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**Working Paper 2014/33**

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# **Spatial and annual variation in fecundity of yellowtail flounder in U.S. waters**

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## Abstract

We applied a semiautomated image analysis method to estimate potential annual fecundity (PAF) over 3 years (2010-2012) for individuals from the 3 U.S. stocks, and additional samples from 2013 for Georges Bank (GB) are included. Variation in fecundity was modeled by stock area, sampling year, fish size, age, relative condition, and mean oocyte diameter. Fish length and condition explained about 70% of the variation in fecundity, and inclusion of mean oocyte diameter (as a proxy for time to spawning) accounted for some of the seasonal down-regulation of fecundity. Within a given year, females collected in the Gulf of Maine (GOM) typically had lower fecundity at length than females collected from the southern New England (SNE) stock. Interannual differences were evident: fecundity was high in 2010 for both these stocks, low in 2012 for both, but 2011 showed no synchronization between these two stocks. The sample size of females from GB was low, but the limited results suggest that patterns in PAF and condition were intermediate to SNE and GOM stocks and more similar to the GOM stock in most years. For most females sampled from GB in 2011, both fecundity at size and relative condition were among the lowest values observed from all stocks and years. Lower condition (at the time sampled) leads to lower fecundity, therefore given the variable and poor condition observed for females from GB, condition may have a greater impact on fecundity for this stock. Previous studies and results herein suggest a latitudinal gradient, of decreasing fecundity with increasing latitude.

## Introduction

Measuring fecundity, and the factors that influence it, is a necessary first step to estimating total egg production of fish stocks, which can improve our understanding of stock-recruitment dynamics; however, these data are limited for many species in the northwest Atlantic (reviewed in Tomkiewicz et al., 2003). A few studies have estimated fecundity in yellowtail flounder, *Limanda ferruginea* (Pitt, 1971; Howell and Kesler, 1977; Rideout and Morgan, 2007), but these investigations differed in methodologies, geographic locales (with a single study on only one of the three U.S. stocks), and years. Given the paucity of estimates of fecundity for yellowtail flounder; there remains a need for data across all three U.S. stock areas. Furthermore, fecundity can vary temporally, thus requiring multiple years of data to try to understand inter-annual dynamics and help bound uncertainty in annual reproductive potential. This working paper addresses this reproductive data need.

In this study, we evaluated the size-specific PAF of individual yellowtail flounder and generated estimates of Potential Annual Fecundity (PAF) for individuals from the three stocks of yellowtail flounder in United States' waters (Gulf of Maine [GOM], Georges Bank [GB], and Mid-Atlantic-Southern New England [SNE]) across multiple years. The latter stock is referred to as just SNE as that is the only subregion from which samples were acquired.

## Methods/Results

### Fish collection and processing

Yellowtail flounder were sampled from January 2010 through May 2012 during the winter and spring months ( $n = 385$ ). Additional data for fish collected in April 2013 from Georges Bank have been processed and are included ( $n = 37$ ). Fish were collected primarily by commercial fishing vessels participating in the Northeast Fisheries Science Center, Northeast Cooperative Research Program's (NEFSC-NCRP) Study Fleet ( $n = 357$

females). Supplemental samples were acquired from other NEFSC-NCRP research studies ( $n = 44$ ), as well as from the Massachusetts Division of Marine Fisheries trawl survey ( $n = 21$ ). Fish were obtained from all three stock areas in United States' waters. To ensure a high quality of the tissues, fish were requested from the last day of a fishing trip, iced during transport, and processed upon arrival at the laboratory. Fish total length (TL, mm), body mass ( $\pm 0.1$  g), and ovary mass ( $\pm 0.001$ g) were measured, and an approximately 1 cm<sup>3</sup> piece of tissue from the middle of the right ovarian lobe was fixed in 10% neutral-buffered formalin. A few fish were sampled while at sea ( $\pm 0.001$  kg,  $n = 21$ ). Scales were removed and subsequent fish age determination followed methods of Penttila and Dery (1988).

