume estimate.

Eggs were incubated at $6.1 \pm 0.5^\circ\text{C}$ (mean \pm SD) until they were visually assessed to be at 50% hatch (15 ± 0.6 days, mean \pm SD). When tanks were at 50% hatch, genetic samples were obtained for 50 individual larvae. Each whole larva was preserved individually in micro tubes containing $250\mu\text{L}$ of ThermoFisher RNAlater for future genetic analyses. Over the course of the 94-day spawning season, daily larval genetic samples totaling 4500 individuals were collected from a free-spawning population of 73 wild adults.

Separate from the genetic samples preserved in RNAlater, an additional 50 larvae were collected daily, individually frozen in liquid nitrogen, and stored at -80°C . Although these larvae were initially sampled for a lipid composition analysis that never occurred (because of logistical constraints), they served as useful back-up samples for the genetic analyses.

2.1.4 Adult Sampling

Adults were sacrificed via a knock-out blow to the head. Otoliths were then extracted and morphological traits measured one month after the completion of spawning in five separate batches over a 19-day period. Morphological traits measured were: total standard length (mm); median fin length (mm); total weight (g); liver weight (g); gonad weight (g); and stomach weight with and without contents (g). The gonadosomatic index ($GSI = \text{gonad weight/body weight}$) and the hepatosomatic index ($HSI = \text{liver weight/body weight}$) were both calculated as proxies for body condition (Lambert and Dutil 1997). Due to poor health, two adults were sampled early in the spawning season, at which time only length, weight, otolith, and sex were recorded.

2.1.5 Age Determination

Age estimates were obtained from otoliths for 72 of the 73 adults. One otolith from each individual was embedded in a black polyester resin and transversally sectioned at the Otolith Research Laboratory at the Bedford Institute of Oceanography, Canada, using equipment and protocol described at http://www.bio.gc.ca/otoliths/methods-methodes/annuli_age-age_anneaux-eng.php. Images of sectioned otoliths were then obtained under reflected light, using an Axiocam Mrm camera mounted to a Zeiss SteREO Lumar v12 stereomicroscope. All images were processed to enhance local contrast between the opaque and translucent zones, after which ages were estimated by counting annuli along transects starting from the nucleus in the centre of the otolith, proceeding until the edge.

2.1.6 Parentage Analysis

2.1.6.1 DNA extraction and microsatellite amplification

DNA was extracted, according to manufacturer's protocols, from parental fin clips in individual tubes, 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*, *Tch13*) and one tetranucleotide repeat locus (*Gmo34*) (Brooker et al. 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 electrophoresis instrument, 3130xl Genetic Analyser (Applied Biosystems). Allelic sizes were calculated with instrument-specific software and the program GeneMapper (Applied Biosystems). To ensure absolute accuracy in parental genotypes, 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.

2.1.6.2 Family reconstruction

Family reconstruction of the allelic data from both offspring and parents was performed with the program COLONY v2.0.6.1 (Jones and Wang 2010). Larvae were run in batches of 10 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 (see sample colony input file in Appendix A).

2.1.7 Statistical Analysis

2.1.7.1 Individual variation in reproductive success

For both males and females, an individual's reproductive success was quantified as the number of offspring fertilized. Cumulative rank curves were used to visualize skews in reproductive success. The proportion of offspring fertilized was plotted against the rank of the individual in terms of highest number of offspring produced. A deviation from a 1:1 ratio line was indicative of a skew in reproductive success.

2.1.7.2 Morphological correlates of reproductive success

Generalized linear models were used to examine morphological correlates of reproductive success. Models were run separately for each sex, such that the number of offspring produced (R_s) was a function of population identity, length prior to spawning, weight, HSI, GSI, age, and the residual mean pelvic fin length (calculated from the residuals of linear regressions between pelvic fin length and body length *sensu* Skjæraasen et al. 2006):

$R_s \sim \text{Population} + \text{Length} + \text{Weight} + \text{HSI} + \text{GSI} + \text{Age} + \text{pelvic fin length}^*$

Due to a high skew in the number of sired offspring, the model for males incorporated a quasi-poisson error structure. The model for females was run under the assumption of a normal distribution. Model selection was performed following Zuur et al. (2009), using the stepwise model reduction. Residual plots were examined to ensure the model was fitting the data. To examine the robustness of the model selection process and final models, stepwise forward model selection was also performed. All analyses were conducted with R version 3.1.0 (R Core Team 2014).

2.1.7.3 Temporal variation in reproductive success

Temporal variation in reproductive success was examined by looking at the seasonal trend in the mean length of fish spawning on a given day. Linear models were run separately for each sex, such that the day of the spawning season was a function of the mean length of fish participating in spawning:

$\text{Day} \sim \text{Mean length of spawning fish}$

Mean length was calculated two ways: 1) the mean length of fish spawning, and 2) the weighted mean length of spawning fish (i.e. the mean length of fish spawning, weighted by the relative number of offspring produced by individuals in a day.)

2.2 Results

2.2.1 Spawning Population

The sex ratio in the spawning basin was 1:1.6 (males : females), with 45 females and 28 males. Of the outer fjord fish, 24 were females and 12 were males where as for the inner fjord, 21 were females and 16 were males. For both males and females, the size

frequency distributions for each population were relatively similar. That being said, the outer fjord fish were on average slightly larger, and for both males and females, the largest fish were from the outer fjord population (Fig. 6 and Fig 7.).

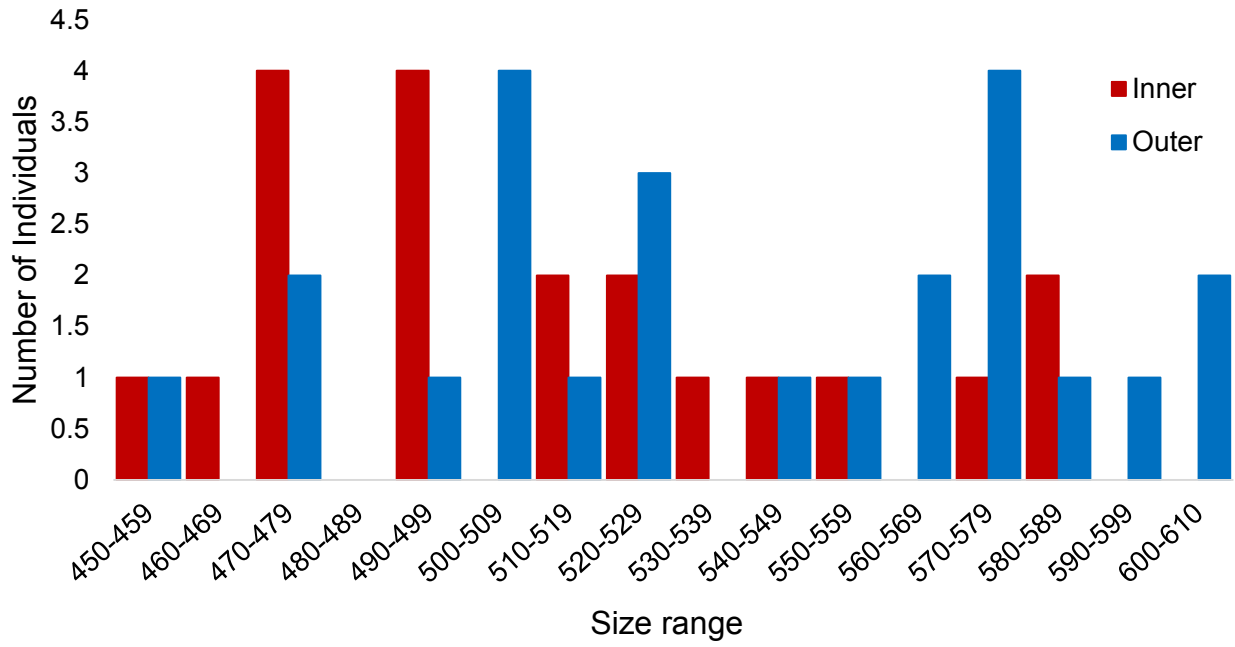


Figure 6: Size frequency distribution of females at the beginning of the spawning season. Blue bars are fish from the outer fjord population and red bars are from the inner fjord population.

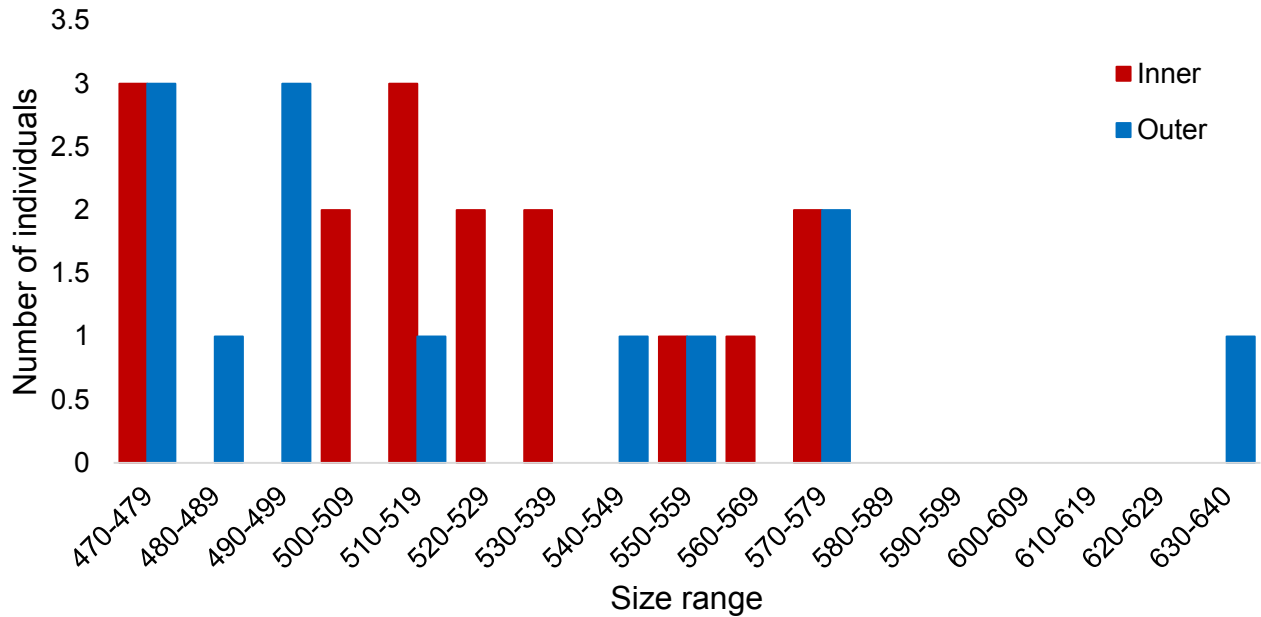


Figure 7: Size frequency distribution of males at the beginning of the spawning season. Blue bars are fish from the outer fjord population and red bars are from the inner fjord population.

2.2.1 Parentage Analysis

2.2.1.1 Microsatellite genotyping

Microsatellite genotypes were successfully obtained for all adults, with a minimum of two successful replicate amplifications per locus per adult. In the outer fjord population one marker, GMO 19, exhibited evidence of potential null alleles, whereas in the inner fjord population, TCH 11, exhibited evidence of potential null alleles. These loci were retained given the lack of consistency of null alleles between the populations. All other markers exhibited no evidence of scoring error, large allele dropout or null alleles.

Microsatellite genotypes were obtained for 4489 of 4500 larvae (99.8%). Of the larvae successfully genotyped, 3508 of 4489 (78%) were comprised of all eight loci and 4459 of 4489 (99%) were comprised of four or more loci (Fig. 8).

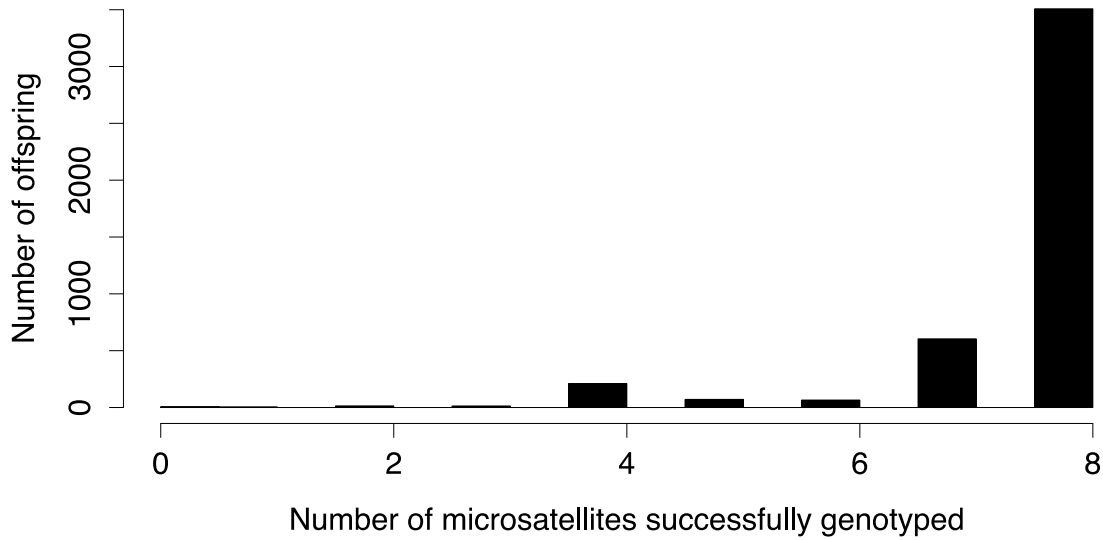


Figure 8: The frequency distribution of the number of microsatellites successfully genotyped per individual in the final dataset.

