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ARTICLE

Histological Analysis Reveals Larger Size at Maturity for Southern Flounder with Implications for Biological Reference Points

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Abstract

For fish stocks managed using biological reference points based upon spawning biomass, it is critical to have accurate maturity schedules. We investigated size- and age-dependent patterns in maturity for southern flounder *Paralichthys lethostigma*, a flatfish supporting valuable coastal fisheries in North Carolina and throughout its range. We evaluated both macroscopic and histological methods over two consecutive reproductive seasons. Histological analyses revealed that maturity occurred at larger sizes and older ages than previously estimated. Length at 50% maturity (L_{50}) was estimated at 408 mm total length (TL), which was more than 60 mm larger than currently assumed, and was relatively stable between study years. We found that only 44% of age-1 southern flounder were mature compared with an estimated 74% in an earlier study. We suspect that most of the differences in maturity timing of southern flounder between our findings and previous studies stem from macroscopic assignment error. During this study, only 61% of fish staged macroscopically as developing were found to be mature based on histological analysis. Assuming incorrectly that all of these fish were mature would have resulted in an L_{50} of 375 mm TL, which is closer to previous estimates. Analysis of spawning stock biomass per recruit demonstrated that biological reference points (e.g., F_{SPR}) could be affected considerably by shifting maturity schedules, and the effects could be magnified at larger sizes at entry and higher harvest rates. Given the life history strategy of southern flounder and the lack of a developed offshore fishery or sampling program, which combine to prevent access to fish on the spawning grounds, it is probably most judicious to routinely analyze reproductive tissue samples histologically to ensure accurate information on the timing of maturity.

The influence of individual reproductive biology on the productivity and resilience of fish stocks has received much recent attention (Kjesbu 2009; Lowerre-Barbieri et al. 2011a). Basic information on the timing and extent of reproductive output such as spawning seasonality and fecundity can refine estimates of stock production and biological reference points (Marshall 2009). More specifically, an accurate understanding of the timing of maturity is essential for effective fisheries management (King and McFarlane 2003). The size and age at which fish become mature is a critical element of a species' life history (Roff 1982; Dieckmann and Heino 2007), and variation in fish maturity scheduling can directly affect annual and lifetime reproductive output.

Due to resource limitations, many contemporary biological sampling programs still use traditional approaches to assign individual fish to maturity stages based on macroscopic features of the gonads. Unfortunately, macroscopically based maturity assignments often lack agreement with histological assessments of maturity, which can contribute to erroneous conclusions about the status of the stock (Saborido-Rey and Junquera 1998; Tomkiewicz et al. 2003; Costa 2009; Ferreri et al. 2009). For instance, Vitale et al. (2006) revealed that

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the use of macroscopic staging alone overestimated female spawning-stock biomass in Atlantic cod *Gadus morhua* in the Kattegat by up to 35%. These results highlight the need for more routine application of histological analyses (e.g., West 1990) that require more time and expense, but yield the most accurate reproductive information (Hilge 1977; Hunter and Macewicz 1985; Murua and Saborido-Rey 2003).

Southern flounder *Paralichthys lethostigma* occur in estuarine and marine waters of the U.S. southern Atlantic Ocean and the Gulf of Mexico, supporting valuable commercial and recreational fisheries throughout their range (Gilbert 1986). Southern flounder are spawned offshore and settle in shallow estuarine and freshwater habitats during late winter and early spring (Wenner et al. 1990; Lowe et al. 2011). Juveniles grow quickly (0.35–1.5 mm/d; Fitzhugh et al. 1996) and some individuals may recruit to commercial and recreational fisheries before reaching age 1. Southern flounder are sexually dimorphic, and females attain larger sizes and contribute disproportionately to fishery landings. Immature southern flounder remain in estuarine habitats for about 2 years or until they mature, at which time they migrate offshore to spawn. The prevailing view maintains that, after spawning, southern flounder return to estuaries, although the fraction of fish returning and the extent of nearshore habitat use have recently been questioned (Wenner et al. 1990; Watterson and Alexander 2004; Taylor et al. 2008).

In North Carolina, southern flounder has been the most valuable finfish resource for much of the past two decades (North Carolina Division of Marine Fisheries [NCDMF] commercial harvest statistics 1991–2010; http://portal.ncdenr.org/web/mf/marine-fisheries-catch-statistics). Currently, the North Carolina stock is considered to be "depleted" due to a long period of elevated harvest rates beginning in the early 1990s. The initial fishery management plan (NCDMF 2005) established several new harvest restrictions in an attempt to lessen the impact on the stock by reducing fishing mortality rates. However, recent findings indicate that fishing mortality rates may still be higher than management targets, at least in the river-based gill-net segment of the fishery (Smith et al. 2009). Indeed, the most recent stock assessment determined that the stock remains both overfished (spawning-stock biomass [SSB] below target) and is undergoing overfishing (fishing mortality [F] above target) (Takade-Heumacher and Batsavage 2009). Observations by Smith and Scharf (2010) also revealed that the commercial gill-net harvest comprised mostly young (primarily age 1) fish, many of which were probably immature.