Relative condition ( $K_n$ ) was calculated as the ratio of the observed weight over the predicted weight (Le Cren, 1951) using an overall length-weight equation determined from all females sampled for fecundity. This was calculated using least-squares regression of log-transformed TL and ovary-free body mass. Ovary-free mass was used to examine changes in condition independent of ovarian development. Differences in  $K_n$  among stocks and years were tested by ANOVA with a Tukey HSD post-hoc test if overall significant differences were found.

#### Fecundity sample processing

The fixed ovarian tissue was processed with standard histological methods, and stained with Schiffs-Mallory trichrome. The most advanced oocyte stage (MAOS), presence of postovulatory follicles, and occurrence and stage of atresia were assessed for each histology section. The histology scheme was adapted from previous studies of this species (Howell, 1983; Zamarro, 1991). Fecundity analysis included only females with a MAOS in late vitellogenesis or germinal vesicle migration. Females were excluded if there were signs that spawning had commenced (germinal vesicle breakdown, hydrated oocytes, or postovulatory follicles) or if there were any indications of significant cell damage.

Fecundity estimation followed a semi-automated image analysis protocol described by McElroy et al. (2013). An autodiometric curve for yellowtail flounder was developed using a subset of females ( $n = 178$ ), and this equation was then used to estimate oocyte density for an additional 244 females. To ensure individual fish were at an appropriate development stage for fecundity analysis, the size distribution of measured oocytes for each fish was assessed with the following two criteria to remove individuals too early in development. The minimum oocyte diameter rule verified the vitellogenic cohort of oocytes for the imminent spawning season had achieved a clear hiatus from the reservoir of smaller primary growth oocytes for future years spawning. Specifically, fish with  $> 5\%$  of the measured vitellogenic oocytes smaller than 250  $\mu\text{m}$  OD were excluded. The second threshold confirmed the leading cohort (largest 10% of oocytes) had reached a minimum size threshold of development within the entire vitellogenic cohort. Specifically, individuals with a leading cohort  $< 375 \mu\text{m}$  were considered too early in development and excluded from further analysis.

When scaling up the oocyte density (oocytes/g; NG) of the subsample to estimate the fecundity of a whole fish we accounted for the mass of the tunica (gonad wall), which contributes to the whole ovary mass ( $M_o$ ). A correction factor for the weight of the tunica was determined from 57 females whose whole, wet ovary was weighed and stripped of all oocytes, and the remaining tunica tissue weighed. The tunica mass expressed as

a percentage of whole  $M_0$  (4.721 %; SE = 0.169) was used to adjust  $M_0$  for calculation of PAF as follows:  
 $PAF = NG (0.9528 M_0)$ .

### Fecundity modeling

We related PAF to fish size (TL) or age (FA) by:  $\ln(PAF) = b \ln(TL \text{ or } FA) + a$ , where  $a$  and  $b$  are coefficients determined by least-squares fit regression. Additionally, model selection evaluated the addition of stock and year as factors, and relative condition ( $K_n$ ) and mean oocyte diameter as continuous variables. Models including stock:year and stock:age interactions were also tested. Model selection was based on AICc criteria (Anderson, 2008), and the coefficient of determination ( $r^2$ ) was also calculated.

The range of fish length and age varied among years and stocks. Therefore, regression of PAF estimates was limited to a range of values that overlapped for females sampled from the SNE and GOM stocks (328 – 450 mm TL [  $n = 321$ ] and 3 – 6 yr [  $n = 322$ ]). Samples from the GB stock were excluded from most analyses due to low sample size and a narrow length range. The form of the final reported PAF regressions was determined based on the model testing, and slopes of the final regressions were tested against an isometric slope ( $b = 3$  for length,  $b = 1$  for age) using a Wald test.

### Fecundity results (all stocks)

The AICc model comparisons for the overlapping length ranges for GOM and SNE females indicated that the best model included all main effects (TL,  $K_n$ , stock, mean oocyte diameter, year) and a stock-year interaction relative to all the simpler models ( $\Delta AICc = 10.18$ ). The best one parameter fecundity model was with TL. Length and stock alone explained the greatest amount of the variation in PAF ( $r^2 = 0.70$ ), and the stock and stock-year interactions only explained an additional 2% of the variation in PAF. Models for fish age had similar results, although the fit to the data was weaker. Therefore, final stock-specific regressions include terms for length or age along with mean oocyte diameter and relative condition (Table 1). The inclusion of the mean oocyte diameter term in PAF models accounts for down-regulation and individual differences in the progression of clutch development, and it improved the explanatory power of the PAF model.

