Title

Fecundity Estimation of Atlantic Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) of Georges Bank: Application of the Autodiametric Method.

Authors

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Abstract

Haddock, *Melanogrammus aeglefinus (L.)*, and Atlantic cod, *Gadus morhua* (L.), are species of major commercial interest in the Northwest Atlantic and their spawning strategy is one characterized by group-synchronous oocyte development and determinate fecundity. Recent advances in image analysis systems and the development of the autodiametric method have led to rapid assessment and accurate estimation of the annual egg production of an individual or its potential fecundity. In this study we estimated the corresponding autodiametric calibration curves for Atlantic cod and haddock of Georges Bank and used these to estimate potential fecundity of these two

species near the southern limits of their geographic distribution. In addition, we explored the relationships between potential fecundity and different condition indices for these populations and found the hepatosomatic index to significantly increase the explanatory power of the fecundity-length relationship which is in agreement with previous studies on the influence of nutritional state on egg production.

Keywords: Atlantic cod, haddock, fecundity, autodiametric method, Georges Bank.

1. Introduction

In fishes, annual egg production of individuals is considered to be a key factor to understanding variations in population size and hence is a life history trait very relevant to fishery management (Hilborn and Walters, 1992; Marshall et al., 2003). Essential to its incorporation in fishery management is the routine estimation of fecundity which permits a better understanding of observed fluctuations in reproductive output and enhances our ability to estimate recruitment and population growth rate (Roff, 1992; Kraus et al., 2002; Lambert, 2008).

Atlantic cod, *Gadus morhua* (*L.*) and haddock, *Melanogrammus aeglefinus (L.)* are species of major interest in the Northwest Atlantic that either currently or historically have supported significant commercial fisheries. Both species are characterized by group-synchronous oocyte development (Clay, 1989; Kjesbu et al., 1990, Kjesbu and Holm, 1994) and determinate fecundity (Trippel et al., 1998; Trippel, 1998; Murua and Saborido-Rey, 2003) such that the number of vitellogenic oocytes in the ovary just prior to spawning corresponds to potential annual fecundity, which through oocyte atresia is adjusted to the actual number of eggs spawned (Hunter et al., 1992; Murua and

Saborido-Rey, 2003). Recent advances in image analysis systems have led to the rapid assessment and accurate estimation of potential fecundity (i.e., number of yolked oocytes) (Klibansky and Juanes, 2008; McCarthy et al., 2008) which was a laborious and time consuming task involving the manual or automatic counting of individual oocytes of ovarian subsamples, i.e., the gravimetric method (Bagenal and Braum, 1978; Kraus et al., 2002; Murua et al., 2003). Recently, Thorsen and Kjesbu (2001) using image analysis developed and tested a new method to estimate potential fecundity based on an oocyte density-diameter relationship. This approach requires ovarian weight and mean diameter of oocytes of a pre-spawning individual to estimate its potential fecundity, precluding the need to count eggs of weighed subsamples.

To date, calibration curves have been developed for a number of fish species and stocks (Witthames et al., 2009). However, in order to apply the autodiametric method one needs to verify a calibration curve for each stock as one cannot be certain whether the calibration curves are comparable within or among species. Inter-population differences may exist in ovarian structure or in gonadal growth (differences in the volume occupied by oocytes in gonads) that can lead to inaccurate and biased estimates of fecundity when using published calibration curves which are unsuitable for the species/stock of interest (Witthames et al., 2009).

In the Northwest Atlantic, traditionally there has been a marked absence of published fecundity-length curves for commercial fishes (Trippel, 1999; Tomkiewicz et al., 2003a). The development of the Northwest Atlantic Fisheries Organization (NAFO) Working Group on Reproductive Potential helped to focus attention on many of the missing elements of basic reproductive biology that if known would improve the biological understanding of fish stocks managed in Atlantic Canada and elsewhere (Tomkiewicz et al., 2003a). Establishment of routine fecundity estimates of marine fish

stocks in general has been hindered due to insufficient technical support and consequently annual levels of egg production have not appeared in stock status reports (Trippel, 1999; Marshall, 2009). To date, an oocyte size-density curve has yet to be developed for a cod stock in the Northwest Atlantic nor has one been generated for haddock of any stock, despite this latter species widespread occurrence and commercial value.

The objective of this study was to estimate the calibration curves for Atlantic cod and haddock of Georges Bank (NAFO Sub. 5Ze, Ruzzante et al., 1998; Van Eeckhaute et al. 1999), and to use these to estimate fecundity by applying the autodiametric method (Thorsen and Kjesbu, 2001). Moreover, we explored the relationships between potential fecundity and various somatic attributes for these two populations situated near the southern limit of their geographical distribution.

2. Materials and Methods

2.1 Ovary sampling

Atlantic cod and haddock were captured on Georges Bank during the first two weeks of March by western style IIA otter trawl during surveys conducted in 2006, 2007 and 2008 by Fisheries and Oceans Canada (R.V. *Needler, Teleost and Templeman*).(Table 1, Fig. 1). Spawning of Atlantic cod on Georges Bank is nearing completion at this time of year, whereas haddock spawning is just beginning (Smith, 1985). These collection periods enabled sampling of mature, unspawned females for fecundity estimates, particularly for haddock. Georges Bank collections were conducted during annual stock assessment surveys and were based on a random stratified design. All samples used for this study were collected from ripe females, stage 3, which did not contain hydrating

oocytes, i.e. prespawning gonadal development stages (Tomkiewicz et al., 2003b) and possessed vitellogenic oocytes in their secondary growth phase (Greer Walker et al., 1994; Murua and Motos, 2006). Details of sampling effort of each survey are described in Table 1. Total length, total body weight, carcass weight (i.e., body weight – weight of organs), liver weight and ovarian weight (± 1 g) of females containing prespawning stage ovaries were recorded. The ovary sample selection was designed to span females of the entire length distribution pending availability. Samples (~ 5-7 g) of ovaries were preserved in 4% buffered formaldehyde in 100 ml glass vials and returned to the St. Andrews Biological Station.