2.2.1.2 Parentage assignments

Short and medium runs in COLONY v2.0.6.1 produced varying parentage results whereas results from long and very long runs were nearly identical. Thus, the results from long runs were used for parental assignment. The maximum likelihood was clearly obtained during long runs, providing further indication that the long run provided sufficient time for the program to reach the best configuration (Fig. 9). In the instance where an offspring was assigned to an unknown parent, the genotype of ‘unknown’ parents was compared to the known parental genotypes. If an unknown parental genotype matched at least 5 of 8 loci of a known parent, the unknown parent was re-assigned as the known parent. Since I am certain that I have all of the parental genotypes, I can assume any mismatches in genotype are a result of either mutation or scoring error. The final parentage analysis resulted in successful paternal assignment to 94.0% of the larvae, 4221 of 4489, and successful maternal assignment to 93.5%, 4198 of 4489 (Fig. 10).

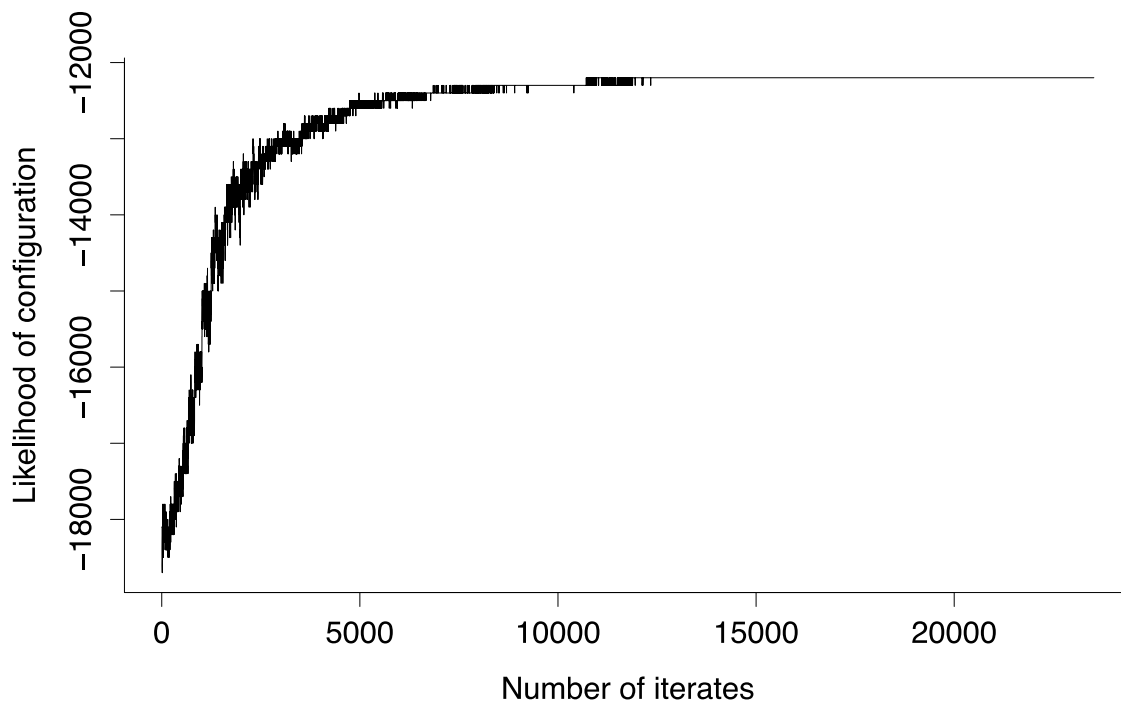


Figure 9: Example of the change of log likelihood as a function of the number of iterates from a long run.



Figure 10: The best pedigree configuration of sampled adults and larvae as determined by COLONY v2.0.6.1 (Jones and Wang 2010). Parental identifications (IDs) are indicated at the top and offspring IDs at the bottom of the figure. Black lines indicate males and grey lines indicate females. Adult IDs are sorted from left to right, beginning with inner fjord cod (F#) and ending with outer fjord cod (R#).

2.2.2 Variation in Reproductive Success

Of the 73 fish in the spawning basin, offspring was detected from 57 individuals over the course of the spawning period (33 females and 24 males). Overall, males exhibited a higher skew in reproductive success than females (Fig. 11). Out of the 24 males who spawned, the top ranked male sired 23.0% of the offspring and the top three males were responsible for 50.5% of the offspring. Females exhibited less of a skew in reproductive success. Among the 33 females who spawned, the top ranked female

produced only 7.5% of the offspring, and the top three females were responsible for 20.2% of the offspring, substantially less than the male equivalent.

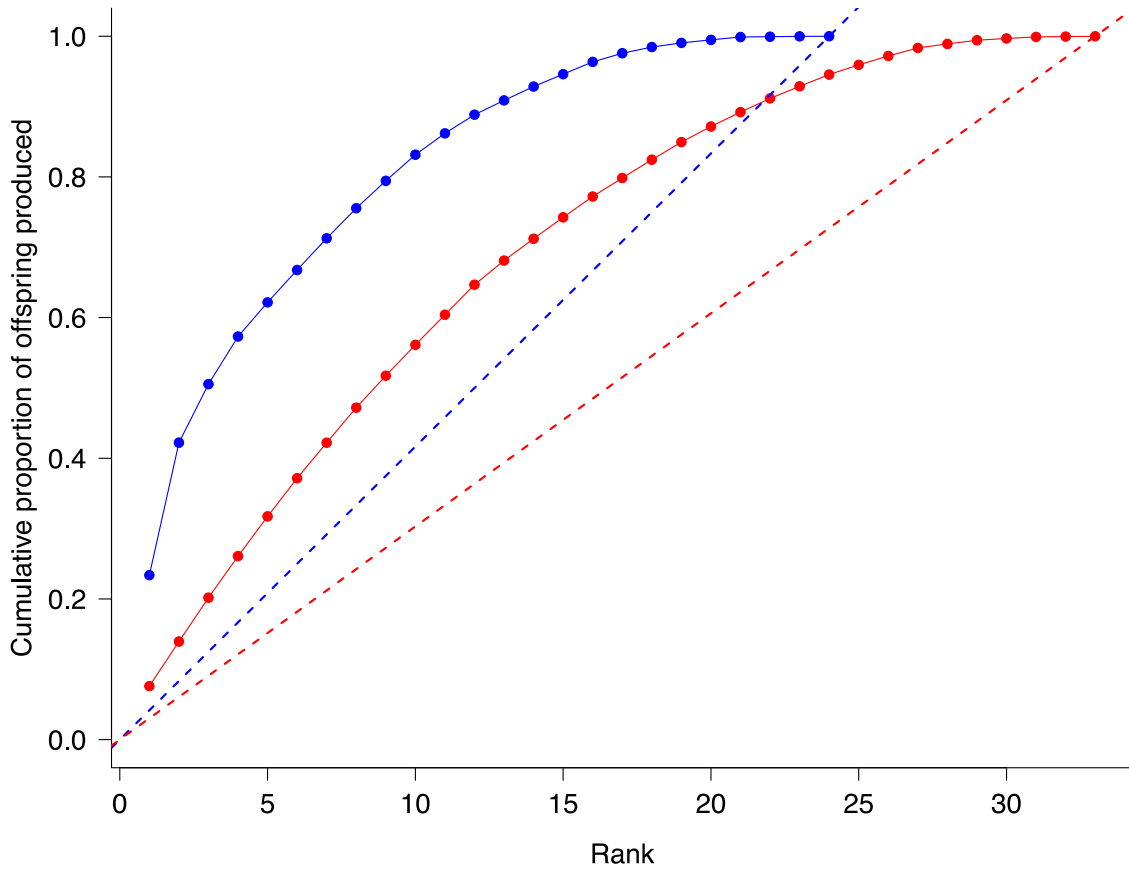


Figure 11: Cumulative proportion of offspring produced by male and female Atlantic cod ranked from most to least successful. Dashed lines represent the relationship if all individuals contributed equally. Blue curve for males. Red curve for females.

When the cumulative reproductive success curves were examined with the populations separated, the males and females exhibited skews similar to those evident when the data were pooled (with the exception of the outer fjord females, who exhibited a much stronger skew; Fig. 12).

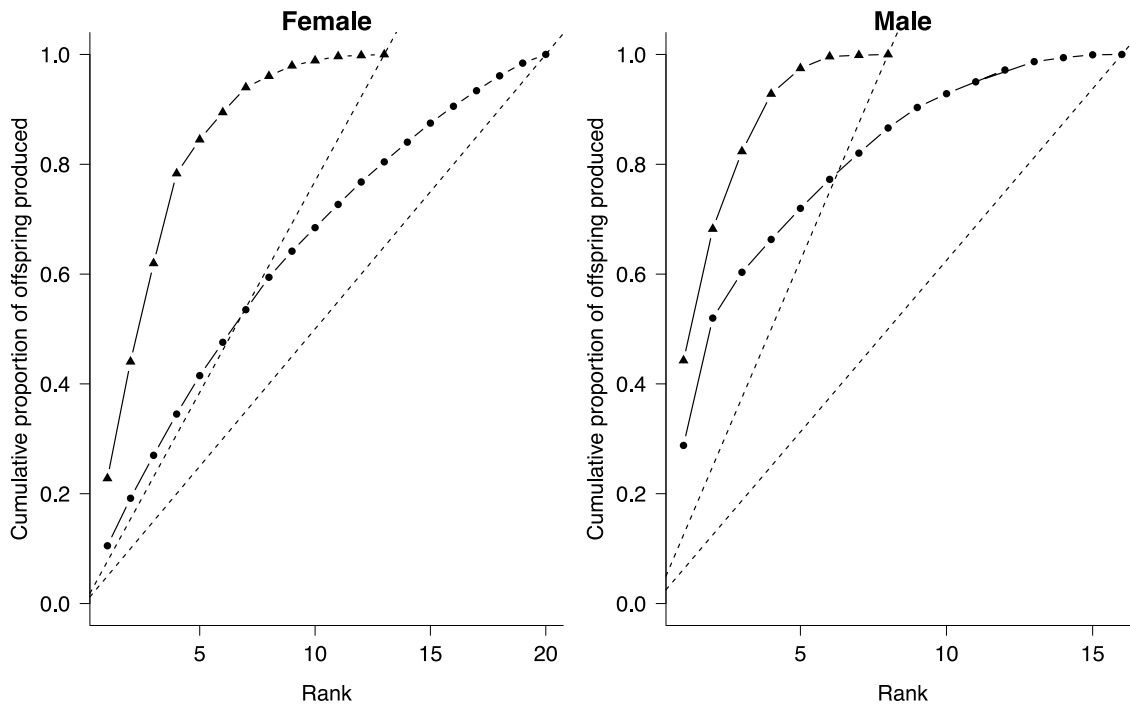


Figure 12: Cumulative proportion of offspring produced by male and female Atlantic cod ranked from most to least successful (\blacktriangle : Outer Fjord; \bullet : Inner Fjord). Dashed line represents relationship if all individuals contributed equally.

2.2.3 Morphological Correlates of Reproductive Success

Following model selection for males, the best model included weight, population and GSI (Table 1). Weight was the most significant predictor with a slightly positive coefficient (0.002), indicating that increases in weight had a positive additive effect on the number of offspring sired. The second most significant predictor was population origin. Males from the outer population had a lower reproductive success than males from the inner population. The GSI was the least significant predictor. Its negative regression coefficient indicates that lower GSI at the end of the spawning season was associated with higher number of offspring sired.

Table 1: Generalized linear model for reproductive success in males, assuming a quasi-poisson distribution. The response variable, the number of offspring sired, was considered to be a function of population identity, length, weight, hepatosomatic index, gonadosomatic index (GSI) and residual mean pelvic fin length. The significant variables ($P < 0.05$) were Weight (body weight), PopOuter (outer population), and GSI.

	Effect	Estimate	SE	t-value	P-value
Males	Weight	0.002	0.000	4.752	0.0001
	PopOuter	-1.759	0.419	-4.203	0.0005
	GSI	-0.336	0.160	-2.102	0.0491

Interestingly, the regression coefficient for weight, the most significant variable in the model, was barely positive with a regression coefficient value of 0.002. Upon examination of the relationship between the number of offspring sired and weight, it is evident that the three most successful males were among the largest in the population, indicative of a strong positive correlation. However, beyond these three top males, there was little to no relationship (Fig. 13). This mixture of a strong positive correlation among comparatively heavy males and no correlation among moderately heavy and small males is likely the cause of the slightly positive, albeit significant, regression coefficient. The pattern in these data indicates that there is not a continuous pattern of association between male body size and male reproductive success. Rather, there is a strong positive correlation between being the heaviest in the population and the number of offspring sired; however, if a male is not among the heaviest individuals, then weight no longer affects its reproductive success.