In terms of stock, PAF at length was higher for females from SNE than from the GOM within all years, but comparison across years indicated the range of PAF models overlaps between the stocks (Fig. 1) and was also evident in the range of the individual data points (Fig. 2). This was particularly the case in 2010, the year with the highest PAF estimates for GOM females, which were comparable with the estimates in the lowest year for SNE females, 2012. Synchrony across stocks was evident in two of the years, with both stocks at the higher end of their observed range in 2010 and at the lower end in 2012. However, model estimates for 2011 varied between the two stocks. Age was a weaker predictor than length (Table 1), and variation in fecundity at age may be partially attributed to the observed differences in size at age among years and stocks (Fig 2c).

Analysis of the regression slopes, when tested against isometric null slopes, indicated a greater contribution to egg production by large females than expected based on their size alone (Table 1). The earlier yellowtail fecundity studies by Pitt (1971) and Howell and Kesler (1977) included larger fish up to 54 cm TL and had higher slopes than the current study ( $b = 4.69$  and  $3.86$ , respectively).

Relative condition varied significantly among stocks and years overall (Fig. 3;  $F = 10.23$ ,  $n = 347$ ,  $p < 0.01$ ), and contributed to explaining the individual variation in both the age and length PAF models. Only two stock-year combinations were found to be significantly different in post-hoc testing (Tukey HSD,  $p < 0.05$ ),

excluding GB samples from this analysis. The  $K_n$  of females in SNE in 2011 was significantly greater than GOM in all years, and  $K_n$  was significantly lower for GOM females in 2011 than all other stock-year combinations. In 2011 the lower PAF of GOM samples was consistent with the generally low condition of most of the females. However, condition and fecundity patterns were not always directly related. Condition, as measured here, only represents a point estimate of a continuous annual pattern and does not account for periods of low or high condition that may have occurred during other portions of the year. Therefore, fish condition could contribute to fecundity to a greater extent than was explained by this single seasonal estimate.

The sample size of fish from GB was too low to include in the model analysis, but the patterns in the available data suggest that fecundity of this stock was intermediate to the other two U.S. stocks or closer to the lower values observed in GOM during 2010-2012. The low fecundity estimates for GB fish in 2011 were consistent with the very low values of relative condition observed in 2011 (Fig. 3), and the lower condition and fecundity also observed in GOM females that year.

#### Results with additional Georges Bank samples (2013)

Samples from 2013 for only Georges Bank individuals have been processed. This doubled the sample size for the stock ( $n = 74$ ). Preliminary results for the four years of data for this stock are presented.

The PAF for this stock still exhibited high variability (Fig. 4), but much of this comes from the low fecundity at size estimates for most individuals in 2011. PAF at length for 2013 females from GB was comparable to the samples collected in 2010 and 2012. Relative condition in 2013 was similar to or higher for GB individuals from earlier years (Fig. 5).

The range of condition observed for GB females was broader than the other two U.S. stock regions, and PAF increased at a faster rate with increasing  $K_n$  than in the models from the other two stocks (Table 1). Predicted fecundity at length was determined for all GB individuals using a model including just mean oocyte diameter and log transformed TL (Fig. 6). The standardized residuals for fecundity at length had a significant positive relationship with  $K_n$  for all 4 years of data combined (slope = 2.7095,  $r^2 = 0.32$ ,  $n = 74$ ,  $p < 0.01$ ).

While estimates of fish condition based on ovary-free fish mass provide independence from reproductive investment, this is rarely available in most fisheries dependent and independent datasets. Therefore, for application of a GB fecundity model to other fisheries data, a model was also determined with a relative condition parameter determined from fish total mass and with Fulton's K (Table 2). Fulton's K is not dependent on the length-weight regression used, as with  $K_n$ , but it assumes an isometric slope ( $b = 3$ ).