2.2 Enumerating oocytes

Three subsamples each weighing ~0.050-0.100 g (± 0.001 g) were removed from each ovary sample for oocyte counting and size measurements during March-May 2008 (each sample had been stored for a minimum two month period in formaldehyde). Subsamples from cod were in the size range of 0.051-0.096 g and haddock subsamples 0.050-0.098 g. Egg counts among subsamples of the same female did not exceed a coefficient of variation (CV) of 10%. All enumerated oocytes were in the cortical alveolus or vitellogenic stage (Tyler and Sumpter, 1996) having a yellow or pale orange colour. A homogeneous oocyte size distribution occurred in both species (Fig. 2) simplifying estimation of mean egg diameter of each sample.

2.3 Procedures for counting and measuring oocytes

Ovary subsamples were withdrawn from the sample using a Pasture pipette and ejected into a watch glass pre-filled with distilled water. This aided in separation of oocytes from connective tissue to generate a distribution pattern conducive to enumeration and

126 measurement. Image analysis Image-Pro Plus v.4.5.1. in combination with a MZ95
127 Leica Microscope and Olympus SZH camera and Q-Imaging MicroPublisher 3.3 RTV
128 software were used to record images of each ovarian subsample (~200-300 oocytes)
129 which were saved in TIFF file format. Microscope light settings for measurements were
130 performed using best fit to enhance feature and increase the contrast using gamma
131 correction (nonlinear operation used to code and decode luminance in the image). After
132 the colour scale was changed to a grey scale a threshold value for black and white (255
133 refers to black and 0 to white) was fixed for each image of selected oocytes. A
134 contouring algorithm was applied to eliminate edges from the oocytes. The system was
135 length-calibrated (mm units) and the measurements were performed using gray scale
136 images.
137
138 **2.4 Data handling**
139 After ~100-150 'oocytes' were measured, the data were examined in order to eliminate
140 particles that were not considered to be individual oocytes. This was done by filtering
141 data based on roundness and diameter threshold ranges that were estimated to be valid
142 for oocytes (Thorsen and Kjesbu, 2001). The roundness threshold was set from 1.0-1.2
143 which effectively removed unwanted particles, which were mostly connective tissue or
144 damaged oocytes. Similarly, the oocyte diameter range was set from 200-1000 µm to
145 eliminate immature and hydrated oocytes based on knowledge of the size distribution of
146 vitellogenic oocytes of these species (Clay, 1989; Kjesbu, 1994; Thorsen and Kjesbu,
147 2001). Also we investigated the likely affect on potential fecundity of different
148 condition indices and included these variables using multiple linear regression.
149 Hepatosomatic index (HSI) and condition factor (K) were estimated as follows:
150 $HSI = (LW / W) \times 100$ (1)

$K = (W / L^3) \times 100$ (2)

Where L is total length (cm), LW is liver weight (g) and W is carcass weight (g).

It is known that the apparent error rate tends to underestimate the true error rate (Efron, 1986) and since models with good predictive qualities should have error measures close to zero (Power, 1993) a Cross-Validation for Generalized Linear Models was used to estimate the corresponding prediction errors of the calibration curves (Stone, 1974; Efron, 1986). Considering the relatively low number of samples the leave-one-out cross-validation procedure was utilized. Chow test (Chow 1960) was used to compare nonlinear power models of calibration curves among species, cod and haddock from Georges Bank and Northeast Arctic cod (Thorsen and Kjesbu, 2001). Multiple linear regression models were performed for fecundity relationships using a backward stepwise multiple regression model for variable selection. Log transformation was used when it was needed to achieve model assumptions. Akaike's information criterion and ANOVA model comparisons were used for model selection. Variance inflation factors permitted removal of collinear explanatory variables during variable selection. Residuals were plotted to check there was no systematic pattern in the residuals for model validation in each case. Statistical analyses were conducted using R Statistical Computing free-software (R version 2.7.2).

3. Results

Oocyte density, defined as the number of vitellogenic oocytes per gram of ovary (OG), formed significant relationships with oocyte diameter (OD in μm) in a non-linear power model for both species (df=27, r^2=0.95, $p<0.01$ for cod and df=34, r^2=0.90, $p<0.01$ for haddock). Parameter estimates for both relationships are listed in Table 2 and fitted

models are shown in Figure 3. The average estimated prediction errors for both calibration curve models (log transformed), assessed using the leave-one-out cross-validation procedure, were very close to zero, 0.0091 and 0.0129 for cod and haddock, respectively and were just slightly above those in the original models (0.0079 for cod and 0.0126 for haddock).

Chow test was used for curve comparison among cod and haddock of Georges Bank and no significant difference was detected (df=53, P=0.796). Also we compared our results with Thorsen and Kjesbu's (2001) calibration curve for Northeast Arctic cod and found no significant difference for cod (df=53, P=0.17), but for haddock the difference was significant (df=64, P=0.03). Prior studies found some differences in parameters estimated among species and the variation accounted for by each curve was also variable, for example curves for asynchronous ovarian development species (*Merluccius merluccius* and *Scomber scombrus*) exhibited a relatively poor explanatory power in comparison with curves developed for synchronous spawners and this discrepancy was likely due to the greater range in oocyte diameters observed in asynchronous spawners (Witthames et al., 2009).