Size- and age-based maturity schedules for female southern flounder were first estimated for North Carolina fish captured during the mid-1990s by Monaghan and Armstrong (2000). Their findings resulted in an estimated L_{50} (length at which 50% of females are mature) of 345 mm, and that 18.1% and 73.5% of age-0 and age-1 fish, respectively, were mature. However, all but 2 of 19 running-ripe females collected in ocean waters were greater than 414 mm, and age-1 females failed to demonstrate a defined peak in their gonadosomatic index (GSI) during late fall, which was observed for age-2 and older fish (Monaghan and Armstrong 2000). More recent studies have provided new evidence suggesting that maturation of southern flounder may occur at larger sizes and older ages. Smith and Scharf (2010) examined a small number ($n = 31$) of southern flounder gonads histologically and estimated that only 56.6% of age-1 females were mature, with no observations of mature age-0 fish. Taylor et al. (2008) found that many southern flounder demonstrated chemical signatures in their otoliths consistent with estuarine residency until just before the deposition of the third annulus (i.e., age-2 fish about to turn age 3), suggesting delayed participation in offshore spawning.

Here, we used histology to estimate the size and age at maturity for southern flounder from estuarine waters throughout North Carolina. We compared histologically validated maturity estimates to those generated using only existing macroscopic criteria, and we examined seasonal and size-dependent patterns in GSI as an indicator of maturity. Last, we explored the impact of variable maturity schedules on estimates of southern flounder spawning stock biomass and management reference points.

METHODS

Fish collection.—Adult female southern flounder were collected between September 2009 and December 2010, allowing maturity schedules to be estimated during two consecutive reproductive seasons. Fish were collected throughout estuarine waters of North Carolina (Figure 1) in three ways: from seafood dealer sampling, from directed trips with commercial

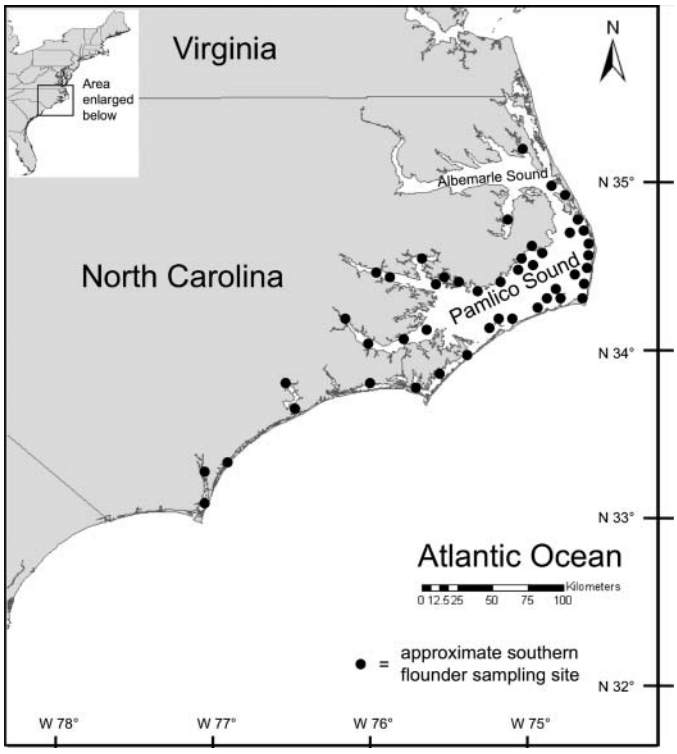

FIGURE 1. Study location in coastal North Carolina with an inset to indicate position relative to the U.S. east coast. Approximate locations where southern flounder were collected for this study are indicated by filled symbols.

TABLE 1. Stages used to assess maturity in North Carolina female southern flounder. Each stage and the description of its macroscopic features follow guides used by the North Carolina Division of Marine Fisheries. The most advanced oocyte stages are based upon histological examination of female gonad sections and follow Brown-Peterson et al. (2011). CA = cortical alveolar, OM = oocyte maturation, PG = primary growth, POF = postovulatory follicle complex, Vtg1 = primary vitellogenesis, Vtg2 = secondary vitellogenesis, Vtg3 = tertiary vitellogenesis.

Stage	Most advanced oocyte stage	Macroscopic features
Immature	PG	Ovaries small and thin; no oocytes visible.
Developing	CA, Vtg1, Vtg2	Ovaries rotund, yellowish-orange, and turgid.
Fully developed	Vtg3	Same as developing, but with oocytes visible.
Ripe (running)	Vtg3, OM, POF	Ovaries large and soft with many large, free-flowing (with slight pressure), hydrated oocytes.
Spent	POF, few Vtg3	Ovaries small and bloodshot; few, if any, hydrated oocytes.
Resting	PG	Ovaries small, flaccid, and translucent with no visible oocytes.