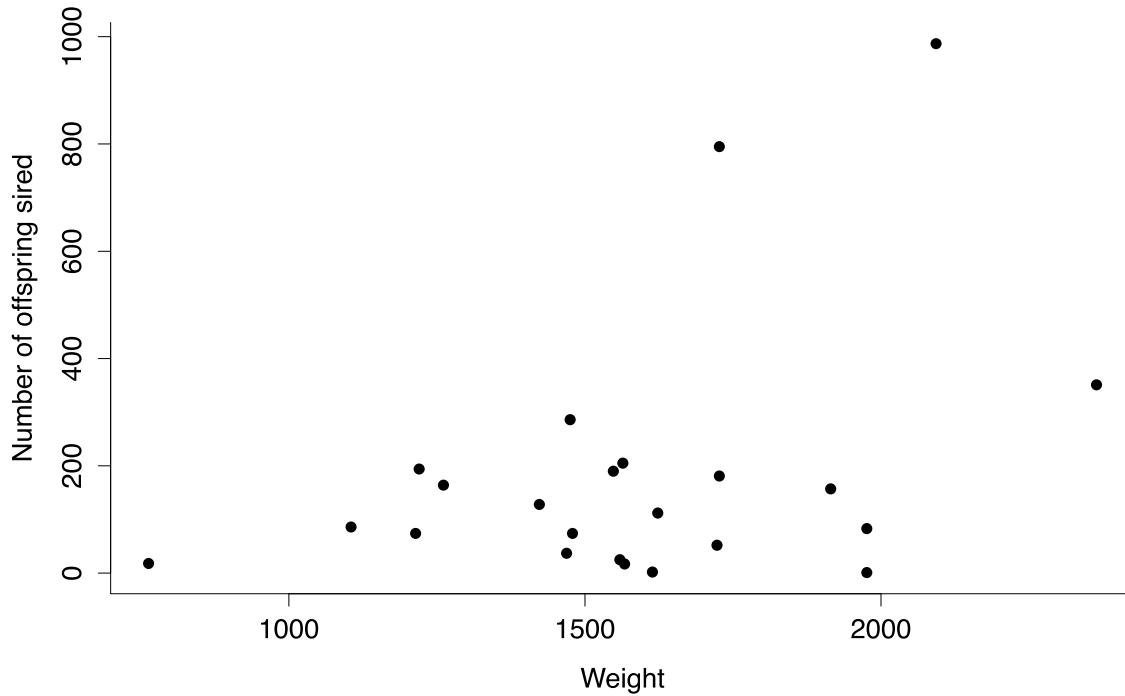


Figure 13: The number of offspring sired as a function of body weight (g) in male Atlantic cod.

Following model selection for females, the best model included length prior to spawning, population identity and body weight (Table 2). Length had the largest effect on the model, indicating a positive additive effect of length on the number of offspring produced. Weight was the second most important variable, and had a negative additive effect on the number of offspring sired. The least important variable was population identity. As observed for the males, outer females were less successful than inner females.

Table 2: Generalized linear models for reproductive success in females, assuming a normal distribution. The response variable, the number of fertilized offspring, was considered to be a function of population identity, length, weight, hepatosomatic index (HSI), gonadosomatic index and residual mean pelvic fin length. Including all data, the significant variables ($P < 0.05$) were Pre.Length (length prior to spawning), Weight (body weight), and PopOuter (outer population). Excluding data for the two largest females, the significant variables were Pre.Length, PopOuter, and HSI.

	Effect	Estimate	SE	t-value	P-value
Females (all individuals)	Pre.Length	1.206	0.390	3.096	0.004
	Weight	-0.126	0.044	-2.890	0.007
	PopOuter	-74.496	28.30	-2.632	0.014
Females (outlier females excluded)	Pre.Length	0.76	0.30	2.52	0.018
	PopOuter	-105.83	29.32	-3.61	0.001
	HSI	-27.01	9.01	-3.00	0.006

A negative correlation between weight and reproductive success was unanticipated. However, when the number of offspring sired was plotted against body weight, it was clear that the negative relationship was heavily influenced by the two largest females both of whom had very low reproductive success (Fig. 14). Exclusive of these two females, there appears to be no relationship between the weight of a female and the number fertilized offspring.

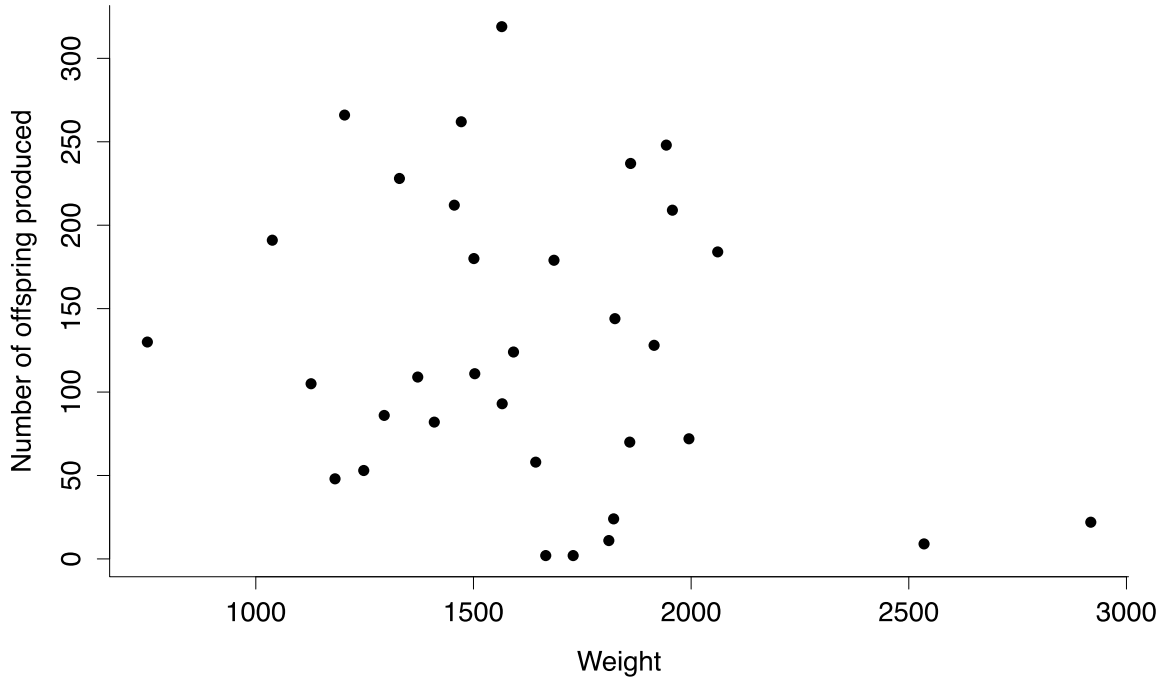


Figure 14: The number of fertilized offspring as a function of body weight (g) in female Atlantic cod.

To verify that these two females were in fact driving the negative regression coefficient for weight, they were removed from the dataset and the model was re-run. In the new model, weight, as expected, was reduced from the model as a non-significant variable, leaving length, population identity and HSI as the remaining significant variables (Table 2). While there were small shifts in the relative importance of each factor, the general trend in coefficients for length and population identity remained unchanged in the new model: the outer population had a negative additive effect on reproductive success whereas length had a positive additive influence. There was the addition of HSI to the model such that reduced HSI was associated with increased reproductive success.

2.2.4 Temporal Variation in Reproductive Success

Among males, there was a significant negative relationship between the day of the spawning period and the mean length of spawners for both the regular mean and the mean length weighted by the relative number of offspring sired by individuals on a given day ($[p = 0.001, r^2 = 0.10]$ and $[p = 0.0025, r^2 = 0.08]$, respectively). In both models, larger males dominated spawning at the beginning of the spawning period (Fig. 15).

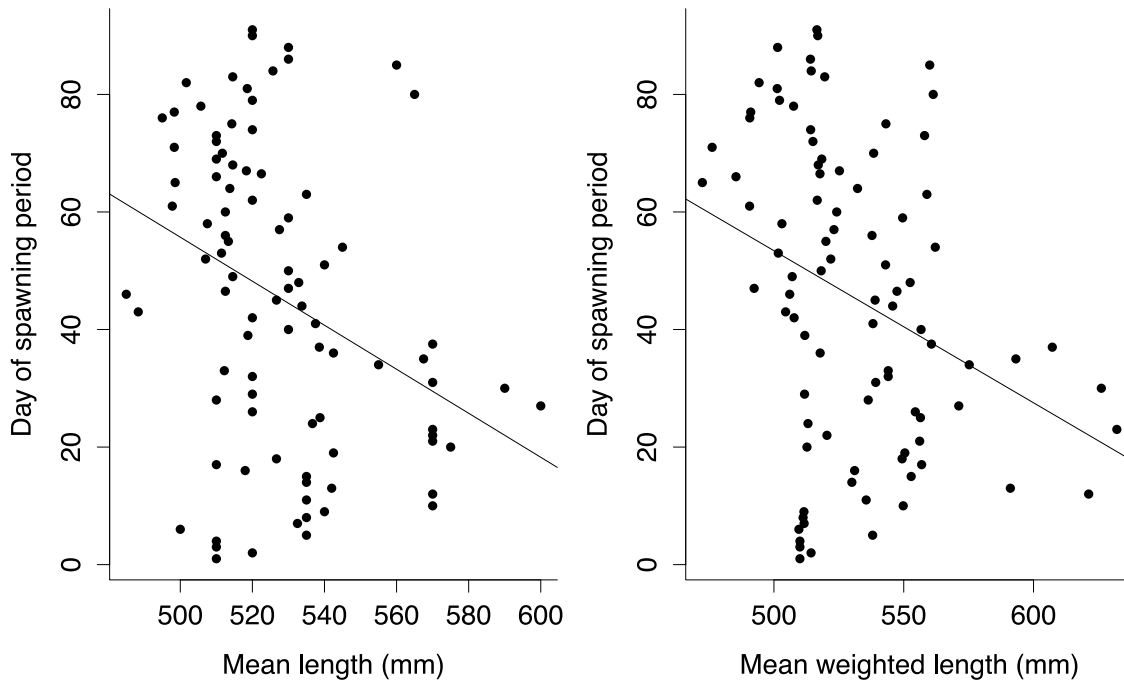


Figure 15: Day of spawning period versus mean length (mm) of reproductively successful male Atlantic cod. Left plot is the mean length. Right plot is the mean length proportionally weighted by the relative number of offspring produced on a particular day.

For females, the relationship was opposite to that of males as there was a positive relationship between the day of the spawning season and the mean length of spawners (Fig. 16). Both models suggest that smaller females were comparatively more active at the beginning of the spawning season. The level of significance of the relationship,

significant or marginally significant, was dependent on whether the mean was weighted or not ($[p = 0.036, r^2 = 0.04]$ and $[p = 0.076, r^2 = 0.02]$, respectively). Regardless of the model, the correlation coefficients for the relationship were very low: 0.02 and 0.04.

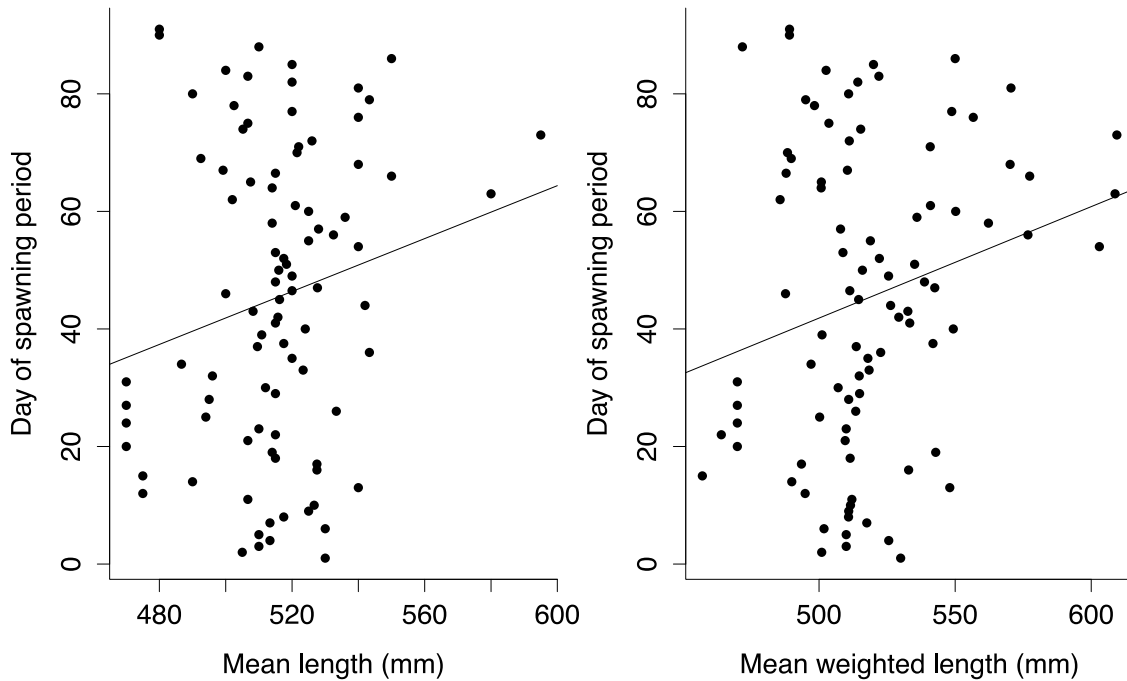


Figure 16: Day of spawning period versus mean length (mm) of reproductively successful female Atlantic cod. Left plot is the mean length. Right plot is the mean length weight proportionally weighted by the relative number of offspring produced on a particular day

2.3 Discussion

I examined reproductive success in a broadcast-spawning marine fish at unprecedented temporal resolution, spatial scale, and sample size. The parentage analysis of Atlantic cod consisted of 4489 reproductive success samples, from 73 wild-caught adults, obtained daily over a 91-day period. Several broad-scale patterns emerged from the present study. Firstly, reproductive success was skewed within both sexes, albeit

much more so among males than females. Secondly, the influence of body size on reproductive success differed between sexes. Among males, body size was generally not a strong predictor of success, representing a positive correlate of fertilization probability only for the very largest of males. Among females, body length (although not weight) was positively associated reproductive success. Thirdly, size-related temporal variability in reproductive success suggested that comparatively large males spawn relatively early during the spawning period, but that comparatively large females do so relatively late in the spawning period. Fourthly, despite the very small distance separating the inner and outer populations, cod from the outer fjord were less successful than those from the inner fjord, a finding consistent for both males and females. Lastly, and perhaps unsurprisingly, reproductive success was negatively associated with metrics of body condition for both males (GSI) and females (HSI, upon exclusion of two presumed outliers).