Using corresponding calibration curves for Georges Bank cod and haddock respectively, Potential Fecundity, PF, was estimated as:

$$PF = OW \times OG = OW \times (7.1e{+}10 \times OD^{-2.526}) \quad \text{Cod} \quad (2)$$

$$PF = OW \times OG = OW \times (2.4e{+}11 \times OD^{-2.703}) \quad \text{Haddock} \quad (3)$$

where OW is total ovary weight (g). No bimodal distribution was found in oocyte diameter distribution, thus, no correction was applied to the equations. Fecundity estimates using the gravimetric and autodiametric methods were highly correlated (r^2= 0.99, $P<0.01$, df = 63).

Potential fecundity estimates for cod and haddock were derived using Eqs. 2 and 3, respectively, for 2006, 2007 and 2008 and were deployed to obtain fecundity-length and fecundity-weight (carcass weight) relationships (Fig 4). For fecundity-length and fecundity-weight relationships a simple linear regression was performed on natural log transformed data. The relationship between fecundity and body size (length and weight) was estimated for cod using pooled samples (n = 29) from surveys 2006, 2007 and 2008 while for haddock these relationships were developed for specimens captured in 2006 (n=37), 2007 (n = 36) and 2008 (n =16) with all relationships being significant ($p<0.001$ for all linear regressions). Since no significant interannual differences were detected for haddock in either intercept or slope (ANCOVA, df=83, $P>0.05$) we pooled data to generate single fecundity-size relationships for each trait (Table 3); low samples sizes for cod precluded ability to generate annual curves. Previously published potential fecundity-body size relationships for cod and haddock from stocks of the Scotian Shelf and Georges Bank (NAFO Subdivisions 4X, 4V, 4W and 5Z, Fig. 1) are presented in Table 4 to facilitate comparisons with those derived in the present study (Table 3).

We explored the relationships of condition indices HSI (1.86-9.96 for cod and 1.56-8.46 for haddock) and K (0.55-1.90 for cod and 0.70-1.31 for haddock) with potential fecundity. After exploration of our data set we decided to exclude carcass weight from our initial model due to its high correlation with length and its representation in K and HSI. No significant correlations were found among explanatory variables of the model, i.e., length, K and HSI. Fecundity and length were log transformed with all years pooled for each species. The initial full model includes the following variables using the pooled samples from 2006-2008:

$$\mathrm{Ln(PF)} = \mathrm{Ln(Length)} + \mathrm{HSI} + \mathrm{K} \qquad (4)$$

The inclusion of both condition indices (Model 4, Table 5), HSI and K, in the model resulted in significant improvement of potential fecundity – length relationships. K was shown as the most influential condition factor for cod increasing the explained variation of potential fecundity relationships by 6.9% (Model 3, Table 5), whereas inclusion of HSI improved it by 4.4% (Model 2, Table 5). In the case of haddock, HSI increased the explained variation by 2.4% (Model 2, Table 5) meanwhile K provided only 1.2% of improvement. Even though the improvements of explanatory power were low (Table 5). ANOVA nested model comparisons showed they were significant ($p<0.01$).

4. Discussion

Methodology described in the present study could be applied to rapidly estimate fecundity from historical sampling of preserved gonads as well as to generate new fecundity data sets for cod and haddock of Georges Bank. This, in turn, would provide the estimation of a fundamental key parameter incorporated in stock reproductive potential that has application to development of biological reference points for fishery management (Mace and Sissenwine 1993**;** Marshall et al., 2003; Marshall, 2009). We found significant differences between oocyte density-size calibration curves among cod and haddock of Georges Bank as well as with Northeast Arctic cod (Thorsen and Kjesbu 2001) and thus recommend the application of species and stock-specific curves when available. Several factors should be evaluated prior to using a general species calibration curve (Witthames et al., 2009). One of the factors which might limit the widespread use of the calibration curves developed in the present study is the small range of oocyte diameters used in their formulation. However, although this may at first appear as a disadvantage it can under certain circumstances have advantages. For

example, it has been demonstrated that down-regulation of potential fecundity due to atresia happens as fish approach their spawning season (Kjesbu et al., 1991; Kurita et al., 2003; Kennedy et al., 2007; Witthames et al., 2009). Consequently, recommendations have been made to collect samples for fecundity studies as close as possible to the onset of a stock's spawning season (i.e., January-March for cod and haddock of Georges Bank; Morgan et al., 2003). This is because if one collects samples very early in the season when the oocyte diameter is still small (i.e., in the very left part of the calibration curve) there still remains a substantial time period during which atretic down-regulation of fecundity can result in overestimation of potential fecundity (Kennedy et al., 2007). In this sense, our calibration curve has clear advantages as it is suited to fishes with an oocyte mean diameter range between 575 to 995 μm for cod and 520 to 820 μm for haddock; which are very close to the onset of the spawning season. Similarly, timing of sampling is crucial to reduce sources of variation due to atretic down-regulation (Witthames et al., 2009); and in the case of Georges Bank cod and haddock stocks it appears that the annual survey times for Canada's assessment of Georges Bank demersal fish stocks are appropriate to permit fecundity estimation of these two gadoids, i.e., close to the onset of their spawning periods (Morgan et al., 2003). Our equations will be valuable to the beginning of routine collections of necessary ovarian samples on research surveys followed by technical analysis in the laboratory to generate annual fecundity-length predictive equations.