pound-net and gill-net fishers, and through the NCDMF fishery-independent gill-net sampling program. The NCDMF sampling program fishes multipanel gill nets (7.6–16.5-cm stretch mesh in eight 27.4-m sections) in the estuarine waters throughout the state during February–December (see Takade-Heumacher and Batsavage 2009 for additional programmatic details). All fish captured with commercial fishing partners were also collected from estuarine locations. All fish purchased from seafood dealers exceeded the minimum legal size (356 mm total length [TL]), while scientific collecting permits enabled the retention of some sublegal size fish during directed trips. Fish collected during fishery-independent sampling encompassed a wide range of sizes due to the use of multiple mesh sizes in the gill nets. In each study year, southern flounder were collected during nearly all months through fishery-independent sampling. Seafood dealer sampling and directed trips were conducted during September–December, immediately prior to offshore spawning, in order to reduce bias associated with sampling resting mature individuals before or after the period of peak reproductive activity. Individuals collected outside of this primary sampling window were investigated to confirm the absence of reproductive activity.

Upon capture, fish were placed on ice for no longer than 24 h (usually <2 h) prior to tissue extraction. All fish were measured for TL (mm) and weight (g). Both otoliths were extracted for aging, which was completed using whole otoliths following NCDMF procedures. Whole gonads were removed and staged macroscopically (Table 1), then weighed and stored in 10% neutral-buffered formalin for histological analysis. A subset of individuals was randomly selected (samples were first stratified by prespawn month and broad geographic region) for histological analysis. Sample preparation followed traditional wax paraffin embedding techniques. Briefly, tissues were rinsed in a series of ethanol dilutions and toluene and then placed in liquid paraffin wax. After drying, 5-¯m-thick sections were removed from the embedded samples, mounted on glass slides, and stained with Gill's hematoxylin #2 and eosin-Y. Histological samples were staged based on the most advanced oocyte stage present (Table 1; Figure 2), using the presence of cortical alveolar stage oocytes (or more advanced developmental stages) to indicate maturity within the present season (Murua and Saborido-Rey 2003; Brown-Peterson et al. 2011).

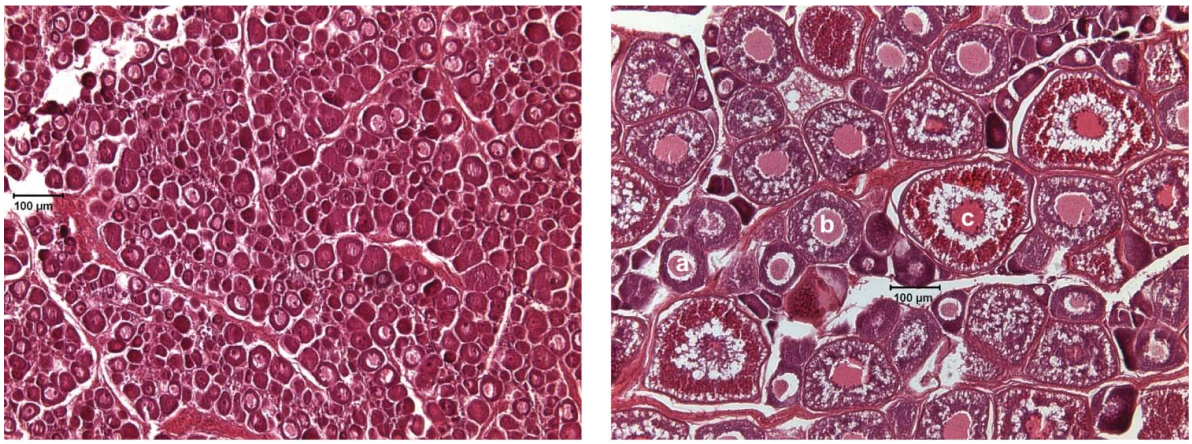

FIGURE 2. Representative histological sections of female southern flounder ovaries for (A) an immature fish exhibiting only primary growth and (B) a maturing fish exhibiting asynchronous oocyte development. a = primary growth oocyte, b = cortical alveolus oocyte, c = vitellogenic oocyte.

TABLE 2. Parameter values, L_{50} estimates, Akaike information criterion (AIC) scores, and relative weights (w_i) for each of the three binary regression models evaluated in the present study. The logistic model received the most support given the data, while the probit model was also plausible. The Hazard model was not well supported.

Model	Equation	Link function	α	β	L_{50}	AIC	ΔAIC	w_i
Logistic	$p_i = \frac{1}{1+e^{-(\alpha+\beta x)}}$	logit	−11.092	0.027	408	487.0	0.0	0.7
Probit	$p_i = \varphi(\alpha + \beta x)$	probit	−6.367	0.016	409	488.6	1.6	0.3
Hazard	$p_i = 1 - e^{\{-e^{(\alpha+\beta x)}\}}$	cloglog	−7.419	0.017	437	496.0	9.0	0

Data analysis.—We initially used three models—logistic, cumulative normal, and Hazard (Table 2)—to estimate the probability of southern flounder maturity as a function of TL. The models were fit using the glm procedure in R with the argument family = binomial and link = "logit," "probit," or "cloglog" depending on the model (R Development Core Team 2009). All models generated estimates for two parameters (α and β) that were used to predict the length at which 50% of individuals were mature ($L_{50} = -\alpha/\beta$) and the standard error for L_{50}, calculated based on the variance of a ratio (Stuart and Ord 1994). Model fit was compared using the Akaike information criterion (AIC; Table 2).