#### Comparison to previous studies

Our fecundity estimates are generally higher at length than previous reports for yellowtail flounder in the northwestern Atlantic Ocean (Table 5), particularly for SNE individuals. There was some overlap in estimates between our 2011 GOM samples and the earlier SNE study (Howell & Kesler, 1977). Pitt (1971) in the 1960's and more recently, Rideout and Morgan (2007), reported lower yellowtail PAF for Grand Bank yellowtail than observed in U.S. waters. This later study also found lower fecundity for females on the eastern side (3LNO NAFO region) of the Grand Bank than the western portion (3Ps).

Results from the present study suggest that a latitudinal trend in fecundity exists for yellowtail flounder. Fecundity at length estimates for GOM females are intermediate to reported estimates for Grand Bank (Pitt, 1971) and SNE individuals, further supporting a latitudinal trend. Evidence for this latitudinal trend in yellowtail flounder fecundity was first identified by Howell and Kesler (1977, sampled in SNE) through comparison to Grand Bank fish sampled by Pitt (1971). Comparisons in fecundity estimates across studies are complicated by temporal separation in sampling and differences in methodology. However, results within the current study, combined with the earlier SNE study, and the multiple Grand Bank studies collectively provide support for decreasing fecundity with increasing latitude.

## Discussion

Fish size and condition are the factors that explain the most variation in fecundity (~70%), and these account for most of the observed differences in fecundity among the U.S. stocks and years studied.

Environmental conditions influence condition and therefore the amount of energy available for growth, and fecundity. Due to inherent trade-offs between growth and reproduction, these traits are dynamic and not always direct linear responses to environmental gradients. Fecundity and condition could theoretically covary in response to similar factors, but it is probable that the energetic condition of individuals has some direct impact on the surplus energy available for egg production.

The positive allometry observed indicates the value of large females, whose egg production is greater than expected for their size alone, and especially when a broader range of female sizes is considered than that recently observed in the GB population.

The analysis presented here focused on egg numbers alone; studies on other species have indicated that egg size and quality can also vary with female size, with implications for larval size and survival. The only investigation we are aware of on egg quality for yellowtail flounder found no relationship with female size, but was limited by small sample sizes ( $n = 10-11$  fish per year), a limited size range, and high variation among individuals (Manning and Crim, 1998). This issue warrants further investigation for this species.

Both condition and fecundity of most yellowtail flounder sampled from GB in 2011 were among the lowest values observed in all stocks and years. The three other years were relatively similar. Low condition and fecundity was also observed for GOM females in 2011.

The stronger positive relationship between condition and fecundity and wider range in condition observed for GB females than for individuals from the other two U.S. stocks resulted in more variable fecundity for GB fish, in the years studied. The observed lower condition found here for GB fish in 2011 was consistent with weight at age estimates from the larger trawl survey dataset, and condition in recent years has remained below the long term average (TRAC 2013).

Results of the present study and comparison with previous work both suggest a trend of decreasing fecundity with increasing latitude.

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Table 1. Stock-specific regression coefficients presented for the natural log-transformed linear regressions of potential annual fecundity of yellowtail flounder relative to mean oocyte diameter (OD) and either total length (TL, Equation 7) or fish age (FA, Equation 8) with standard errors (in parentheses) for the intercept,  $a$  and the slopes:  $b$ ,  $c$ , and  $d$  for samples collected in 2010-2012. The relative condition ( $K_n$ ) parameter was determined for ovary-free fish mass. Wald  $t$ -tests were for an isometric slope of  $b = 3$  for TL and  $b = 1$  for FA. Regressions were determined over full range of FA or TL except rare lengths (TL < 300 mm or TL > 500 mm) and ages (FA = 2) were excluded ( $n = 7$ ).