With regard to the oocyte size models developed in the present and other studies, there exist a number of other sources of variation affecting their formulation. For example, one must be cognizant of the variation of oocyte diameter attributed to size differences among fresh and preserved samples (West, 1990). Both preservation fluid (Joseph, 1963) and duration of preservation (Witthames and Greer Walker, 1987) could have

274 significant influences on oocyte shrinkage which, in turn, can affect their accuracy when
275 applied. In our case, use of buffered formalin with ample tissue storage time prevented
276 any introduction of bias due to oocyte shrinkage. Homogeneity in size distribution of
277 oocytes should be checked to avoid bias in oocyte density estimation in relation to
278 tissue sampling location within the ovary (Kennedy et al., 2007; Witthames et al.,
279 2009). In this aspect, previous studies on cod and haddock did not detect differences in
280 oocyte density in relation to sampling location within the ovary (Kjesbu and Holm,
281 1994). Different image analysis configurations can also potentially lead to different
282 estimates of fecundity of the same sample, though this source of error has proven to be
283 minimal (Witthames et al., 2009). Consequently, due to the limitations described above
284 when a calibration curve does not exist for a particular species or stock one should
285 proceed with caution when using a general species-wide calibration curve or one
286 initially developed for another species or stock.
287 A number of perspectives have been put forward to predict fecundity using body traits.
288 Potential fecundity estimates using the autodiametric method provided good fits to body
289 length and weight data in our study. Carcass weight resulted in the best predictor of
290 potential fecundity, explaining 83% of the total variation for cod and 93% for haddock,
291 however due to the high correlation between length and weight we decided to include
292 only length in the models; 76% of variation explained for cod and 91% for haddock.
293 Although carcass weight accounted for a large portion of the variation in potential
294 fecundity it undergoes greater seasonal variation than length during a yearly cycle and
295 therefore it is considered less reliable than length as the main predictor of fecundity
296 (Thorsen et al., 2006). Body weight is also highly correlated with K and this introduces
297 conceptual redundancy when including body weight with K in the same fecundity
298 model (Blanchard et al., 2003). Conversely, the use of length also requires some

299 consideration as it could overestimate correlations between fecundity and fish condition
300 (Koops et al., 2004) but the application of weight-based relationships tended to
301 overestimate potential fecundity at low K (Thorsen et al., 2006). Condition factor has
302 been used to forecast a stock's potential energy content and nutritional state (Lambert
303 and Dutil, 1997b; Marshall et al., 1999) and the condition indices K and HSI have also
304 been used to improve fecundity predictions and reproductive success (Kjesbu et al.,
305 1991; Marshall et al., 2003; Trippel and Neil 2004). Timing for potential fecundity
306 determination is critical for determinate fecundity species, like cod and haddock, and is
307 a function of available energy reserves and onset of vitellogenesis (Skjaeraasen et al.,
308 2006). In our study, we used condition indices measured shortly before spawning has
309 commenced to predict fecundity, and one may argue that other periods of the year may
310 lead to improvements in fecundity prediction, however sampling so close to
311 commencement of spawning minimizes the down-regulation effect on fecundity
312 (Kennedy et al., 2007; Witthames et al., 2009). Nonetheless, the inclusion of condition
313 indices in our fecundity relationships improved the explanatory power of the models.
314 Our results agree with previous studies on cod where HSI was a good indicator of lipid
315 energy reserves (Lambert and Dutil, 1997b) with its positive influence on egg
316 production (Kjesbu et al., 1991; Marshall et al., 1998; Marshall et al., 1999). Condition
317 factor, K, significantly increased the total explained variation of fecundity for cod and
318 haddock (Blanchard et al., 2003) and is a correlate of energy storage in cod (Lambert
319 and Dutil, 1997a). The combined effect of both condition indices added to the same
320 potential fecundity-length relationship resulted in significant improvements that could
321 reflect two difference sources of storage energy for egg production. While HSI reflects
322 the energy stored in the liver due to lipid accumulation, K is more related to protein
323 accumulation in the muscle (Lambert and Dutil, 1997b) and both, lipids and proteins,

are related to vitellogeneic processes during oocyte maturation (Wiegand, 1996; Patiño and Sullivan, 2002). Although relationships for cod and haddock in our study did not show significant differences between years one cannot infer from this that annual differences may not exist for other time spans (Murua et al., 2003) and the incorporation of condition indices could serve to account for some interannual variability in egg production (Blanchard et al., 2003).

Prior to the present study, potential fecundity estimates for Georges Bank haddock were scarce and dated (1970-1973) (Lough et al., 2008) (Table 4). In the case of cod, the amount of fecundity data of stocks near the species southern distribution is also limited (McIntyre and Hutchings, 2003) (Table 4). Only a few have been published for Canadian cod and haddock stocks along the Scotian shelf and Bay of Fundy (Clay, 1989; Waiwood and Buzeta, 1989; Blanchard et al., 2003; Trippel and Neil, 2004) (Table 4). Moreover, within-species differences exist in the parameter estimates for potential fecundity-length relationships between our study and those of others. The source of variation for these differences may be due to interannual variability (Murua et al., 2003), stock differences (Ruzzante et al., 1998) or the methodological approach used for fecundity estimation. Therefore, it may be difficult to relate fecundity changes over time with changes in population dynamics of these very important species. It is clear that monitoring fecundity using a consistent technique and assessing fecundity-somatic relationships every year is fundamental because it enables the development of an extensive data base that will permit one to follow possible changes in reproductive potential for a given species or stock. This, in turn, would be very helpful for evaluation of stock dynamics since fecundity can be considered a direct measurement of reproductive potential in determinate group-synchronous species like cod and haddock (Marshall et al., 1998).