The skew in fertilization probability observed here is well within previously reported estimates of male reproductive success. The top 3 males (out of 28 males) in our study fertilized 50% of the total number of eggs produced during the spawning period, an estimate that falls within the range (48-93%) for the top 3 males (range in number of males: 18-37) reported among four Northwest Atlantic spawning groups (Rowe et al. 2008).

Cod exhibit a complex mating system that includes male mate competition, male dominance hierarchies, male courtship displays, and quite possibly female mate choice. The release of gametes by a spawning pair is preceded by a ventral mounting of the female by a single male (Brawn 1961, Hutchings et al. 1999). Males have also been observed to adopt a satellite strategy and release milt alongside a spawning pair

(Hutchings et al. 1999, Rowe et al. 2008). Studies suggest that males who participate in paired spawning events are afforded this opportunity because of their rank within a dominance hierarchy, established by factors such as size and aggressive behaviour (Brawn 1961, Hutchings et al. 1999).

The present study lends firm support to the existence of this duality of male spawning strategies in cod. It is highly probable that the top three males were the top-ranked males within the dominance hierarchy and, thus, dominated paired spawning events throughout the spawning period. The small to moderately heavy males were likely to be lower ranked males who, failing to obtain paired spawning events, would be more likely to adopt the satellite male spawning behaviour, resulting in lower, but non-trivial, levels of fertilization success. Based on work by Bekkevold et al. (2002) and Rowe et al. (2008), the fertilization success of individual satellite males might range from 1 to 20%.

Those responsible for a disproportionate amount of offspring sired were the larger, heavier males, a finding consistent with previous work (Rowe et al. 2008). However, while I did find a positive correlation between the number of offspring sired and weight, this correlation was only true among the heaviest males. For individuals not among the top-ranked males, body size had little to no effect on reproductive success. This mixture between a positive correlation among comparatively heavy males and no correlation among moderately heavy to small males lends further support to the hypothesis that dominant males are those who can monopolize paired mating and do so on the basis (at least in part) of their body size. Thus, for males, being among the largest is critical in achieving high reproductive success as it is a key component in establishing one's rank within the dominance hierarchy. If you are not able to establish yourself as one

of the top-ranked males, size seems to have little to no effect on one's reproductive success. This is likely due to the lower rank males resorting to more opportunistic spawning strategies such as satellite mating, where size has a much less of a significant role.

For females, a skew in reproductive success was evident, though much less so than that observed in males and the length of a female had a positive effect on the number of offspring produced. The variance in reproductive success among females likely represents variance in a female's reproductive capacity (e.g. fecundity), as opposed to differences in mating behaviour, as fecundity is intrinsically linked with female size (Buzeta and Waiwood 1982, Kjesbu et al. 1996, Marteinsdottir and Begg 2002). Thus, it is not surprising that longer females correlated with higher numbers of offspring produced.

An unanticipated finding in the present study is that the two largest spawning females had unexpectedly low reproductive success, so much so that they were solely responsible for a negative relationship between weight and the number of offspring in the initial model. Not only were these two females among the three heaviest fish in the spawning basin (2918g and 2535g), the only other fish within their size range was also a female (2686g) and had zero reproductive success. Thus, the three largest females in the spawning basin, who were also the three largest fish in the spawning basin, all exhibited uncharacteristically low or zero reproductive success.

This surprising pattern among large females could be a result of stressful conditions that caused them to alter their spawning behaviour. That said, the three females exhibited no physical indicators of stress. Furthermore, even if the females had

been unduly stressed, this does not necessarily mean that this would have affected their reproductive success. Morgan et al. (1999) for example, found that under stressful conditions while the courtship sequences are altered, spawning and fertilization rates remained comparable to unstressed cod. It is possible that the eggs from the potentially stressed large females were of poor quality, did not hatch, and thus were not present in the larval reproductive success samples. However, again females exhibited no physical indicators of stress, and offspring quality analyses (Appendix C and Chapter 3) indicate that offspring produced by the two successful females were not of poor quality.

The lack of success among the largest females might also be attributable to a lack of suitably sized males for paired spawning. The weights of these three females were clear outliers among all adult cod (Fig. 17). Whether it be due to a physical limitation from males failing to grasp the females during the ventral mount, or a behavioural choice on the female's behalf, either way, females may have failed to fertilize offspring due to the lack of suitably sized mates. It is also possible that females choose not to participate in paired spawning events, as Brawn (1961) observed unaccompanied females releasing eggs on the bottom of an experimental spawning enclosure. It is further possible that these unaccompanied spawning events are due to mate size mismatches, as Brawn (1961) also attributed one such event to a size mismatch between male and female. Further supporting this hypothesis, Bekkevold et al. (2002) found reproductive success in females to decrease when paired with smaller males and Rakitin et al. (2001) found male reproductive success was highest when paired with similarly size females. Thus, it is possible that the lack of suitably size mates negatively affected reproductive success among the largest females.

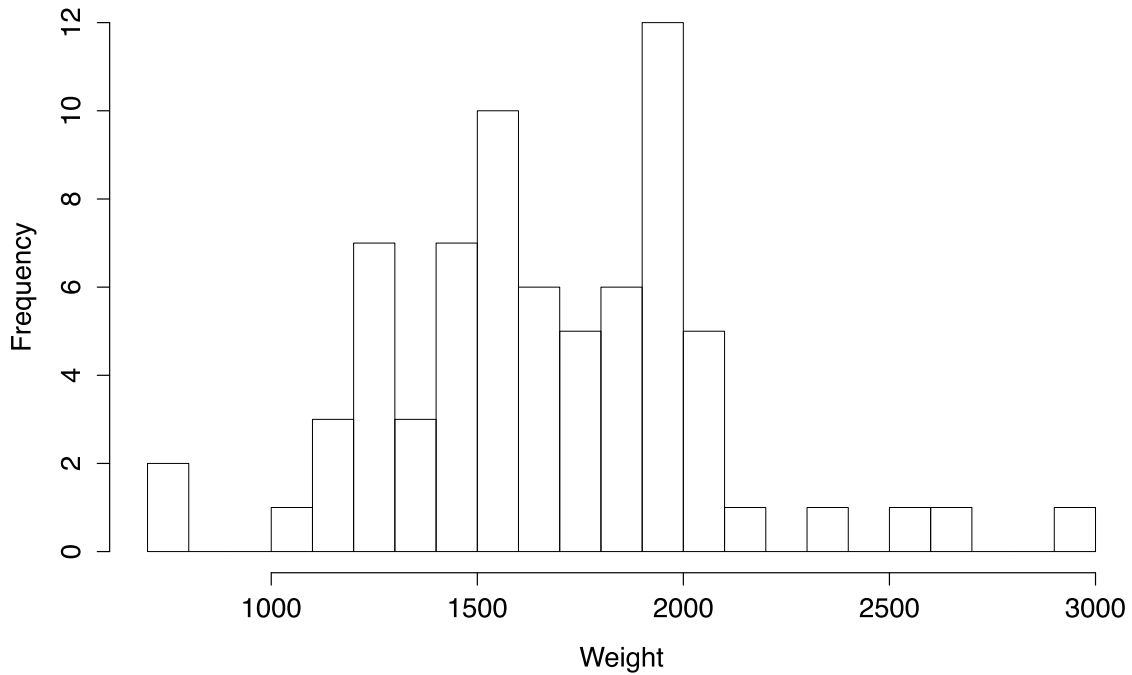


Figure 17: Weight frequency distribution of all adult male and female Atlantic cod in the spawning basin.

The mean size of reproductively successful males decreased throughout the spawning period, indicating that the larger males were dominating mating earlier on in the spawning season. As the spawning season progressed and the larger dominant males began to exhaust their energy and sperm reserves, smaller males were then able to become more reproductively successful. Bekkevold et al. (2002) noted similar temporal shifts in size ranks of reproductively successful males among groups of spawning males (<4 males); later on in the spawning period lower ranked males began to be more successful. Skjæraasen and Hutchings (2010) also noted a similar temporal shift in dominance hierarchies. Hutchings and Myers (1993) reported contrasting results, finding via analyses of fisheries-independent survey data that younger males initiated spawning earlier than older males. With the exception of Hutchings and Myers (1993), it appears as

though larger more successful individuals are dominant at the beginning of the spawning period. As time progresses the mean size of successful male's declines, likely indicative of shifts in dominance hierarchy as energetic limitations and sperm depletions begin to occur among top-rank males.

Though a weak correlation, the mean size of reproductively successful females increased throughout the spawning period, indicating that smaller females tended to spawn earlier in the spawning period. Previous studies provide conflicting support for our findings. Similar to our findings, Hutchings et al. (1999) found that smaller females initiated spawning earlier than larger females; whereas Marteinsdottir and Bjornsson (1999) found the opposite, with evidence indicating that larger females began spawning earlier (both studies were based on relative prevalence of females in spawning condition on spawning grounds).

Unexpectedly, I found small-scaled variance in reproductive success, as after controlling for body size, there was a difference between the reproductive success of the inner and the outer fjord population. For both males and females, individuals from the outer fjord had poorer reproductive success relative to the inner fjord population, and it is unclear what is driving this difference between populations. The difference in the relative reproductive success between populations was particularly unexpected as the distance that separates these putative populations is extraordinarily fine scaled, tens of kilometers. Females from the outer fjord population had poorer reproductive success even though the outer fjord fish were, on average, longer (mean \pm SD: 53.4 \pm 4.5 cm) than the inner fjord (mean \pm SD: 50.8 \pm 4.0 cm). Based on these size differences and standard body size-fecundity relationships, the outer fjord females would be expected to have higher

fecundity and subsequent reproductive success (Buzeta and Waiwood 1982, Kjesbu et al. 1996, Kuparinen et al. 2012, 2015). Though the outer fjord fish were on average larger, they had a slightly younger age structure than the inner fjord population, with a mean age of 4.6 ± 1.2 years compared to 5.4 ± 0.6 years, the difference being driven by differences in growth rates between populations (Kuparinen et al. 2015). Therefore, even though age was not found to be a significant predictor of individual reproductive success, it is possible that the difference in age structure is a factor driving the variation in reproductive success between populations. Further investigation into the correlates of this small-scale variance in reproductive success is necessary

It is often hypothesized that older individuals, presumed to be more experienced spawners, are more likely to have higher reproductive success when compared to their younger counterparts (Cardinale and Arrhenius 2000, Berkeley et al. 2004a, Palakovich Carr and Kaufman 2009, Hixon et al. 2013). Contrary to this hypothesis, for both males and females, I did not find any correlation between the age of a spawner and its respective reproductive success. In our study, there was a limited range of age classes in the spawning population (mean \pm SD: 5.04 ± 1.02), limiting the power at which I could examine the relationship. The size variation among individuals was much greater than the variation in age, thus it is perhaps not surprising that I did not find a relationship. This does not mean that older spawners do not have higher reproductive success, but rather within a small range of age classes, the size of a fish is a better predictor of reproductive success. Given that size is highly correlated with age, the consequences of size-dependent reproductive success will likely still translate to age effects at a broader range of age classes.

The condition indices in our study, HSI and GSI, were both negatively correlated with reproductive success, indicating that individuals in poorer condition, or with lighter gonads relative to body size, fertilized more offspring. Since the morphological measurements used to calculate these indices were taken at the end of the experiment, they are representative of the post-spawning condition and are, thus, consequences of one element of reproductive effort (extrusion of eggs and (or) sperm) rather than causal predictors. For example, females who had higher reproductive success, expended more energy producing eggs throughout the spawning season and were thus in poorer condition at the end of the spawning period. Similarly, for males, spawners who sired more offspring, participated in more spawning events, and maximized sperm extrusion resulting in a lower gonadosomatic index.

I cannot discount the possibility that the spawning basin might have altered spawning behaviour and subsequent levels of reproductive success. Environmental cues in the spawning basin were muted (e.g., changes in temperature) and this ‘constancy’ might conceivably have had an effect on spawning behaviour. The spawning density in the basin was $\sim 2 \text{ fish m}^{-3}$ which is higher than previously recorded natural spawning densities ($[0.28-0.31 \text{ m}^{-3}$ [Morgan et al. 1997] and 0.90 m^{-3} [Rose 1993]). However, while it was notably higher than naturally occurring densities, fish were monitored throughout the spawning season for signs of stress or poor health, and only two fish were culled due to health concerns, one of which occurred very soon after placement in the spawning basin. The poor health could have resulted from numerous other factors such as trauma during capture, transport or pre-existing health issues. Furthermore, as previously discussed, even if fish had been stressed to a greater extent than they would be in the

wild, it is not clear that this would have affected reproductive success results. Studies on spawning cod under stressful conditions have noted either changes in courtship sequences (Morgan et al. 1999) or changes in egg quality (Kjesbu 1989), but have not found stress to influence relative fertilization rates. Finally, it is possible that the sampling regime did not accurately sample offspring and, thus, reported reproductive success in proportion to the actual reproductive success. However, with the fine-scaled temporal and spatial coverage, and large sample size, used in the present study, I believe this is unlikely to be an issue and at minimum broad scale trends would have been accurately captured.