TL	Stock	$a$	$b$ (TL)	$c$ (OD)	$d$ ( $K_n$ )	$r^2$	$n$	TL range	$t$ ( $b=3$ )	$p$ ( $b=3$ )
	GOM	-9.0950 (1.2212)	3.8938 (0.2059)	-0.0025 (0.0004)	1.1697 (0.1991)	0.66	204	313 - 459	4.34	< 0.01
	GB	0.9458 (7.7246)	1.8699 (1.2468)	-0.0004 (0.0011)	2.2474 (0.6487)	0.27	37	340 - 430	-0.91	0.37
	SNE	-7.9751 (1.1352)	3.6975 (0.1783)	-0.0014 (0.0004)	0.9780 (0.1910)	0.78	136	315 - 465	3.91	< 0.01
FA	Stock	$a$	$b$ (FA)	$c$ (OD)	$d$ ( $K_n$ )	$r^2$	$n$	FA range	$t$ ( $b=1$ )	$p$ ( $b=1$ )
	GOM	12.8914 (0.4394)	0.4827 (0.1165)	-0.0015 (0.0006)	1.1402 (0.3281)	0.14	196	3 - 7	-4.44	< 0.01
	GB	11.4169 (0.8466)	0.6414 (0.3022)	-0.0003 (0.0011)	1.9425 (0.5572)	0.31	37	3 - 6	-1.19	0.24
	SNE	13.3675 (0.4257)	0.9104 (0.0772)	-0.0018 (0.0006)	0.5792 (0.2773)	0.54	133	3 - 8	-1.16	0.25

Table 2. Model coefficients for Georges Bank yellowtail were determined for total length-fecundity regressions from four years (2010-2013) of data with parameters for mean oocyte diameter (OD) and relative condition ( $K_n$ ). Relative condition was calculated with length and mass data from all stocks, but fish total mass was used to produce regressions applicable to other datasets lacking ovary mass data. Fecundity model parameters were also determined using Fulton's K ( $K$ ) to facilitate comparison to datasets with a different length-weight relationship.

	$a$	$b$ (TL)	$c$ (OD)	$d$ ( $K$ )	$r^2$	$n$	TL range
$K_n$	-5.0344 (2.8152)	2.9640 (0.4595)	-0.0024 (0.0006)	2.6042 (0.2253)	0.69	74	336 - 448
$K$	-3.3817 (2.7746)	2.6875 (0.4543)	-0.0024 (0.0006)	2.8513 (0.2466)	0.69	74	336 - 448

Table 3. Potential annual fecundity estimates were calculated for previous studies using reported regressions for fish of two total lengths within the size range of all studies. Rideout and Morgan (2007) estimates are presented separately for both Grand Bank regions reported. Estimates for the current study were calculated for each stock and year independently, except all years combined for Georges Bank (GB), and using an oocyte diameter of 450  $\mu\text{m}$  and relative condition of 1 ('average' condition).

Study	Location	Sampling year (s)	<i>n</i>	Fecundity (millions)	
				370 mm	420 mm
Pitt (1971)	Grand Bank	1966 - 1967	51	0.80	1.46
Rideout & Morgan (2007)	Grand Bank (3LNO)	1993 - 1998	444	0.73	1.06
	Grand Bank (3Ps)	1993 - 1998	102	0.92	1.36
Howell & Kesler (1977)	SNE	1976	64	1.11	1.80
Current Study	GOM	2010	57	1.42	2.30
	GOM	2011	62	1.09	1.72
	GOM	2012	85	1.13	1.69
	GB	2010 - 2013	74	1.23	1.79
	SNE	2010	57	1.59	2.51
	SNE	2011	47	1.60	2.50
	SNE	2012	33	1.47	2.32

Fig. 1. Potential annual fecundity (PAF) model estimates at age (a) and length (b) were determined for yellowtail flounder sampled in each year (2010-2012) from the SNE and GOM stocks. Estimates were calculated from linear regressions determined over a range of ages (3 - 6 yr,  $n = 323$ ) and total lengths (328 - 450 mm,  $n = 322$ ) sampled from both stocks in all years (both age and length on a log scale). Constant values were used for the mean oocyte diameter ( $OD = 450 \mu\text{m}$ ) and relative condition ( $K_n = 1$ ) terms for all. Predicted length at age was plotted for each stock-year combination as determined from the same data subset using least-squares linear regression (c).