In summary, we provide for the first time, a calibration curve between oocyte mean diameter and ovarian oocyte density which can be applied within the autodiametric method to estimate potential fecundity for cod and haddock of Georges Bank. We suggest adopting this methodology as a reliable tool for fecundity estimation and continuing with data acquisition, which can be used to augment scientific advice in the fishery assessment process in light of the establishment of biological reference points for fishery management.

Acknowledgements

The authors would like to thank the crews of Fisheries and Oceans Canada Research Vessels *Needler, Teleost and Templeman* for providing samples and data necessary for our study. We also thank M.X. Rodríguez Álvarez for helpful statistical advice. Funding was provided by a grant from Xunta de Galicia, within the INCITE program to A. Alonso-Fernández and DFO's International Governance Strategy targeted for Canada-Spain scientific collaboration.

References

Bagenal, T. B. and Braum, E., 1978. Eggs and early life history. Methods for Assessment of Fish Production in Fresh Waters. Blackwell Scientific Publications. p. 165–201.

371 Blanchard, J. L.; Frank, K. Y.; Simon, J. E., 2003. Effects of condition on fecundity and
372 total egg production of eastern Scotian Shelf haddock (*Melanogrammus*
373 *aeglefinus*). Can. J. Fish. Aquat. Sci. 60(3), 321-322.

374 Clay, D., 1989. Oogenesis and fecundity of haddock (*Melanogrammus aeglefinus* L.)
375 from the Nova Scotia shelf. ICES J. Mar. Sci. 46(1), 24-34.

376 Efron, B., 1986. How biased is the apparent error rate of a prediction rule? Journal of
377 the American Statistical Association, 81, 461–470.

378 Greer Walker, M.; Witthames, P. R.; Bautista de los Santos, I., 1994. Is the fecundity of
379 the Atlantic mackerel (*Scomber scombrus*: Scombridae) determinate? Sarsia. 79(1),
380 13-26.

381 Hilborn, R. and Walters, C. J., 1992. Quantitative Fisheries Stock Assessment: Choice,
382 Dynamics, and Uncertainty. Chapman and Hall. 570 p.

383 Hunter, J. R.; Macewicz, B. J.; Lo, N. C. -.; Kimbrell, C. A., 1992. Fecundity,
384 spawning, and maturity of female Dover sole *Microstomus pacificus* , with an
385 evaluation of assumptions and precision. Fish. Bull. 90(1), 101-128.

386 Joseph, J., 1963. Fecundity of the yellowfin tuna (*Thunnus albacares*) and skipjack
387 (*Katsuwonus pelamis*) from the eastern Pacific Ocean. Bull. Inter. Amer. Trop.
388 Tuna Comm. 7, 257-292.

389 Kennedy, J.; Witthames, P. R.; Nash, R. D. M., 2007. The concept of fecundity
390 regulation in plaice (*iPleuronectes platessa*) tested on three Irish Sea spawning
391 populations. Can. J. Fish. Aquat. Sci. 64(4), 587-601.

392 Kjesbu, O. S.; Klungsoyr, J.; Kryvi, H.; Witthames, P. R.; Greer Walker, M., 1991.
393 Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation
394 to proximate body composition. Can. J. Fish. Aquat. Sci. 48(12), 2333-2343.

395 Kjesbu, O. S.; Witthames, P. R.; Solemdal, P.; Walker, M. G., 1990. Ovulatory rhythm
396 and a method to determine the stage of spawning in Atlantic cod (*Gadus morhua)*.
397 Can. J. Fish. Aquat. Sci. 47(6), 1185-1193.

398 Kjesbu, O. S., 1994. Time of start of spawning in Atlantic cod (*Gadus morhua*) females
399 in relation to vitellogenic oocyte diameter, temperature, fish length and condition. J.
400 Fish Biol. 45(5), 719-735.

401 Kjesbu, O. S. and Holm, J. C., 1994. Oocyte recruitment in first-time spawning Atlantic
402 cod (*Gadus morhua*) in relation to feeding regime. Can. J. Fish. Aquat. Sci. 51(8),
403 1893-1898.

404 Klibansky, N. and Juanes, F., 2008. Procedures for efficiently producing high-quality
405 fecundity data on a small budget. Fish. Res. 89(1), 84-89.

406 Koops, M. A.; Hutchings, J. A.; Mcintyre, T. M., 2004. Testing hypotheses about
407 fecundity, body size and maternal condition in fishes. Fish Fish. 5(2), 120-130.

408 Kraus, G.; Tomkiewicz, J.; Köster, F. W., 2002. Egg production of Baltic cod (*Gadus*
409 *morhua*) in relation to variable sex ratio, maturity, and fecundity. Can. J. Fish.
410 Aquat. Sci. 59(12), 1908-1920.

411 Kurita, Y.; Meier, S.; Kjesbu, O. S., 2003. Oocyte growth and fecundity regulation by
412 atresia of Atlantic herring (*Clupea harengus*) in relation to body condition
413 throughout the maturation cycle. J. Sea Res. 49(3), 203-219.

414 Lambert, Y., 2008. Why should we closely monitor fecundity in marine fish
415 populations? J. Northw. Atl. Fish. Sci. 41, 93-106.

416 Lambert, Y. and Dutil, J. D., 1997a. Can simple condition indices be used to monitor
417 and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus*
418 *morhua*)? Can. J. Fish. Aquat. Sci. 54(Suppl. 1), 104-112.