The potential for temporal variation in southern flounder maturity was evaluated through development and comparison of annual maturity schedules. A bootstrap randomization test (Manly 2007) was used where two groups (with 2009 and 2010 sample sizes to represent the different years) were randomly assigned individual measures of TL. An L_{50} was then calculated for each group, and the absolute value of the difference between the two L_{50}s was considered the test statistic. This process was repeated 9,999 times. The actual difference in L_{50} based on years (the 10,000th iteration) was then compared with the distribution of L_{50}s. A *P*-value was then assigned to the actual value based on its location in the distribution of test statistics. Monthly GSI distributions were tested for skewness (D'Agostino K^2 test, $\alpha = 0.05$) and temporal patterns in GSI were analyzed using appropriate parametric or distribution-free methods. We evaluated macroscopic and histological staging disagreement error by calculating the percentage of correct maturity classifications (based on histology) for each macroscopic stage encountered during the study. We also estimated a separate length-based maturity schedule using only macroscopic assignments and compared this schedule with a histology-based schedule.

To examine the effect of variable maturity schedules on estimates of stock reproductive potential and management reference points, we evaluated spawning potential ratio (SPR) as a function of fishing mortality rate (F) and length at entry using both our histology-based maturity schedule as well as the currently assumed maturity schedule that was used in the most recent stock assessment. The SPR is a commonly used measure of reproductive potential and was calculated as the ratio of spawning stock biomass per recruit (SSB/R) at a given level of F to maximum SSB/R (i.e., when $F = 0$), and was expressed as a percentage (Goodyear 1993). We chose to model SPR as a function of length at entry rather than the more common age at entry because southern flounder are managed using minimum size limits and the landings are primarily composed of fish of ages 1–3, which would limit contrast in an age-based model. To calculate SSB/R as a function of length at entry, we modeled natural and fishing mortality of flounder as they grew through 10-mm-TL size-groups. We estimated the time to transition between length groups using von Bertalanffy growth parameters and estimated body weights using a species-specific length–weight relationship (NCDMF 2005). Natural mortality (M) was estimated on a weight-specific basis using the Lorenzen (1996) model. Fishing mortality was set at a value of 0.7534 based on the terminal F estimate included in the most recent stock assessment (Takade-Heumacher and Batsavage 2009). Both M and F were applied to each length-group by multiplying the appropriate transition time (i.e., the time each length-group was exposed to a specific rate of mortality before transitioning to the next length-group). We seeded the model with 1,000 pre-recruit individuals and tracked the number remaining alive in each length-group, N_{tl}, as:

$$N_{tl} = N_{tl-1}e^{-(F+M)},$$

where N_{tl-1} = the number alive in the previous length-group, and F and M are instantaneous fishing mortality and natural mortality, respectively. Biomass remaining was calculated by multiplying the number alive by average weight. Spawning stock biomass was estimated from the length-specific maturity schedule and was divided by the initial number of recruits to estimate SSB/R. We estimated F at each of six different lengths at entry necessary to produce SPR values ranging from 20% to 50% and compared these rates between maturity schedules.

RESULTS

Over two consecutive reproductive seasons, we collected a total of 1,174 southern flounder: 423 fish during 2009 and 751 fish during 2010. During both years, fish were collected from directed trips with commercial fishers ($n = 358$, $\overline{\text{TL}} = 375$ mm, SD = 42 mm), seafood dealers ($n = 227$, $\overline{\text{TL}} = 408$ mm, SD = 50 mm), and from the NCDMF fishery-independent gill-net sampling program ($n = 589$, $\overline{\text{TL}} = 385$ mm, SD = 61 mm). We selected 614 individuals for histological analysis: 309 fish from 2009 and 305 fish from 2010. Total lengths of individuals

sampled for histology were approximately normally distributed ($\overline{TL}$ = 392 mm, SD = 52 mm) and fish ranged in size from 214 to 740 mm TL. We completed histological analysis for 102 age-0 fish, 398 age-1 fish, 95 age-2 fish, and 19 age-3+ fish, which included fish of ages 3–5.

Timing of Maturation

We did not expect to encounter any newly maturing southern flounder during the spring and summer, and this was confirmed through histology. From February through August, a subsample (n = 19) of larger individuals (mean TL = 425 mm, SD = 34 mm) was selected for histological processing and none showed any secondary-growth oocytes or other indicators of maturation, such as elevated GSI. Thus, southern flounder reproductive activity was isolated between September and January. During the 2009 reproductive season, we collected fish from 22 September 2009 to 20 January 2010 and developing individuals were detected throughout the entire period. During the 2010 season, we sampled from 1 September to 22 December 2010. The first evidence of developing fish was detected on 8 September, while maturing individuals were still detected when sampling ended in late December due to poor weather and limited fish availability.