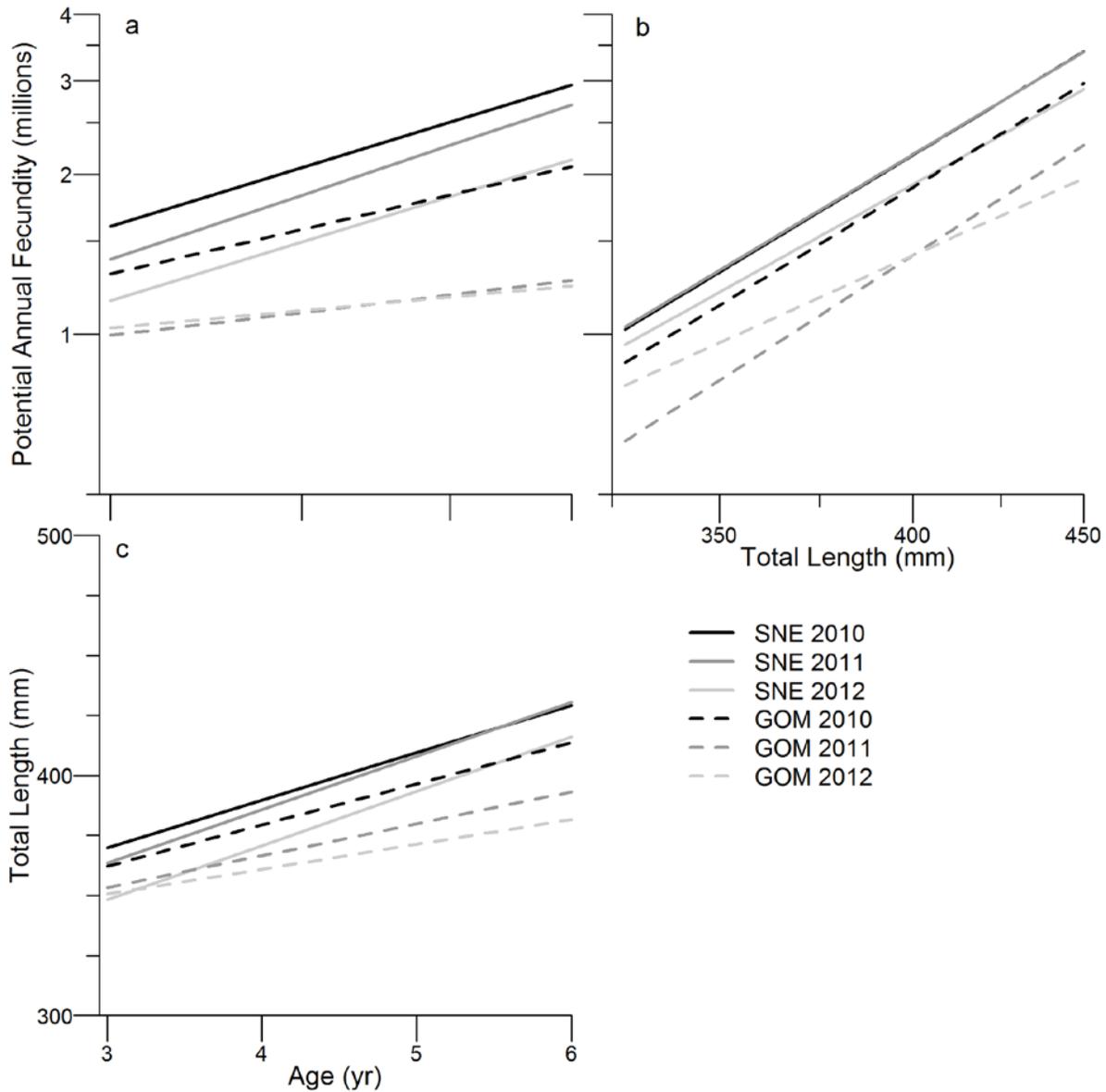


Fig. 2. Yellowtail flounder potential annual fecundity (PAF) data was plotted relative to total length (TL, left column) and fish age (FA, right column) by year and stock on a log-log scale. Lines are predicted PAF at length or age from linear regressions (Table 1) with constant values used for the mean oocyte diameter ( $OD = 450 \mu\text{m}$ ) and relative condition ( $K_n = 1$ ) terms. Georges Bank (GB) regression was for all years combined. Ages jittered to reduce over-plotting. Rare lengths ( $TL < 300 \text{ mm}$  or  $TL > 500 \text{ mm}$ ) and ages ( $FA = 2$ ) were excluded from the regressions.