419 Lambert, Y. and Dutil, J. D., 1997b. Condition and energy reserves of Atlantic cod
420 (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock.
421 Can. J. Fish. Aquat. Sci. 54(10), 2388-2400.

422 Lough, R. G.; O'Brien, L.; Buckley, L. J., 2008. Differential egg mortality of Georges
423 Bank cod and haddock inferred from two independent estimates of seasonal egg
424 production. J. Northw. Atl. Fish. Sci. 41, 119-128.

425 Mace, P.M.; Sissenwine, M.P., 1993. How much spawning per recruit is enough? Can.
426 Spec. Publ. Fish. Aquat. Sci. 120, 101–118.

427 Marshall,C.T., 2009. Implementing information on stock reproductive potential in
428 fisheries management: The motivation, challenges and opportunities. In Fish
429 Reproductive Biology and its Implication for Assessment and Management.
430 Editors : Jakobsen, T., Forgarty, M., Megrey, B.A. and Mokness, E. (eds). Wiley-
431 Blackwell, 440 p.

432 Marshall, C. T.; Kjesbu, O. S.; Yaragina, N. A.; Solemdal, P.; Ulltang, O., 1998. Is
433 spawner biomass a sensitive measure of the reproductive and recruitment potential
434 of northeast Arctic cod? Can. J. Fish. Aquat. Sci. 55(7), 1766-1783.

435 Marshall, C. T.; O'Brien, L.; Tomkiewicz, J., et al, 2003. Developing alternative indices
436 of reproductive potential for use in fisheries management: case studies for stocks
437 spanning an information gradient. J. Northw. Atl. Fish. Sci. 33, 161-190.

438 Marshall, C. T.; Yaragina, N. A.; Lambert, Y.; Kjesbu, O. S., 1999. Total lipid energy
439 as a proxy for total egg production by fish stocks. Nature. 402(6759), 288-290.

440 McCarthy, J. L.; Friedland, K. D.; Brodziak, J., 2008. Enhancement of image-based
441 fecundity methods: Gravimetric sampling at sea and safer sample preservation.
442 Fish. Res. 93(1-2), 47-53.

443 McIntyre, T. M. and Hutchings, J. A., 2003. Small-scale temporal and spatial variation
444 in Atlantic cod (*Gadus morhua*) life history. Can. J. Fish. Aquat. Sci. 60(9), 1111-
445 1121.

446 Morgan, M. J.; Burnett, J.; Tomkiewicz, J.; Saborido-Rey, F., 2003. The Availability of
447 Data for Estimating Reproductive Potential for Selected Stocks in the North
448 Atlantic. Working Group on Reproductive Potential. NAFO Sci. Coun Studies. 37,
449 69-149.

450 Murua, H.; Kraus, G.; Saborido-Rey, F.; Witthames, P. R.; Thorsen, A.; Junquera, S.,
451 2003. Procedures to estimate fecundity of marine fish species relation to their
452 reproductive strategy. J. Northw. Atl. Fish. Sci. 33, 33-54.

453 Murua, H. and Motos, L., 2006. Reproductive strategy and spawning activity of the
454 European hake *Merluccius merluccius* (L.) in the Bay of Biscay. J. Fish Biol. 69,
455 1288-1303.

456 Murua, H. and Saborido-Rey, F., 2003. Female reproductive strategies of marine fish
457 species of the North Atlantic. J. Northw. Atl. Fish. Sci. 3323-31.

458 O'Brien, L., 2003a. Working Group on Reproductive Potential. Atlantic Cod in NAFO
459 Div. 5Z+Subarea 6. NAFO Sci. Coun Studies, 37, 69-75.

460 O'Brien, L., 2003b. Working Group on Reproductive Potential. Haddock in NAFO Div.
461 5Z+Subarea 6. NAFO Sci. Coun Studies, 37, 143-149.

462 Patiño, R. and Sullivan, C. V., 2002. Ovarian follicle growth, maturation, and ovulation
463 in teleost fish. Fish Physiol. Biochem. 26(1), 57-70.

464 Power, M., 1993. The predictive validation of ecological and environmental models.
465 Ecological Modelling, 68(1), 33-50.

466 Roff, D. A., 1992. The Evolution of Life Histories: Theory and Analysis. Chapman and
467 Hall. 535 p.

468 Ruzzante, D. E.; Taggart, C. T.; Cook, D., 1998. A nuclear DNA basis for shelf- and
469 bank-scale population structure in northwest Atlantic cod (*Gadus morhua*):
470 Labrador to Georges Bank. Mol. Ecol. 7(12), 1663-1680.

471 Skjaeraasen, J. E.; Nilsen, T.; Kjesbu, O. S., 2006. Timing and determination of
472 potential fecundity in Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 63(2),
473 310-320.

474 Smith, W.G., 1985. Temporal and spatial spawning patterns of the principal species of
475 fish and invertebrates in the George Bank region. NMFS Sandy Hook Lab., Rep.
476 No. SHL 85-04, 35.

477 Stone, M., 1974. Cross-validation choice and assessment of statistical predictions (with
478 Discussion). Journal of the Royal Statistical Society, B, 36, 111–147.

479 Thorsen, A. and Kjesbu, O. S., 2001. A rapid method for estimation of oocyte size and
480 potential fecundity in Atlantic cod using a computer-aided particle analysis system.
481 J. Sea Res. 46(3-4), 295-308.

482 Thorsen,A.; Marshall,C.T.; Kjesbu,O.S., 2006. Comparison of various potential
483 fecundity models for north-east Arctic cod *Gadus morhua*, L. using oocyte
484 diameter as a standardizing factor. J.Fish Biol. 69(6), 1709-1730.