Although we observed female southern flounder with secondary growth oocytes (cortical alveolus or more advanced stage) during September and January (Figure 3), we restricted our estimation of maturity schedules to fish collected during October–December. We did this primarily to minimize any bias associated with incorrectly classifying resting mature individuals as immature. Even histological classification systems can fail to distinguish between mature females whose ovaries are in a resting or regressed state and immature females with inactive ovaries that have yet to attain maturity and participate in spawning activities (Hunter and Macewicz 2003; Lowerre-Barbieri et al. 2011b). Using guidelines presented in Murua and Saborido-Rey (2003) and Brown-Peterson et al. (2011), we assumed the presence of cortical alveolar oocyte stages to indicate that an individual would continue with oocyte development and participate in spawning during the subsequent breeding season. However, because the developmental time necessary for oocytes to advance from cortical alveolus stages to ovulation in southern flounder is unknown, our aim was to restrict our analysis to a time period when evidence of upcoming spawning was most likely. We elected not to include fish collected in September since we encountered several large individuals during this month that did not possess advanced oocyte stages and were probably in a resting state. However, we could not definitively rule out immature status for these individuals. Similarly, although developing fish were still encountered in January, there were relatively fewer mature individuals compared with those sampled in the fall. We interpreted these data to indicate that most fish collected in January were unlikely to emigrate for spawning and instead represented fish that were very likely to overwinter in the estuary. To avoid the possibility of biasing the maturity schedule with a sample of mostly immature fish collected in January, we omitted them from the analysis.

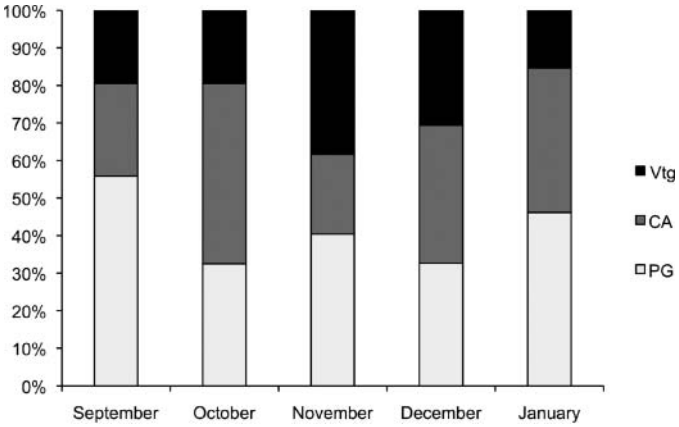

FIGURE 3. The proportion of most advanced oocyte stages (based on histology) encountered among female southern flounder during the reproductive season. Only fish captured during the months (October–December) immediately preceding offshore spawning were included in construction of maturity ogives, although some mature fish were observed in September and January. CA = cortical alveolar, PG = primary growth, Vtg = all vitellogenic oocyte stages.

Gonadosomatic Index

Gonadosomatic index data collected during February–September were each normally distributed and nearly all GSI values were less than 1%. During October–January, GSI data were positively skewed (D'Agostino K^2 test; $\alpha = 0.05$) with mean GSI still below 1%, but included several observations of GSI above 1–2%. Commonly applied transformations did not eliminate skewness during these months, which prevented parametric testing for differences among months (Figure 4).

Size and Age at Maturity

Based on 451 fish captured during October–December of 2009 and 2010, we calculated an overall L_{50} of 408 mm TL (SE = 65.6 mm) using the logistic model. The Hazard model was not supported and, although the probit model was also well supported, the logistic model is applied more conventionally and allows for comparison with other studies. We refer only to logistic model estimates hereafter. Estimates of L_{50} differed by about 17 mm TL between years (Figure 5), but the bootstrap randomization test indicated that this difference was not statistically significant ($P = 0.064$). The proportion of mature female southern flounder at ages 0, 1, and 2 was estimated to be 3%, 44%, and 76%, respectively (Figure 6).

Macroscopic versus Microscopic Maturity Classification

Although several biologists and technicians participated in southern flounder collections and macroscopic gonad staging, all staging personnel used the same NCDMF macroscopic classification guide (Table 1). In total, 427 ovaries collected during October–December of both years were staged using macroscopic features (Table 3; note that sample sizes were different