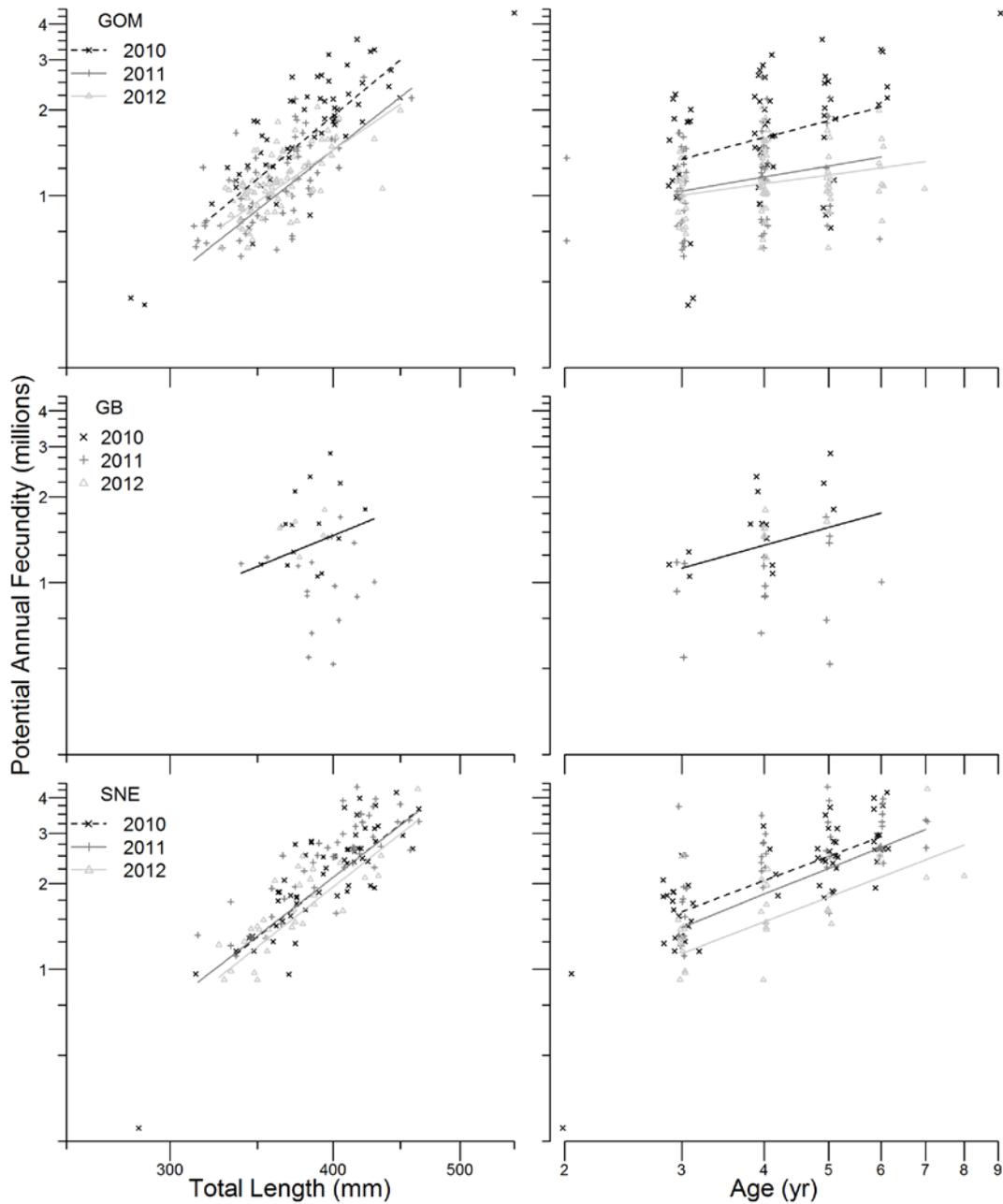


Fig. 3. Relative condition ( $K_n$ ) of female yellowtail flounder was tested among stocks and years, except GB fish were not included in the analysis because of low sample size but are depicted for comparison. Groups with matching letters were not significantly different from each other ( $P > 0.05$ ; Tukey HSD post-hoc test). Sample sizes are shown across the bottom.