485 Tomkiewicz, J.; Morgan, M. J.; Burnett, J.; Saborido-Rey, F., 2003a. Available
486 information for estimating reproductive potential of Northwest Atlantic groundfish
487 stocks. J. Northw. Atl. Fish. Sci. 33, 1-21.

488 Tomkiewicz, J.; Tybjerg, L.; Jespersen, A., 2003b. Micro- and macroscopic
489 characteristics to stage gonadal maturation of female Baltic cod. J. Fish Biol. 62(2),
490 253-275.

491 Trippel, E. A., 1999. Estimation of stock reproductive potential: history and challenges
492 for Canadian Atlantic gadoid stock assessments. J. Northw. Atl. Fish. Sci. 25, 61-
493 81.

494 Trippel, E. A., 1998. Egg size and viability and seasonal offspring production of young
495 Atlantic cod. Trans. Am. Fish. Soc. 127(3), 339-359.

496 Trippel, E. A.; Doherty, C. M.; Wade, J.; Harper, J. R., 1998. Controlled breeding
497 technology for haddock (*Melanogrammus aeglefinus*) in mated pairs. Bull.
498 Aquacult. Assoc. Can. 98(3), 30-35.

499 Trippel, E. A. and Neil, S. R. E., 2004. Maternal and seasonal differences in egg sizes
500 and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*)
501 in relation to body size and condition. Can. J. Fish. Aquat. Sci. 61(11), 2097-2110.

502 Tyler, C. R. and Sumpter, J. P., 1996. Oocyte growth and development in teleosts. Rev.
503 Fish Biol. Fish. 6(3), 287-318.

504 Van Eeckhaute, L.A.M.; Gavaris, S.; Trippel, E.A., 1999. Movements of haddock,
505 *Melanogrammus aeglefinus*, on eastern Georges Bank determined from a
506 population model incorporating temporal and spatial detail. Fish. Bull., 1999, 97(3),
507 661-679.

508 Waiwood, K. G. and Buzeta, M. –I.., 1989. Reproductive biology of southwest Scotian
509 Shelf haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 46(Suppl. 1),
510 153-170.

511 West, Grant, 1990. Methods of assessing ovarian development in fishes: A review.
512 Aust. J. Mar. Freshwat. Res. 41(2), 199-222.

513 Wiegand, M. D., 1996. Composition, accumulation and utilization of yolk lipids in
514 teleost fish. Rev. Fish Biol. Fish. 6(3), 259-286.

515 Witthames, P. R. and Greer Walker, M., 1987. An automated method for counting and
516 sizing fish eggs. J. Fish Biol. 30(3), 225-235.

517 Witthames, P. R.; Thorsen, A.; Greenwood, L. N.; Saborido-Rey, F.; Dominguez, R.;
518 Murua, H.; Korta, M.; Kjesbu, O. S., 2009. Advances in fecundity methodology
519 applied to some marine fish. Fish Bull. 107, 148-164.

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Tables

Table 1. Survey information for ovaries sampled for fecundity estimation from Georges Bank, 2006-2008.

Year	Research Vessel	Dates	Hauls	Latitude range	Longitude range	NAFO Div.
2006	Teleost	21/02/06-02/03/06	46	41°14.34 - 41°42.07	66°46.11 - 67°15.65	5Ze
	Needler	21/02/06-28/02/06	12	40°20.64 - 42°09.95	65°53.91 - 68°41.82	5Ze
2007	Templeman	20/02/07-02/03/07	63	40°08.86 - 42°10.78	65°53.06 - 69°51.25	5Ze
2008	Templeman	04/03/08-20/04/08	33	40°31.18 - 45°17.79	65°04.85 - 69°52.72	5Ze

Table 2. Estimates of parameters for oocyte density (OG) and oocyte mean diameter (OD) relationships for cod and haddock expressed as the power equation ($OG = a \times OD^b$). OG: number of vitellogenic oocytes per gram of ovary. C.I: 95% confidence interval.

Species	Oocyte diameter range (um)	*n*	df	P	Explained Variation (%)	*a*	*b*	C.I. (*b*)
Cod	575 - 995	29	27	<0.01	94.6	7.1e+10	-2.526	(-2.757; -2.295)
Haddock	520 – 820	36	34	<0.01	90.3	2.4e+11	-2.703	(-3.006; -2.400)

Table 3. Parameter estimates for potential fecundity-length and carcass and total body weight relationships for cod and haddock (pooled samples from 2006-2008) of Georges Bank. Parameters correspond to the linear model, log (PF) = $a + b \times$ log (length/carcass w and total w).

Species	Variable	Fish size range	*n*	df	P	Explained Variation (%)	*a*	Std. Error (*a*)	*b*	Std. Error (*b*)
Cod	Ln(length)	46-112 cm	29	27	<0.01	76.3	-3.139	1.784	4.047	0.434
	Ln(carcass w)	825-12050 g	29	27	<0.01	83.0	2.720	0.939	1.426	0.124
	Ln(total w)	955-16100 g	29	27	<0.01	80.1	3.418	0.968	1.305	0.126
Haddock	Ln(length)	28-77 cm	89	87	<0.01	91.0	-1.964	0.502	3.857	0.131
	Ln(carcass w)	174-3616 g	89	87	<0.01	93.4	4.301	0.245	1.264	0.036
	Ln(total w)	196-4418 g	89	87	<0.01	94.1	4.293	0.233	1.233	0.033

Table 4. Parameter estimates for potential fecundity (PF) – body size relationships for cod and haddock collected from NAFO Subdivisions of the Scotian Shelf and Georges Bank.