In conclusion, our results add to the growing body of researched highlighting the influence that the mating system in cod can have on individual reproductive success. Our work further underscores the importance of size on reproductive success for both males and females, but also reveals some intricacies, potentially attributable to female mate choice, that can both negatively and positively affect an individual's reproductive success. If large fecund females have poor reproductive success when suitable mate sizes are not present, this could have negative recovery implications for populations experiencing size truncation from exploitation. Furthermore, large skews in reproductive success during a mating season will lead to lower effective population size. Ultimately, understanding variance in reproductive success among individuals is critical for effective population management.

Chapter 3: Morphological correlates of offspring quality and the timing of reproduction

3.1 Methods

3.1.1 Experimental Design

The adult collection and experimental design were identical to those described in Chapter 2, sections 2.1.1 to 2.1.5. Briefly, the methods are as follows.

Prior to the 2014 winter spawning season, 73 wild adult cod were collected from two genetically distinct populations: inner fjord (n=36) and outer fjord (n=37). Cod were placed in a semi-natural spawning basin where they remained undisturbed for the duration of the experiment. The experiment began when eggs were first present in the egg collector, after which eggs were collected daily. The experiment was terminated when eggs were absent from the egg collector for five consecutive days. The spawning period lasted 94 days, from 20 January to 24 April; eggs were collected on 91 days.

One month following the completion of spawning, adults were sacrificed by a knock-out blow to the head, and the following morphological measurements were recorded: total standard length (mm); median fin lengths (mm); total weight (g); liver weight (g); gonad weight (g); and stomach weight with and without contents (g). The gonadosomatic index ($GSI = \text{gonad weight}/\text{body weight}$) and the hepatosomatic index ($HIS = \text{liver weight}/\text{body weight}$) were both calculated as proxies for body condition (Lambert and Dutil 1997). Respective ages were estimated by the 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.

3.1.2 Larval Collection

Each day between 08:00 and 10:00 hours, eggs present in the egg collector were removed and put into one of 14 incubation tanks. Eggs were incubated at 6.1°C (SD: ± 0.5) until they were visually assessed to be at the 50% hatch development stage (15 ± 0.6 days, mean \pm SD) (Fig. 18). When a tank was at 50% hatch, an ‘offspring-quality’ photo and a genetic sample were taken for 50 individual larvae. To obtain individual offspring quality measurements, larvae were killed in RNAlater and individually photographed on a wet slide, using a Leica DFC425 C camera mounted to a Leica MZ16 A stereoscope at 20X magnification. A standardized length slide etched with a 1 μ m scale bar was photographed at the start of each daily batch of photographs and was later used to calibrate the image analysis software. After the photograph was taken, each whole larva was individually preserved in micro tubes containing 250 μ L of RNAlater for future genetic analysis. In sum, over the course of the spawning period, daily larval samples totaling 4500 individuals were collected over 94 days from a spawning population comprised of 73 wild adults; each of these larvae was sampled for individually specific genetic and morphological data.

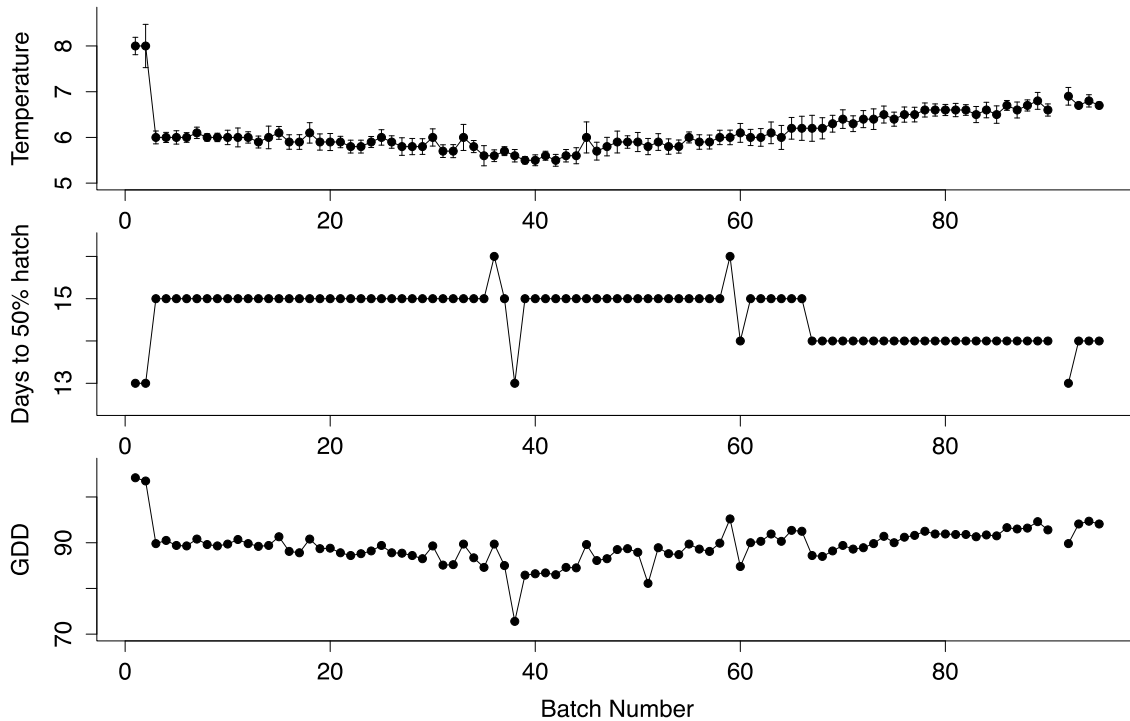


Figure 18: Water temperature and metrics of larval development during the experimental period, as functions of egg batch number (each batch number represents a separate day). Upper panel: mean tank temperature ($^{\circ}\text{C}$); error bars represent standard deviation among tanks. Middle panel: days until 50% of the larvae had hatched. Lower panel: growing degree-days (GDD).

3.1.3 Parentage Analysis

The parentage analysis was identical to that described in Chapter 2, section 2.2.1.

Briefly, the methods are as follows.

DNA was extracted from parental fin clips and whole larvae. All samples were amplified for eight loci, using two separate multiplexes (Multiplex 1: *Gmo8*, *Gmo19*, *Gmo35*, and *Tch11*; Multiplex 2: *Gmo132*, *Gmo2*, *Gmo34*, and *Tch13*). Loci were analysed by capillary gel electrophoreses and allelic sizes were calculated with the program GeneMapper (Applied Biosystems). Adults were amplified three times per multiplex to ensure absolute accuracy in parental genotypes.

Family reconstruction of the allelic data was performed using the program COLONY v2.0.6.1 (Jones and Wang 2010). Larvae were run in batches of 500 using the full-likelihood approach with high precision. Each analysis was repeated using medium, long and very long runs to assess whether maximum likelihood configuration had been reached. (See sample colony input file in Appendix A for input parameters.)

3.1.4 Offspring Quality Indicators

The offspring quality indicators used in the present study were the length of the larvae and the volume of the yolk sac standardized to the length of the larvae. The length of each larva was measured as the distance from the snout to the end of the tail. The $\frac{V}{L^2}-1$ (Uusi-Heikkilä et al. 2010), where L is the length (horizontal measurement; μm) and H is height (vertical measurement; μm) of the yolk sac. All measurements were taken using the image analysis software Fiji (Schindelin et al. 2012).

3.1.5 Batch Verification

Before analyzing trends in offspring quality and reproductive timing, it was necessary that eggs could be confidently ascribed to particular egg batches. There was a low probability that eggs collected on day x might not have been part of the egg batch spawned on day x , but rather have been part of an egg batch produced on a previous day, retained in the spawning basin, and subsequently collected after their initial spawn date. To ascertain the correct egg batch corresponding to each larva, I 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 was an average of 1330 mm² at hatch and would steeply decline to zero after 5-9 days when the larvae had exhausted their yolk sacs and grown in length.

For a given female, if offspring she had produced were present in the egg samples for two consecutive days, offspring collected during the second day were considered to be offspring that had been retained from a prior batch. In order for the second day to be considered a new unique batch, it needed to meet two requirements: 1) the second day must have contained more larvae than the previous day, and 2) the mean volume of the yolk sac, standardized to length, must not have declined on the second day relative to the day prior.

3.1.6 Timing of Reproduction

The number of 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). The duration of spawning for each individual was the difference between the first and last day on which offspring were observed, plus one.

3.1.7 Statistical Analysis

3.1.7.1 Offspring quality

3.1.7.1.1 Pooled offspring quality

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 in the spawning season. This was done for both estimates of offspring

quality: length-at-hatch and standardized yolk sac volume. Residual plots were examined to ensure the model was fitting the data (Appendix D)

3.1.7.1.2 Individual female offspring quality

In order to estimate whether a female consistently produced higher quality offspring throughout the spawning season, I first had to remove 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 (Section 3.1.4.1.1). 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 poor quality. Finally, to obtain mother-specific estimates, I calculated the mean of an individual's residual offspring quality values across the spawning season. 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. The mean residual offspring quality (OQ in the equation below) of a female (either for length or standardized yolk-sac volume) was a function of population identity (inner/outer fjord), length (prior to spawning), 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):

$$\text{OQ} \sim \text{Population} + \text{Length} + \text{Weight} + \text{HSI} + \text{GSI} + \text{Age} + \text{mean pelvic fin length}$$

3.1.7.2 Reproductive timing

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

$$RT \sim \text{Population} + \text{Length} + \text{Weight} + \text{HSI} + \text{GSI} + \text{Age} + \text{mean pelvic fin length}$$

Model selection was performed following Zuur et al. (2009), using stepwise model reduction. 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).

3.2 Results

3.2.1 Parentage Analysis

Microsatellite genotypes were obtained for 4489 of the 4500 larvae. Parental assignment was based on the results of the long runs, given that this was the level at which maximum likelihood was reached and that 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 5 of 8 loci of a known parent, the unknown parent was re-assigned as the known parent. Since I am certain that I have all of the parental genotypes, I can assume that any mismatches in genotype are a result of either mutation or scoring error. The final parentage analysis

resulted in successful paternal assignment to 94.0% (4221 of 4489) of the larvae and successful maternal assignment to 93.5% (4198 of 4489) of the larvae.

3.2.2 Batch Verification

After batch verification, 135 larvae were identified as having been collected after their initial spawning date. All further analyses were conducted without them. 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 in the spawning basin (Fig. 19).

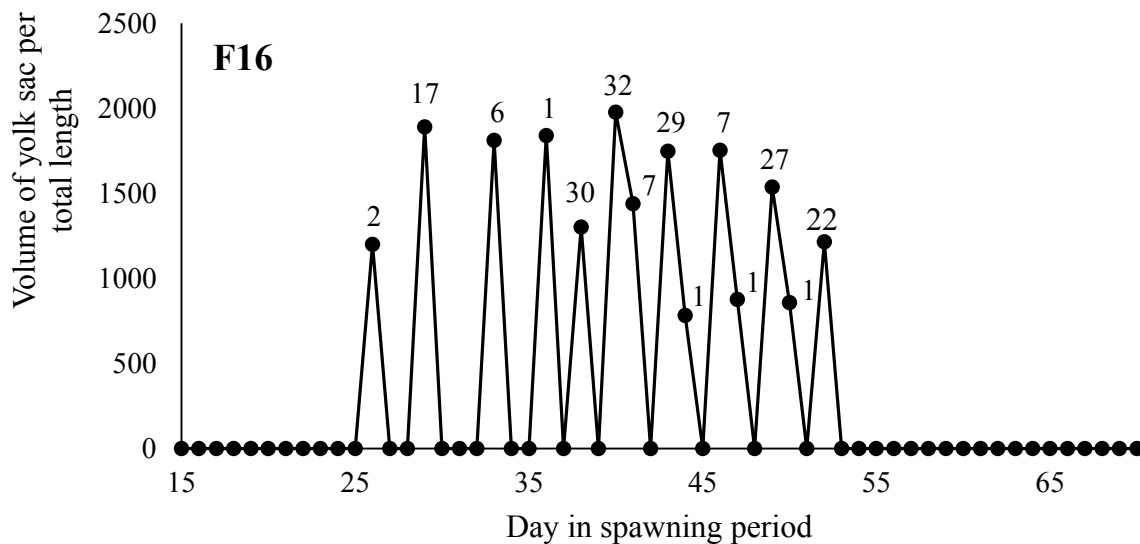


Figure 19: An example of plots used to identify larvae that had been sampled after the actual spawn date (here, female F16). Line represents the volume of yolk sac, standardized to length and numbers above points represent the number of larvae in each batch. In order for the second day of two consecutive batches to be considered a new unique batch, it needed to meet two requirements: 1) the second day must have contained more larvae than the previous day, and 2) the mean volume of the yolk sac, standardized to length, must not have declined on the second day relative to the day prior. In this example, 4 days would have been removed from the analyses.

3.2.3 Offspring Quality

3.2.3.1 Pooled spawning period variation

The total length of larvae declined relatively consistently over the course of the spawning period (Fig. 20, Table 3). Between the first batch and the final batch of the spawning period, the model-estimated length of cod larvae declined 11% (443 μm to 394 μm).