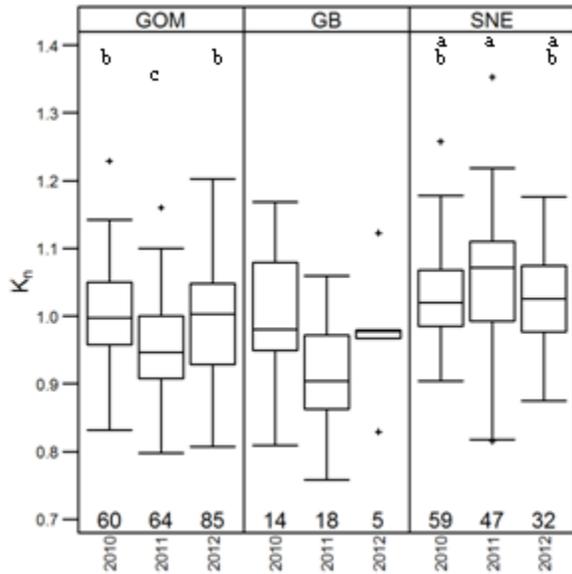


Fig. 4 Yellowtail flounder potential annual fecundity data from fish sampled from Georges Bank (GB) over four years were plotted relative to total length by year on a log-log scale.

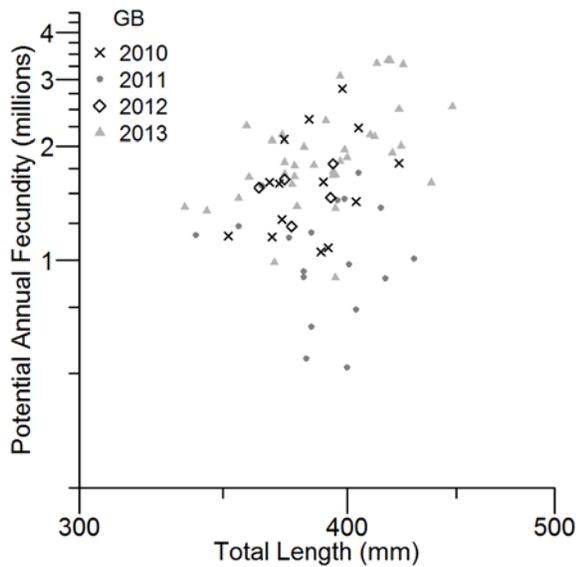


Fig. 5 Relative condition ( $K_n$ ) of Georges Bank female yellowtail flounder sampled for fecundity were plotted by year. Regressions used for length-mass equation were for ovary-free body mass based on samples from females caught in all stock areas and years. Sample sizes are shown across the bottom.

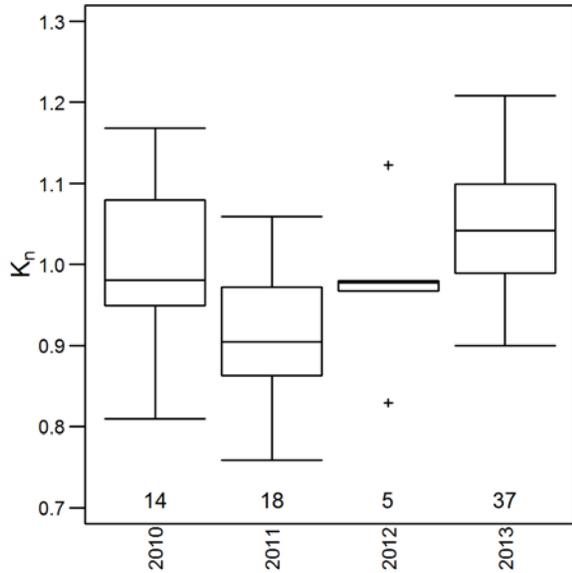


Fig. 6 Standardized residuals for predicted potential annual fecundity of female Georges Bank yellowtail flounder plotted versus relative condition ( $K_n$ ). Predicted fecundity at length values were generated from a stock-specific model that included a mean oocyte diameter term. Line is the least-squares fit linear regression.