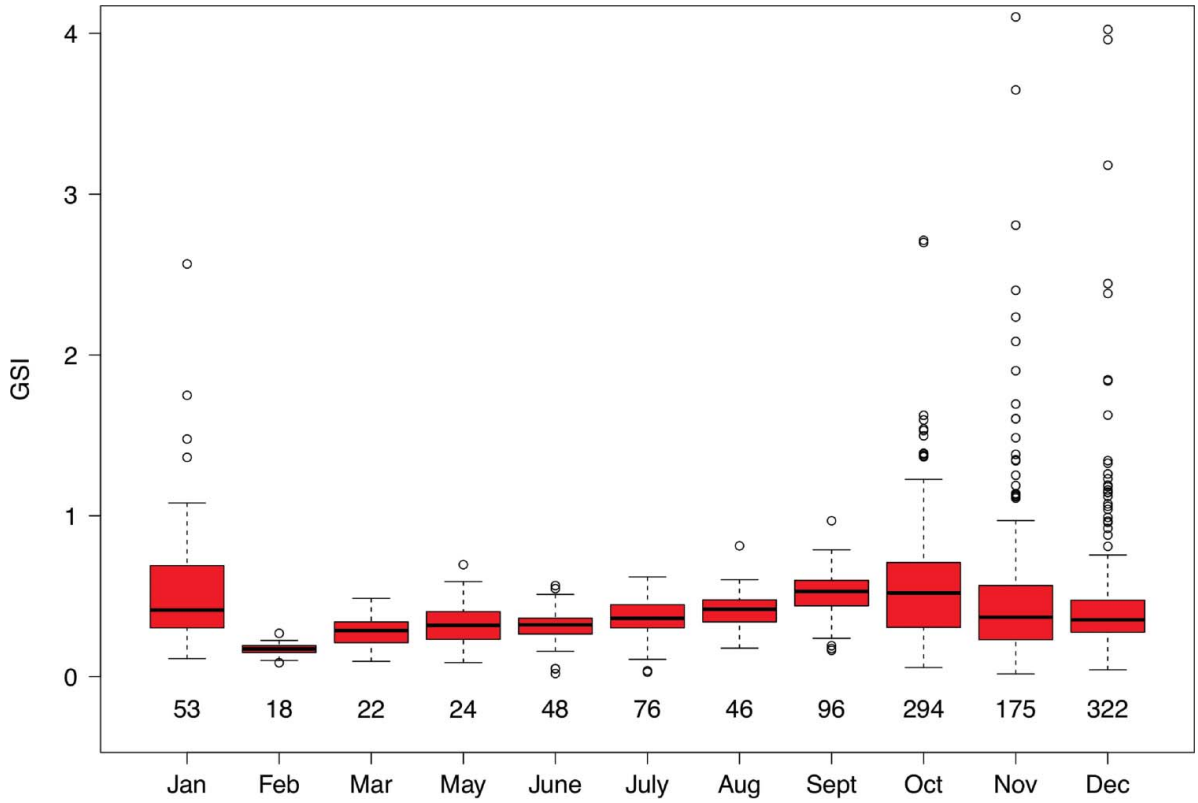

FIGURE 4. Box-and-whisker plot of gonadosomatic indices (GSI) by month for female southern flounder. October–January data were significantly skewed (D'Agostino K^2 test; $\alpha = 0.05$). Monthly sample sizes are reported below the corresponding box-and-whisker plot. No fish were collected in April.

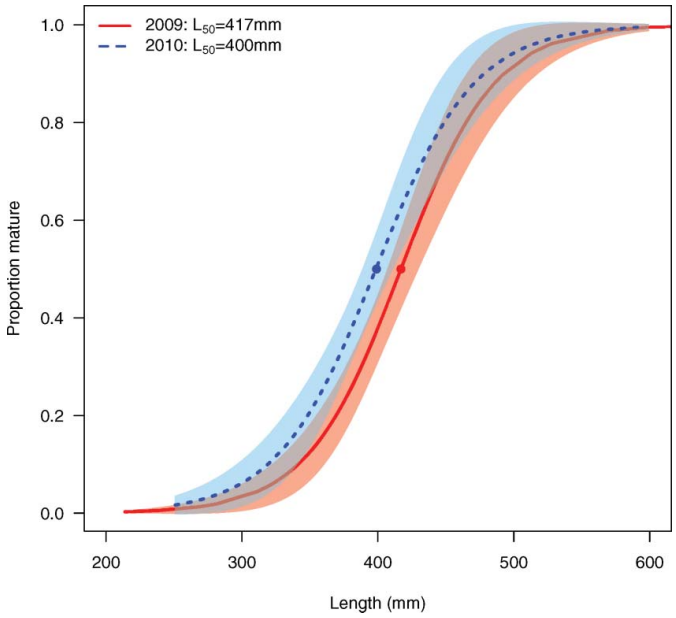

FIGURE 5. Maturity ogives ($\pm$95% confidence intervals) and estimates of L_{50} for North Carolina southern flounder during both years of this study. Dashed lines represent the 95% confidence interval for each estimate. The difference in the L_{50} estimates between years was not statistically significant (see text). For both years combined the estimate of L_{50} was 408 mm TL.

because among the 451 ovaries analyzed histologically, 24 of these were not staged macroscopically). We did not expect to encounter resting, spent, or running-ripe fish during this time period; however, 44 specimens were characterized as resting

TABLE 3. Classification distribution (%) for female southern flounder captured during October–December. Within each macroscopic stage, the fraction of fish assigned to each histological stage (based on most advanced oocyte stage detected) is presented. All macroscopic stages, except immature, are assumed to represent mature fish. For immature, developing, and fully developed macroscopic stages, entries in bold italics indicate fractions of fish that were assigned a correct (either mature or immature) histological maturity designation. Running-ripe, spent, and resting stages were not expected to be encountered in estuarine waters during the fall and thus were not assessed for agreement with histological staging. Histological abbreviations follow those presented in Table 1 with Vtg indicating any of the three vitellogenic stages (Vtg1, Vtg2, or Vtg3).