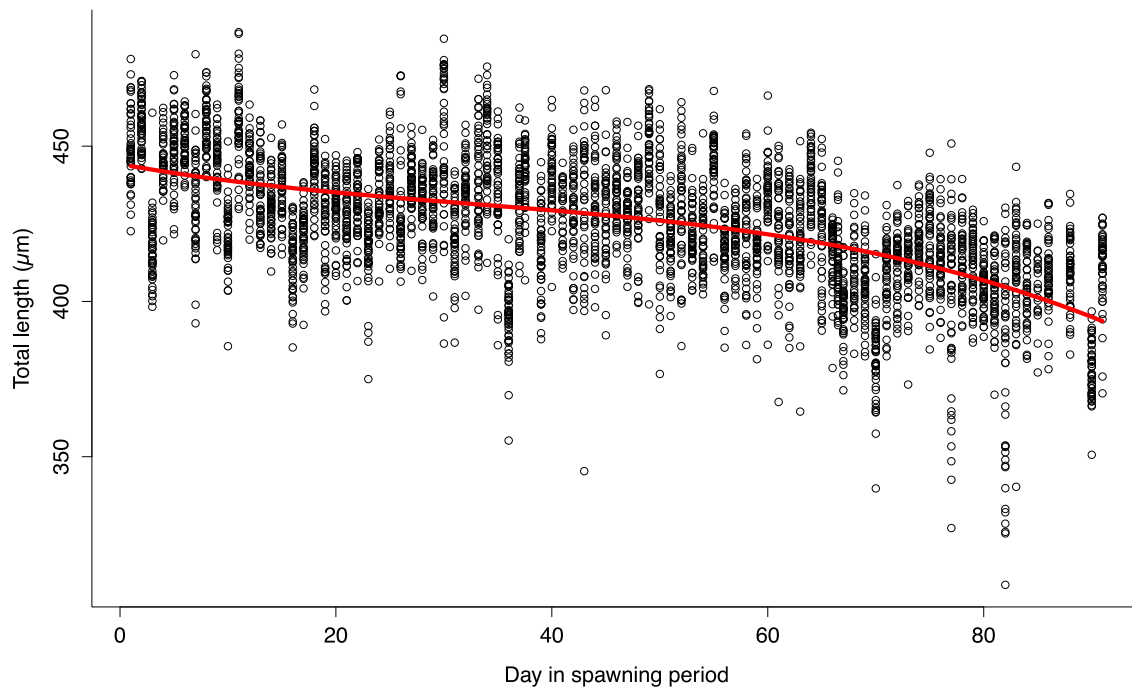


Figure 20: Seasonal trend in the total length-at-hatch of larvae from 73 wild-caught Atlantic cod, *Gadus morhua*, during the course of an entire spawning season. The model fit for the polynomial (red line): $\text{total length} = \beta_0 + \beta_1(\text{batch \#})^2 + \beta_2(\text{batch \#})^3$, computed using the total length versus batch number.

The standardized yolk-sac volume peaked a third of the way into the spawning season, and then declined relatively consistently towards the end of the season (Fig. 21, Table 3). Between the first and final batch, there was a 49% decline in the model-

estimated yolk-sac volume (1222 to $624 \mu\text{m}^3 \cdot \mu\text{m}^{-1}$) with a peak standardized volume of $1635 \mu\text{m}^3 \cdot \mu\text{m}^{-1}$ on the 28th of the 91 batches.

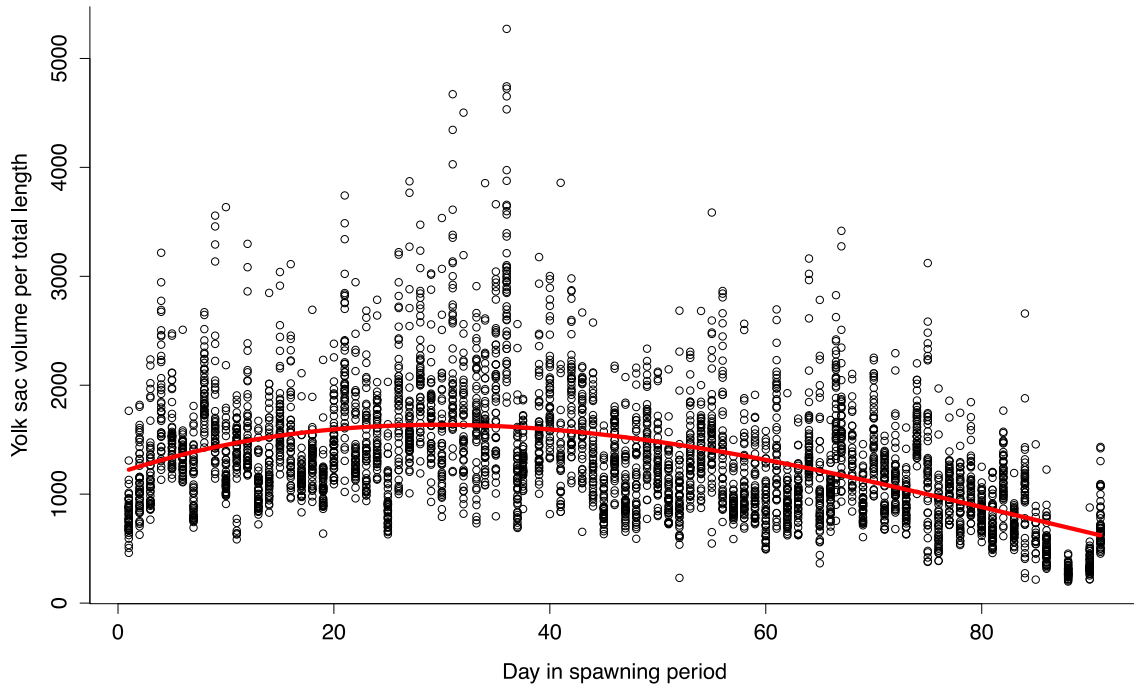


Figure 21: Seasonal trend in the yolk-sac volume standardized to length at hatch of larvae produced during the spawning period by 73 wild-caught Atlantic cod. The model fit for the polynomial: $\text{yolk-sac volume per total length} = \beta_0 + \beta_1(\text{batch \#}) + \beta_2(\text{batch \#})^2 + \beta_3(\text{batch \#})^3$ was computed by using the yolk-sac volume per total length versus batch number.

Table 3: Parameter estimates for the pooled seasonal trend in offspring quality for a free spawning population of 73 wild-caught Atlantic cod. 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 number (SE represents standard error).

Model [$\beta_0 + \beta_1(\text{batch \#}) + \beta_2(\text{batch \#})^2 + \beta_3(\text{batch \#})^3$]	Estimate	SE	P-value
Length ~ Batch #	β_0 4.43 $\times 10^2$	1.08	<0.001
	β_1 -5.12 $\times 10^{-1}$	1.01 $\times 10^{-1}$	<0.001
	β_2 8.02 $\times 10^{-3}$	2.56 $\times 10^{-3}$	0.0017
	β_3 -9.01 $\times 10^{-5}$	1.85 $\times 10^{-5}$	<0.001
Yolk sac volume per total length ~ Batch #	β_0 1.19 $\times 10^3$	3.18 $\times 10^1$	<0.001
	β_1 3.26 $\times 10^1$	2.98	<0.001
	β_2 -6.74 $\times 10^{-1}$	7.55 $\times 10^{-2}$	<0.001
	β_3 2.72 $\times 10^{-3}$	5.45 $\times 10^{-4}$	<0.001

3.2.3.1 Individual variation

Following model selection for the mean residual length of larvae, the best model included female body weight and population identity (Table 4). Weight was the most influential predictor with a positive coefficient (0.011). Increases in the weight of a female had a positive additive effect on mean length of larvae over the course of the spawning season. Of the two populations, outer fjord fish produced longer larvae than the inner fjord fish, though in the model this effect was only marginally significant. While there was not a large difference in the mean size-at-hatch between populations – 428 μm for the outer fjord versus 423 μm for the inner fjord – there was a large difference in the mean residual length between populations: 4.49 μm for outer-fjord fish compared to -1.81 μm for inner-fjord fish. Thus, all else being equal among fish (i.e. same weight), the outer fjord population produced longer larvae relative to their equivalently weighted inner fjord counterparts.

Following model selection for the mean residual standardized volume of the yolk sac, the best model included length and the length difference of a female between the beginning and end of the spawning period (Table 4). Both female length, and the spawning-period length difference, had positive additive effects on the standardized yolk-sac volume.

Table 4: Generalized linear models for offspring quality in females. 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, hepatosomatic index, gonadosomatic index, length difference between the beginning and the end of the experiment, and residual mean pelvic fin length. The best model for residual length included the variables ‘Weight’ (body weight) and ‘PopOuter’ (population). The best model for residual volume of the yolk sac standardized by length included the variables ‘PreLength’ (length prior to spawning) and ‘Lenth.Diff’ (the length difference between the beginning and the end of the experiment).

	Effect	Estimate	SE	t-value	P-value
Residual Length	Weight	0.011	0.004	2.966	0.006
	PopOuter	5.722	3.121	1.833	0.077
Residual Volume/Length	PreLength	2.005	0.875	2.291	0.0297
	Lenth.Diff	4.167	1.838	2.267	0.0313

3.2.4 Timing of Reproduction

3.2.4.1 Males

Following model selection for males, weight and population identity were the only variables significant in the two models (Table 5). For the model predicting the number of batches, weight and population were both included. For the model predicting spawning duration, population was the only predictor and only marginally significant (0.08). Regardless of the model, the outer population had a negative additive effect, meaning that outer-fjord males spawned for a shorter period of time than inner-fjord

males. Weight, on the other hand, exhibited a positive additive effect, indicating that larger males sired offspring in more batches.

Table 5: Generalized linear models for reproductive timing metrics in males: number of batches and spawning duration. The response variable, the spawning metric, was considered to be a function of population identity, length, weight, hepatosomatic index, gonadosomatic index and residual mean pelvic fin length. The best model for number of batches included ‘PopOuter’ (population) and ‘Weight’ (body weight). The best model for the length of spawning included ‘PopOuter’ (population).

	Effect	Estimate	SE	t-value	P-value
Number of Batches	PopOuter	-14.731	6.015	-2.449	0.0237
	Weight	0.018	0.008	2.209	0.0390
Duration of Spawning	PopOuter	-14.948	8.351	-1.79	0.0879

3.2.4.1 Females

Following model selection for females, the best models both included weight, length and population origin (Table 6). Although the absolute value of the regression coefficients varied, the main effect, positive or negative, remained consistent between models. The outer population had a negative additive effect on both metrics of reproductive timing, meaning that cod in the outer fjord spawned fewer egg batches and reproduced for a shorter time period than those in the inner fjord. Length prior to spawning had a small positive additive effect on spawning duration. Finally, weight had a significantly negative additive effect on number of egg batches. Thus, all else being equal (population and size), females who were lighter at the end of the spawning period had produced more batches over a longer period of time.

Table 6: Generalized linear models for reproductive timing metrics in females: number of batches and spawning duration. The response variable, the spawning metric, was considered to be a function of population identity, length, weight, hepatosomatic index, gonadosomatic index and residual mean pelvic fin length. The best model 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), ‘Weight’ (body weight), and ‘PreLength’ (length prior to spawning).

Response	Effect	Estimate	SE	t-value	P-value
Number of Batches	Weight	-0.006	0.002	-2.523	0.0174
	PopOuter	-3.639	1.534	-2.372	0.0245
	PreLength	0.047	0.021	2.223	0.0341
Spawning Duration	PopOuter	-18.561	5.994	-3.097	0.0044
	Weight	-0.016	0.009	-1.752	0.0906
	PreLength	0.146	0.082	1.78	0.0860

3.3 Discussion

I examined offspring quality and reproductive timing in a batch-spawning marine fish at an unprecedented temporal resolution, spatial scale, and sample size. The analysis consisted of 4489 individual larvae taken daily throughout a 93-day period, facilitating an extraordinarily in-depth analysis into individual variation in offspring quality and reproductive timing. Several maternal effects and reproductive timing patterns emerged from the present study. Firstly, there was significant variation in the length-at-hatch of larvae both over the course of the spawning period and among individual females. The pooled length-at-hatch declined relatively consistently throughout the spawning period and, after removal of the temporal trend, the weight of a female had a positive effect on the length of her larvae. Similar trends were observed in the other offspring quality

indicator, the volume of the yolk sac standardized to length. Throughout the spawning period, while there was an overall decline in the standardized yolk sac volume, unlike the temporal trend for length-at-hatch, it initially increased, peaking a third of the way into the spawning, and then declined. After removal of the temporal trend, maternal size had a positive effect; longer females, and surprisingly, those that experienced the greatest growth during the spawning period, produced larvae with larger yolk sacs relative to the size of the larvae. Finally, for both males and females, larger individuals experienced longer spawning periods and participated in more spawning events.

For cod, while no prior studies to our knowledge have examined seasonal variation in larval length-at-hatch, many have examined temporal changes in egg size, a metric highly correlated with size-at-hatch (Pepin et al. 1997, Marteinsdottir and Begg 2002). Thus, the trends in larval length reported here are comparable to previously reported trends in egg size.

The observed decline in larval length reported in the current work (11%) is well within previously reported estimates of individual seasonal declines in egg diameter (0%-12% [Chambers and Waiwood 1996], 2-19% [Trippel 1998]). While overall reductions were similar to ours, the trend throughout the spawning period differed among studies. Similar to our findings, Trippel (1998) reported relatively consistent declines in egg size, whereas Chambers and Waiwood (1996) observed that egg size peaked halfway through the spawning period. Studies examining egg size as a function of an individual's batch number similarly found relatively consistent declines in size (Kjesbu 1989, Kjesbu et al. 1996, Vallin and Nissling 2000).

In addition to the temporal trends in larval length, the present study also found that female size had a positive effect on larval length-at-hatch, with heavier females producing longer larvae. Our findings are similar to those reported by previous investigations, all finding that larval length-at-hatch and egg size are positively correlated with female size (Kjesbu 1989, Kjesbu et al. 1996, Vallin and Nissling 2000, Marteinsdottir and Begg 2002).