Species	NAFO Subdivision	Year	Source	Model	*n*	*a*	*b*	r^2
Cod	4V,4W	1998-2000	McIntyre and Hutchings,2003	PF = a + b x length	29	– 466525	16517	0.28
	4V,4W	1998-2000		PF = a + b x total weight	29	240.86	83046	0.25
	5Z	1999-2000		$PF = e^{(a + b \times length)}$	96	10.03	0.052	0.75
	5Z	1999-2000		PF = a + b x total weight	96	– 153199	336.03	0.80
Haddock	4X,4V,4W	1978-1980	Clay, 1989	Ln(PF) = a + b x Ln(length)	44	962.64	1.64	0.26
	4X,4V,4W	1978-1980		PF = a + b x total weight	44	3.07E+05	0.22	0.33
	4V,4W	1997-1999	Blanchard et al. 2003	$PF = a \times (fork\ length)^b$	405	0.4441034	3.395312	0.32
	4V,4W	1997-1999		$PF = a \times (total\ weight)^b$	401	4965698	1.210043	0.42
	4X	1983-1986	Waiwood and Buzeta, 1989	Ln(PF) = a + b x Ln(length)	405	0.3456	3.1225	0.74
	4X	1983-1986		Ln(PF) = a + b x Ln(total weight)	378	2.38989	1.0452	0.78
	4X	1997-1999	Trippel and Neil, 2004	$PF = a \times (fork\ length)^b$	22	7.54E-06	6.241	0.49
	4X	1997-1999		$PF = a \times (total\ weight)^b$	22	134.913	1.621	0.57
	5Z	1972-1973	Lough et al. 2008	$PF = a \times (fork\ length)^b$	121	3.19	3.15	0.79

Table 5. Multiple linear regression models developed to predict potential fecundity (PF) for cod and haddock of Georges Bank. HSI: hepatosomatic index; K: Fulton's condition facto; AIC: Akaike's Information Criterion.

Species		Model	*n*	df	Explained Variation (%)	AIC	*P*
Cod	1	Ln(PF)=logan(length)	29	27	76.3	46.32	<0.01
	2	Ln(PF)=Ln(length)+HSI	29	26	80.7	42.38	<0.01
	3	Ln(PF)Ln(length)+K	29	26	83.2	38.34	<0.01
	4	Ln(PF)Ln(length)+HSI+K	29	25	86.7	33.67	<0.01
Haddock	1	Ln(PF)Ln(length)	89	87	91.0	46.71	<0.01
	2	Ln(PF)Ln(length)+HSI	89	86	93.4	35.97	<0.01
	3	Ln(PF)Ln(length)+K	89	86	92.2	21.11	<0.01
	4	Ln(PF)Ln(length)+HSI+K	89	85	94.3	10.221	<0.01

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575 **List of Figure Captions**

576 **Figure 1.** Northwest Atlantic Fisheries Organization (NAFO) Subdivisions located
577 along the Scotian Shelf and Georges Bank.

578 **Figure 2**. Vitellogenic oocyte size distributions for an individual cod and haddock from
579 Georges Bank in prespawning condition collected in 2007 (~0.05 g subsample).

580 **Figure 3**. Relationships and fitted curves between oocyte density (number of oocytes
581 per gram of ovary) and oocyte diameter for cod and haddock of Georges Bank (refer to
582 Table 2 for equations).

583 **Figure 4.** Fitted curves of potential fecundity (number of vitellogenic oocytes) - length
584 and weight relationships for cod and haddock of Georges Bank

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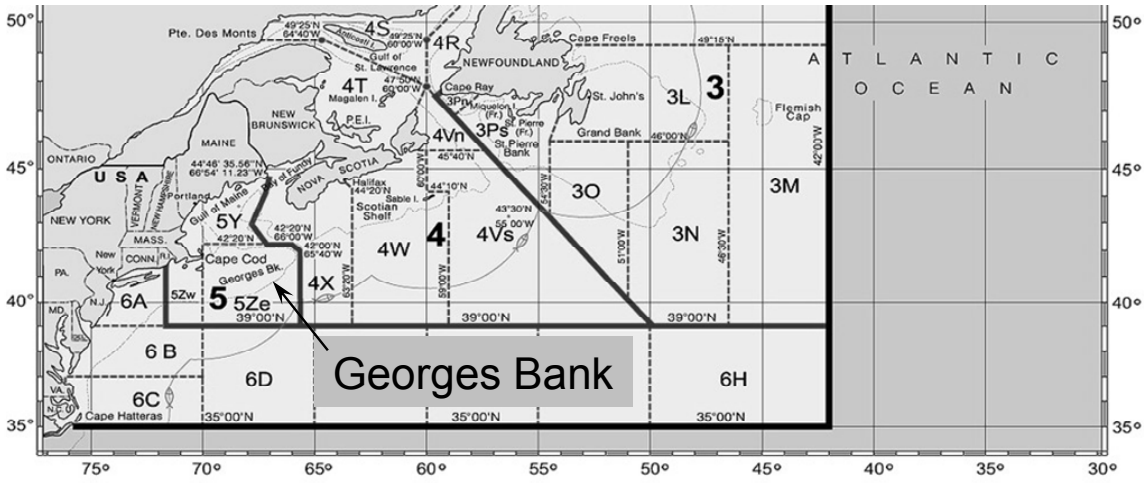

Cod

Haddock

Frequency

0
20
40
60
80
100

0
20
40
60
80
100

200
300
400
500
600
700
800
900
1000

Oocyte diameter, µm