		Histological stage		
Macroscopic stage	*n*	PG (%)	CA (%)	Vtg (%)
Immature	187	***88.8***	10.2	1.1
Developing (mature)	148	38.5	***45.9***	***15.5***
Fully developed (mature)	44	9.1	***29.5***	***61.4***
Ripe (running)	1	0.0	0.0	100.0
Spent	4	75.0	25.0	0.0
Resting	43	79.1	20.9	0.0

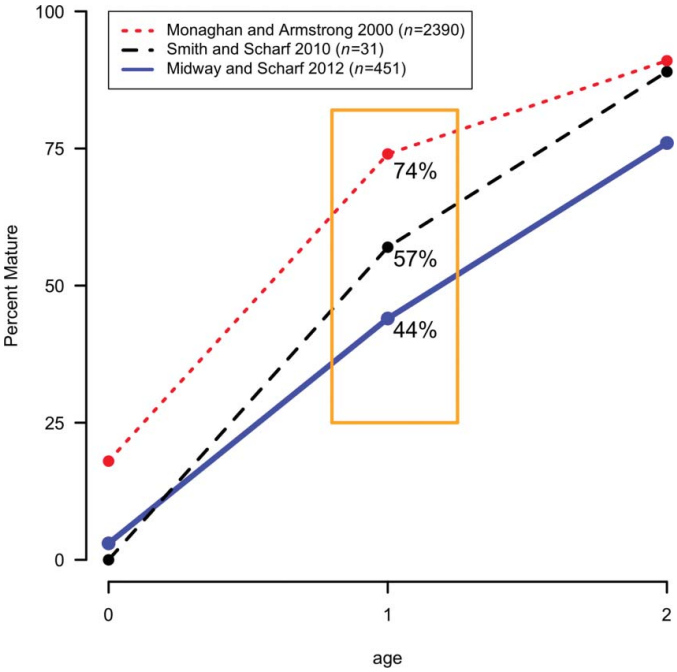

FIGURE 6. Estimates of the proportion of mature fish at age for North Carolina female southern flounder of ages 0–2 based on this study and two previous studies. The estimated proportion of mature fish generated during this study is based only on histologically staged fish and was lower at each age compared with previous estimates.

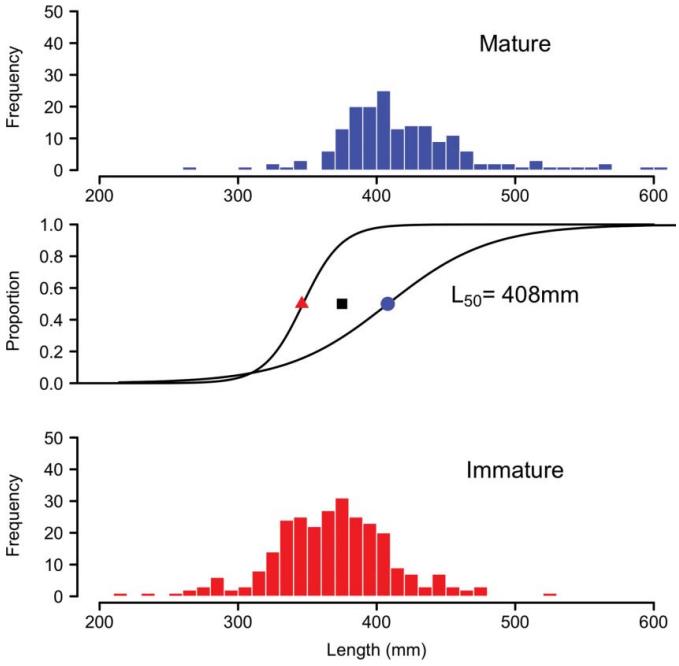

FIGURE 7. The maturity ogive and L_{50} estimate for North Carolina female southern flounder based on both years combined during this study (blue circle) relative to the maturity ogive (red triangle) estimated during a previous study (Monaghan and Armstrong 2000) (center panel). The length distributions of mature and immature fish that were used to generate the model during this study are displayed in the upper and lower panels, respectively. The black square represents the estimate of L_{50} (375 mm TL) from a maturity ogive using data from this study and only macroscopic staging.

based on the criteria outlined in Table 1. Only four fish were staged macroscopically as spent, and one fish was considered as running-ripe. The vast majority of fish were staged macroscopically as fully developed ($n = 43$), developing ($n = 148$), or immature ($n = 187$). Fish assigned to the fully developed stage achieved the highest agreement between macroscopic and histological classifications, with 91% agreement. Fish classified macroscopically as developing were assumed to be mature; however, only 61% of those fish were found to be mature based on histology. Similarly to fully developed fish, flounder that were staged macroscopically as immature were mostly confirmed to be immature through histology, with 89% agreement between methods. When the logistic regression model was used to generate a southern flounder maturity schedule using only macroscopic maturity assignments, the estimate of L_{50} was 375 mm TL, 33 mm smaller than our model built using histologically examined fish (Figure 7).