It was unanticipated that faster maternal growth during the spawning period would have a positive effect on larval length. For females, it is generally hypothesized that there is a trade-off between growth and reproduction, thus, one would expect that the production of better quality offspring, which are more energetically costly, would result in a lower growth rate (Jørgensen et al. 2006). That being said, Chambers and Waiwood (1996) similarly noted that females who grew the most during the spawning period had larger average egg sizes. It is possible that females who had superior growth were those that were in better overall condition and, thus, in addition to increased growth, they were also able to produce better quality larvae. Chambers and Waiwood (1996) hypothesized that females who had increased amounts of energy available to them during spawning resulted in both better quality offspring and better growth. Another point to consider is the question of when, in a female's life, the trade-off between reproduction and growth is actually realized. Our findings suggest that the trade-off is not manifest during the spawning period per se, consistent with the hypothesis that reduced growth attributable to the diversion of tissue from soma to gonads likely occurs considerably earlier than the time of spawning.

It is also possible the feeding regime during the experiment influenced these results. As food was very accessible for cod throughout the spawning season, females may have altered or enhanced their natural eating habits due to the decreased energetic costs of capturing their prey: stationary cooked shrimp. Furthermore, females had ceased spawning for approximately a month before they were sampled. Thus, differences in growth may have occurred during this period.

Size-dependent mortality is an important mechanism influencing the survival of larval marine fish. Depending on the hypothesis, larval length has been found to both negatively and positively affect larval survival (Miller et al. 1988, Pepin 1993). Egg diameter and the size of larvae are positively correlated with numerous indicators of larval viability including: faster swimming speed (advantageous for avoiding predators; earlier success at first feeding; higher probability of producing a swim bladder; and increased specific growth rates later on in development (Marteinsdottir and Steinarsson 1998, Miller et al. 1988). It has been hypothesized that size-selective predation could increase mortality rates in larger larvae, if longer larvae are more vulnerable to size-selective predation (Miller et al. 1988). However, Pepin (1991) suggests otherwise. Using Pepin's (1991) equation for how larval mortality relates to temperature and larval length, I find that the 11% reduction in average larval length throughout the spawning period reported here is predicted to be associated with an 8% increase in daily larval mortality. This suggests that the temporal changes in larval length documented in the present study might be linked with rather significant temporal changes in larval and, thus, maternal fitness.

In addition to temporal shifts in the size of larvae produced by females, there also appear to be shifts in another metric of female reproductive effort: size of yolk sac. Variation in yolk sac size, standardized by larval length, is representative of variation in the amount of energy reserves available to a larva relative to its physiological needs. A higher yolk sac to length ratio is likely to be representative of a higher quality larva, as there are more energetic reserves available to the larva.

For cod, few studies have examined individual maternal effects and seasonal variation of nutritional quality of offspring. Many studies assume that variation in egg size accurately represents variation in the nutritional quality of an offspring, and while egg size has been shown to be correlated with size of yolk sac (Trippel 1998), the results in this study are inconsistent with this assumption as the length of larvae, a metric highly correlated with egg size, and standardized yolk sac volume exhibited very different seasonal trends. Similarly, Clemmesen et al. (2003) found that temporal patterns in larval size did not correspond to variation in the RNA:DNA ratio, a ratio which chemically reflects the nutritional condition of larvae. Nonetheless, the individual trend among females was the same for both metrics of offspring quality: larger females produced both longer larvae and larvae with larger yolk sacs relative to their size.

Our finding that female size correlates with relative yolk sac size (i.e. nutritional reserves) are in concordance with the few studies quantifying variation in the nutritional content of larvae. Solemdal et al. (1993) found a strong correlation between female size and the free amino acid content in eggs. Although Ouellet et al. (2001) similarly found that pre-spawning condition positively influenced the energetic content of eggs, they found no temporal trend across successive batches for individual females.

Yolk-sac larvae begin feeding exogenously before the yolk sac is completely exhausted, resulting in a period of overlap while transitioning from internal to external feeding sources (Neilson et al. 1986, Morrison 1992). The length of time during which larvae can feed both exogenously and endogenously is at most a few days and strongly influenced by temperature (Pepin et al. 1997, Kamler 2007). Increases in energetic reserves (i.e. the size of the yolk sac) could lengthen the period in which larvae can rely on endogenous nutrition while simultaneously feeding exogenously. An increase in the transition period during which a larva is able to feed exogenously, however not yet being fully dependent on it, could subsequently lead to lower mortality rates during this critical period (May 1974). Fiksen and Folkvord (1999) modeled the survival consequences of increases in larval energetic reserves, finding that increases in the yolk/somatic mass ratio at hatching increased survival. Thus, larvae from larger females might have lower mortality rates through the critical first feeding period due to the increased energetic reserves initially provisioned to them.

The final reproductive pattern documented in the present study, for both males and females, was that larger individuals experienced significantly longer spawning periods and participated in more spawning events than smaller individuals. The number of batches a female produced in the current work (range: 1-18, mean: 9) is similar to previously reported ranges: 1-19 (Kjesbu 1989), 4-21 (Kjesbu et al. 1996), 4-11 (Chambers and Waiwood 1996), and 3-11 (Trippel 1998). Notwithstanding three very long individual spawning periods (68, 71, and 78 days), our estimates of individual spawning period (range: 1-76, mean: 31) were within previously documented durations: 47-60 (Kjesbu 1989), 6-48 (Kjesbu et al. 1996), 55 ± 40.2 SD (Chambers and Waiwood

1996),17-51 (Trippel 1998). Our finding that the size of an individual positively influenced the duration of spawning and number of batches is consistent with previous findings (age [Hutchings and Myers 1993; Lawson and Rose 2000; Trippel 1998], and size [Kjesbu et al. 1996]). The exception was the study by Chambers and Waiwood (1996) which found no relationship between female size and any reproductive timing metric.

Although previous studies have documented similar trends in reproductive timing, they were based on paired spawning events or estimated from the spawning condition of fish collected during surveys. Examining reproductive timing within a spawning population at the individual level, with conditions similar to those they would experience in the wild (i.e. a wide range of mate choices), is of particular importance for fish such as cod for which mate choice seems likely to play an integral role in their proposed lek-based mating system (Hutchings et al. 1999, Nordeide and Folstad 2000). This study is the first to do just that, examining these relationships among a large free-spawning population (n=73) with an essentially fine-scaled temporal resolution (daily) and very substantive sample size.

Variation in the duration and number of batches represents the degree to which a female spreads the environmental risk to her offspring. Females that produce offspring in a greater number of batches and over a longer period will subsequently increase the probability that her offspring are matching up with favorable conditions, thus increasing the probability of continued survival of her offspring. One would expect this bet-hedging spawning strategy (Hutchings and Rangeley 2011) to positively affect a female's continued reproductive success and subsequent fitness.

As with any experimental study, I cannot discount the possibility that the experimental conditions may have affected our results. For instance, I cannot exclude the possibility that incubation temperature affected the larval length-at-hatch or yolk sac size; however, the standard deviation in temperature among incubation tanks was only half a degree (mean \pm SD: 6.1 ± 0.5). Studies that document the effect of temperature on size-at-hatch have found differences in length at hatch similar to those reported here (~ 40 - $50 \mu\text{m}$) but across a significantly greater temperature gradient (6 - 10°C) (Pepin et al. 1997, Jordaan et al. 2006). Thus, it seems unlikely that the small variation in temperature experienced by different sampling days in the present study was a primary determinant of the variance in length at hatch.

It is also possible that the sampling scheme did not accurately capture female and male spawning patterns. A sampling bias might have existed towards the detection of trends for individuals that were more reproductively successful. For individuals who had poorer relative daily reproductive success, there is a lower probability that their offspring would have been captured in the reproductive success sample for the day; this would mostly affect the number of batches, resulting in an under-estimation in the number of batches, and potentially affect estimates of individual spawning duration. Our measures of reproductive timing could also be under-estimated because they are based on eggs that had been fertilized, rather than being representative of all of the eggs that a female spawned.

As with Chapter 1, I cannot entirely rule out the possibility that the spawning basin altered spawning behaviour; however, fish were monitored throughout the season and exhibited no external indications of stress.

Our findings highlight fine-scale resolution in the temporal variability in offspring quality and individual variation in offspring quality and reproductive timing in Atlantic cod. I found that offspring quality 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 changes in the environment, such as water temperature. I also found that increasing parental body size positively affected both the quality of offspring and the period over which a female produces those offspring. Thus, larger females not only produce larvae over a longer period, spreading the chance of their offspring experiencing sub-optimal conditions, but they also produce offspring that are better provisioned to survive beyond the yolk-sac stage. These additive maternal influences will positively affect the continued reproductive success of a female and her fitness, if these influences are heritable. Thus, beyond variation in the absolute fecundity of a female, variation in reproductive strategy can also have serious implications on female reproductive success and, as a consequence, population viability.

Chapter 4: Conclusion

4.1 Summary

My research provides a holistic understanding of reproductive success and further underscores the importance of the mating system in cod. Overall, I found that larger individuals correlated with increased numbers of offspring fertilized, higher quality of offspring produced, and increased periods over which offspring were produced.

In Chapter 2, I found that male size positively correlated with the number of offspring fertilized. Among males, male size was a dominant factor driving variance in reproductive success, though this was only true among the largest males, likely a consequence of being top rank males within the dominance hierarchy. For females, while size similarly had a positive effect on the number of offspring fertilized, the top three females in the spawning basin had unexpectedly low reproductive success. I hypothesized this was a result of a size mismatch in equivalent size male fish.

Chapter 3 further underscored the importance of size on reproductive success. I found that larger individuals produced larvae over a longer period, thus spreading the environmental risk to offspring, and furthermore, that larger females produced longer larvae with larger yolk sac sizes, thus better provisioned to survive the critical first feeding period.

Empirically examining individual correlates of the number of offspring fertilized, the quality of offspring produced, and reproductive timing is critical to estimating in situ variance in reproductive success. Variation in these reproductive strategies among fish will have an additive effect on the continued reproductive success of individuals and thus,

influencing per capita population growth rate, gene flow, and ultimately, population viability.

4.2 Implications

Worldwide, marine fish species are experiencing unprecedented environmental and anthropogenic pressures (Hutchings and Reynolds 2004). Sustained heavy fishing pressure and environmental change during recent decades have resulted in dramatic declines in population sizes, reduced individual growth rates, decreased sizes at maturation, and severe truncations in the age structure of populations (Hutchings and Baum 2005, Sharpe and Hendry 2009). The unintended negative consequences of these pressures have resulted in an increase in the uncertainty of recovery and a decrease in the resilience of populations to future pressures (Hutchings and Reynolds 2004, Kuparinen and Hutchings 2012, Kuparinen et al. 2014). Consequently, gaining an in-depth understanding as to how these changes will affect population stability is critical for future effective population management.

The relationships documented in this study will be integral in understanding how changes in population structure might affect population stability. Our research gains a holistic understanding of individual correlates of factors affecting the continued reproductive success of an individual: variation in the number of offspring fertilized, quality of offspring produced, and reproductive timing. I documented that larger individuals are not only responsible for a higher proportion of offspring produced, but they also produce offspring that are better provisioned nutritionally, over a wider temporal window. The consequences of such additive parental effects being that larger

individuals have a superior reproductive strategy that increases their likelihood of reproductive success. Thus beyond variation in the absolute gametic contribution of individuals, variation in reproductive strategies can also have serious implications on an reproductive success and ultimately the stability of a population.

While it is well established that age and size generally have a positive effect on correlates of reproductive success in fish (Berkeley et al. 2004a, 2004b, Hixon et al. 2013), strong empirical relationships documenting these effects are lacking (Scott et al. 2006, Fitzhugh et al. 2012). Coupled with recent truncations of age and size structures among fishes, establishing defensible parameters will be integral in accurately modeling how the changes in age and size structures affect the stability of systems. The few modeling studies have quantified how changes in demography might affect population stability, have all noted the lack of basic empirical relationships at an individual level necessary in parametrizing modeling studies (Scott et al. 2006, Wright and Trippel 2009, Fitzhugh et al. 2012). Thus, developing empirically defensible relationships at the individual level, such as those document in this study, will be critical to accurately estimating the consequences at the population level. Without the holistic understanding of all factors affecting an individual's reproductive success (i.e. offspring quality and reproductive timing) modelers and managers are at risk of under or overestimating expected reproductive success and subsequent population stability.

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Appendix A – Sample COLONY Input File with Parameters:

```

'm1-21-31-d1-10es'      !Dataset name
'm1-21-31-d1-10es'      !Output file name
499                      ! Number of offspring in the sample
8                        ! Number of loci
1234                    ! Seed for random number generator
1                        ! 0/1=Not updating/updating allele frequency
2                        ! 2/1=Dioecious/Monoecious species
0                        ! 0/1=No inbreeding/inbreeding
0                        ! 0/1=Diploid species/HaploDiploid species
0 0                      ! 0/1=Polygamy/Monogamy for males & females
1                        ! 0/1=Clone inference =No/Yes
1                        ! 0/1=Full sibship size scaling =No/Yes
1 1.0 1.0              ! 0,1,2,3=No,weak,medium,strong sibship size prior; mean paternal & maternal
                        sibship size
1                        ! 0/1=Unknown/Known population allele frequency
23 8 31 20 24 14 8 25  !Number of alleles per locus

124  129  133  137  141  145  149  153  158  162  166  170
174  178  182  186  190  192  196  200  203  205  216
0.0822 0.0068 0.0068 0.0205 0.0479 0.1438 0.1096 0.0753 0.1301 0.0753 0.0548 0.0205
0.0137 0.0137 0.0137 0.0205 0.0342 0.0479 0.0205 0.0274 0.0137 0.0068 0.0137

122  125  128  131  134  137  140  148
0.0753 0.2945 0.1712 0.1575 0.1507 0.1027 0.0411 0.0068

111  115  119  123  127  131  135  140  144  148  153  157
161  165  169  173  177  181  185  189  207  217  221  223
225  228  248  251  264  288  308
0.0205 0.0068 0.0137 0.2055 0.0685 0.0274 0.0342 0.0616 0.0479 0.0890 0.0890 0.0137
0.0685 0.0411 0.0548 0.0205 0.0274 0.0068 0.0068 0.0068 0.0068 0.0068 0.0068 0.0068
0.0137 0.0137 0.0068 0.0068 0.0068 0.0068 0.0068

131  143  147  152  156  160  164  168  172  176  180  184
188  192  196  200  204  208  212  216
0.0068 0.0274 0.0205 0.0137 0.0479 0.0685 0.0548 0.0479 0.1370 0.0890 0.0753 0.1027
0.0616 0.0685 0.0342 0.0411 0.0822 0.0068 0.0068 0.0068

115  116  118  121  124  131  133  135  137  139  142  144
146  149  151  153  155  164  166  168  174  176  212  236
0.1918 0.0205 0.0068 0.0342 0.0753 0.0274 0.0274 0.0959 0.1849 0.0890 0.0548 0.0479
0.0479 0.0205 0.0068 0.0068 0.0068 0.0068 0.0068 0.0068 0.0068 0.0137 0.0068 0.0068

105  107  109  111  113  115  117  119  121  124  128  130
134  143
0.0342 0.2603 0.1370 0.1096 0.1575 0.0685 0.0479 0.1027 0.0137 0.0137 0.0205 0.0137
0.0068 0.0137

90  94  98  102  106  110  113  117
0.0274 0.1438 0.5411 0.1644 0.0548 0.0274 0.0342 0.0068

```

78	80	82	84	86	88	91	93	95	97	100	102
104	106	108	110	112	114	116	118	122	124	139	144
174											
0.0479	0.0274	0.0068	0.0137	0.0205	0.0274	0.0068	0.1236	0.1027	0.1236	0.1507	0.0479
0.0822	0.0274	0.0342	0.0205	0.0205	0.0274	0.0342	0.0068	0.0137	0.0068	0.0068	0.0137
0.0068											

```

1      ! Number of runs
3      ! Length of run  1,2,3,4=short,med,long,very long
0      ! 0/1=Monitor method by Iterate#/t/time in second
100000 ! Monitor interval in Iterate#/t/time in seconds
0      ! non-Windows version 0= for other eks Linux
1      ! Fulllikelihood
3      ! 1/2/3=low/medium/high Precision for Fulllikelihood

```

GMO19	GMO35	GMO8	TCH11	GMO132	GMO2
GMO34	TCH13	!Marker names			
0	0	0	0	0	0
0	0	!Marker types, 0/1			
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.0000	0.0000	!Allelic dropout rate			
0.020	0.0200	0.0200	0.0200	0.0200	0.0200
0.0200	0.0200	!false allele rate			

Appendix B – Residual plots from Chapter 2:

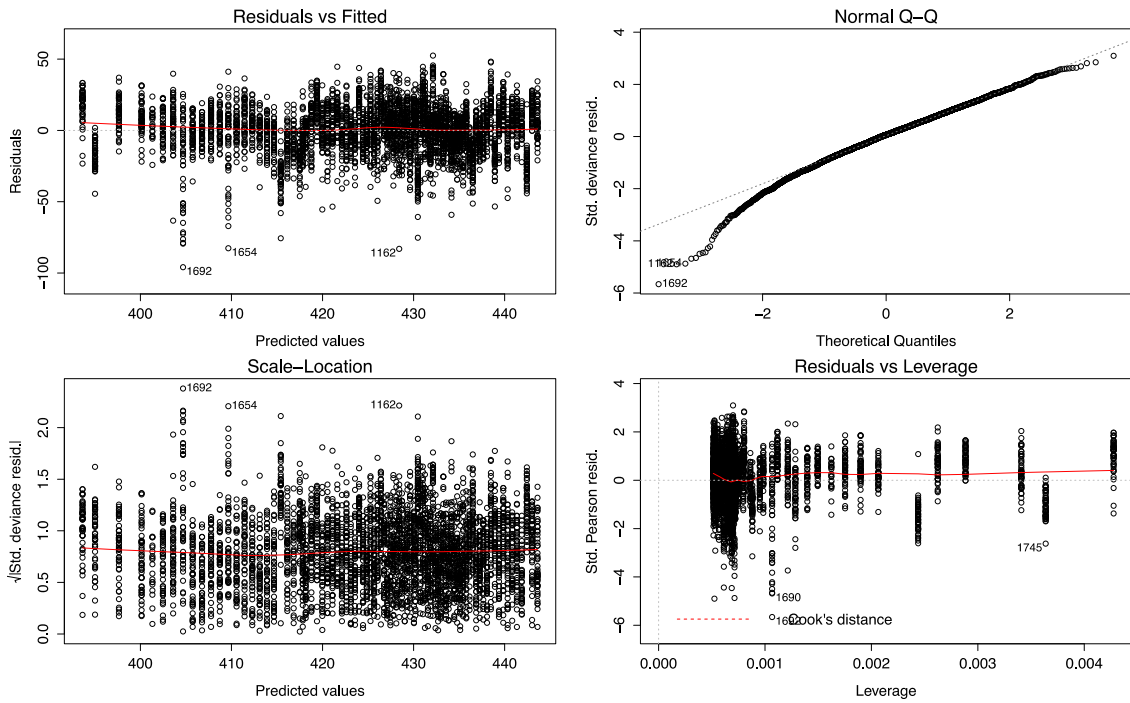


Figure B1: Residual plots from length vs. batch number linear model.

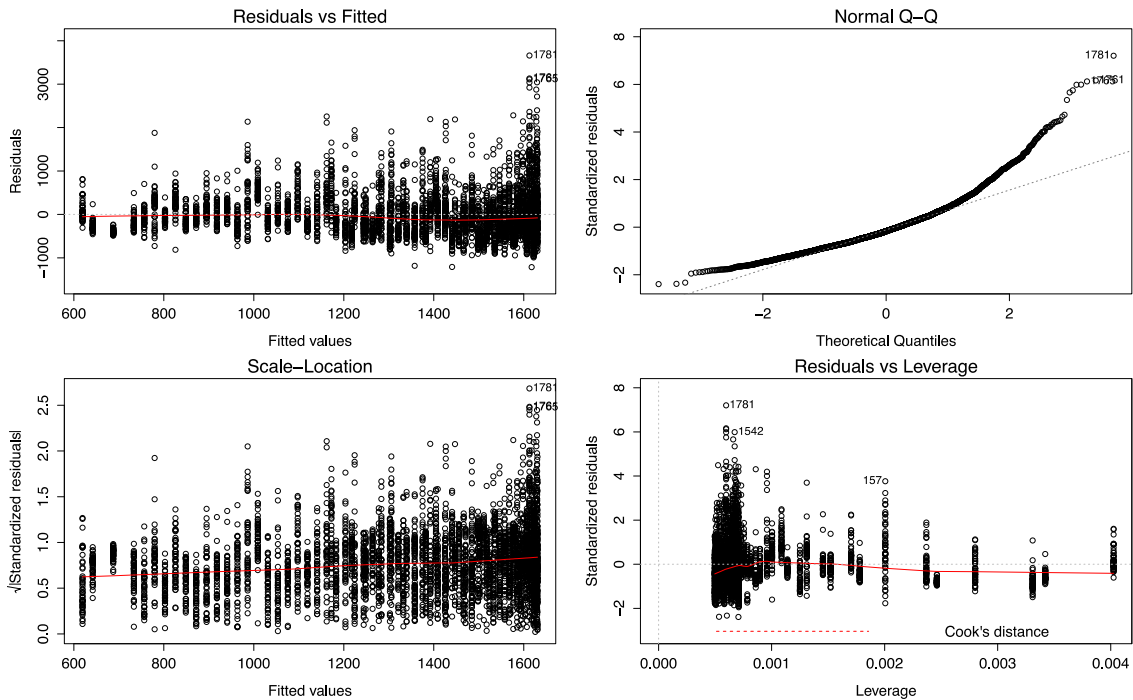


Figure B2: Residual plots from yolk sac volume per total length vs. batch number.

Appendix C – Female weight vs offspring quality plots:

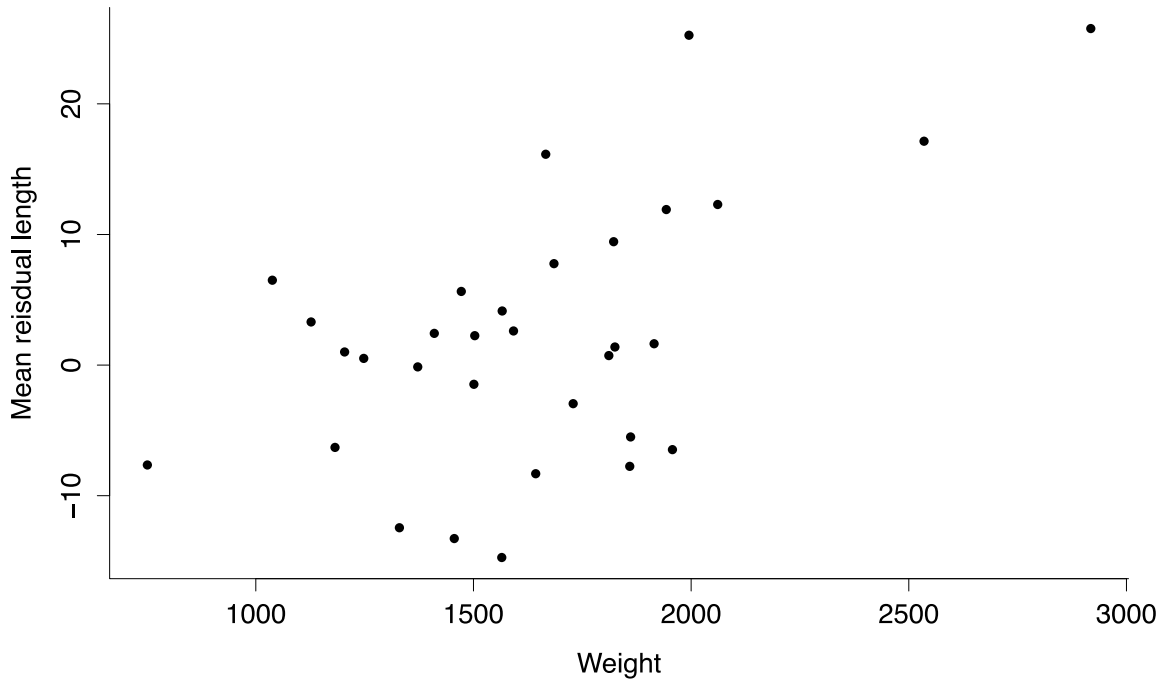
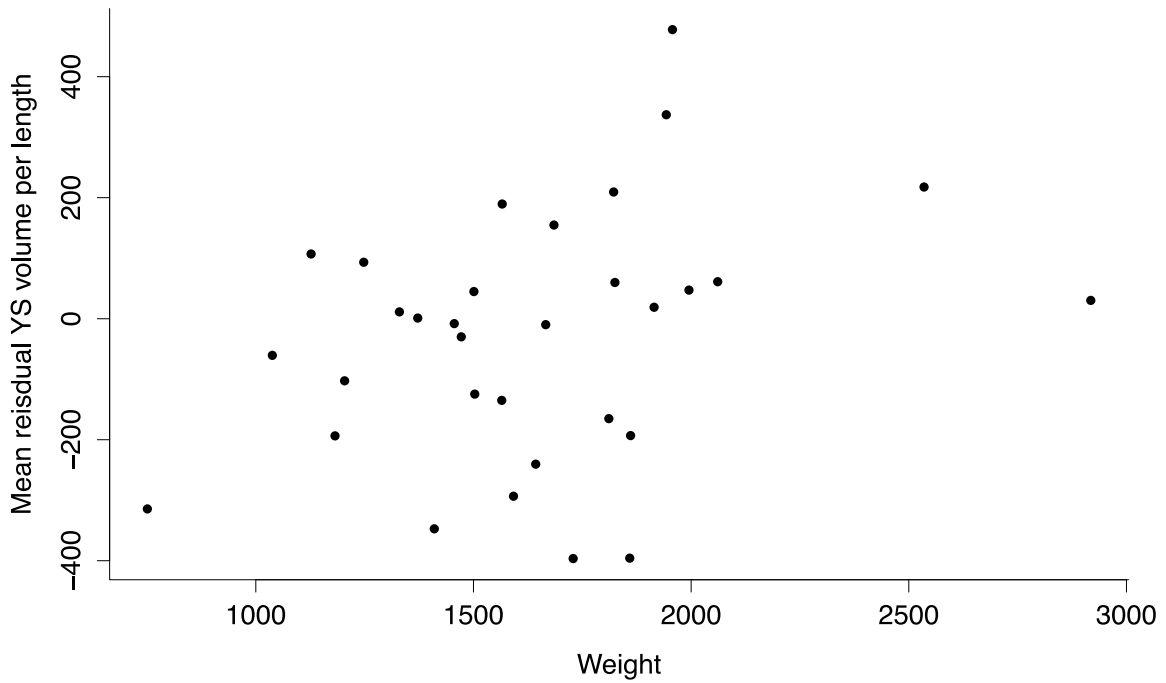


Figure C1: Mean residual length of larvae versus female weight.



FigureC2: Mean residual yolk-sac (YS) volume per length vs female weight.