Spawning Potential Ratio Analysis

When the histologically validated maturity schedule was incorporated, SPR isopleths for southern flounder were shifted by approximately 10% relative to the currently assumed maturity schedule (Figure 8). At the present length at entry of 356 mm and the most recent estimate of $F = 0.7534$, SPR was estimated at 36.5% and 26.6% when using the currently assumed maturity schedule and the updated schedule from this study, respectively. In addition, the disparities in SPR between maturity schedules were magnified at larger lengths at entry. Our analysis predicts that to achieve an SPR of 40%, F would need to be reduced by between 26% and 74%, depending on length at entry, when incorporating the histologically validated maturity schedule. The effects of the maturity schedule are magnified when the stock is more depressed (i.e., at SPR values $< 40\%$).

DISCUSSION

Size and Age at Maturity

During this study, North Carolina southern flounder matured at larger sizes and older ages than previously estimated and assumed in current management efforts. Our size-at-maturity estimate was over 60 mm larger than the L_{50} previously calculated by Monaghan and Armstrong (2000), and our estimates of the fraction mature at age suggest that many individuals do not mature until ages 2 and 3, rather than maturing at age 1. Our findings are aligned with recent studies (Taylor et al. 2008; Smith and Scharf 2010) that have also presented evidence suggesting maturity at older ages and larger sizes for southern flounder in North Carolina. Between study years we detected a modest, although not statistically significant, difference in southern flounder maturity schedules. Observing a difference as large as 17 mm (nearly 5%) between L_{50} estimates during two consecutive years could imply that interannual variability in the size at

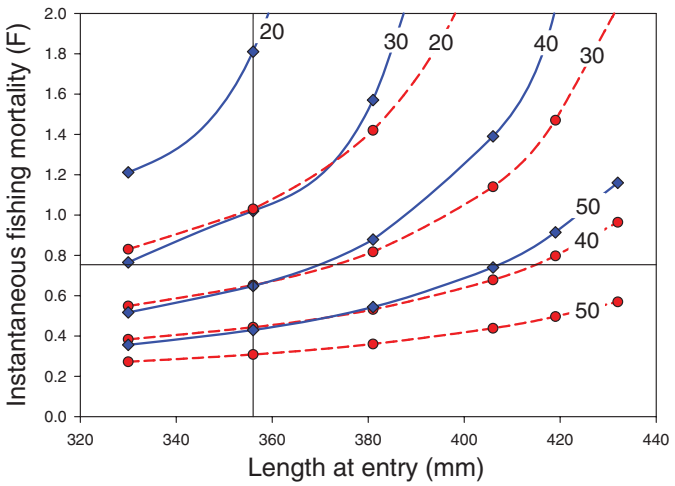

FIGURE 8. Spawning potential ratio (SPR) isopleths (20–50%) for North Carolina southern flounder across a range of lengths at entry and instantaneous fishing mortality rates (F) using two different maturity schedules (blue solid line with diamonds = currently assumed maturity schedule; red dashed line with circles = the histologically validated maturity schedule estimated in this study). Combining the current length at entry of 356 mm and the most recent estimate of $F = 0.7534$ (each represented by a solid black reference line) results in SPR = 36.5% when using the currently assumed maturity schedule and 26.6% when using the updated schedule. The symbols are located at hypothetical lengths at entry in 25.4-mm (1 in) increments between 330 and 432 mm (13 and 17 in), with the exception of the symbols located at 419 mm (16.5 in).

which maturity is attained is considerable for this species, owing to fluctuations in environmental conditions affecting early growth. Temporal variability in maturity schedules has been widely reported for other commercially exploited marine fishes (e.g., Morgan and Bowering 1997; Cardinale and Modin 1999; Cooper et al. 2010). Our findings at least suggest that interannual variation in maturity scheduling of southern flounder should be quantified.

Based on the currently assumed maturity schedule, the increased minimum size limit that was included among recent (2005) regulatory changes was hypothesized to result in lower harvest rates of immature fish. However, our histologically validated maturity schedule indicates that few southern flounder probably have a chance to spawn prior to recruiting to the fishery. Fish of age 2 and under still make up nearly 90% of the commercial harvest, regardless of gear type, and the modal sizes captured range from 360 to 380 mm TL (Takade-Heumacher and Batsavage 2009). Yet, fewer than one-half (44%) of age-1 individuals are mature based on histological examination, and many of those fish are harvested during the summer and fall before successfully emigrating to offshore spawning grounds. Furthermore, the age structure of the landings means that very few fish reach age 3, which is the first age when nearly 100% maturity can be expected. These findings point to a high likelihood of recruitment overfishing in the southern flounder fishery.

Southern flounder are inaccessible on the winter spawning grounds; thus, our sampling was concentrated during the estuarine period prior to emigration. Throughout their range, mature southern flounder are believed to migrate toward the continental shelf edge for spawning in winter. Sparse data for southern flounder larvae collected during broad ichthyoplankton surveys supports this assertion (Smith et al. 1975; Walsh 2007), but to our knowledge, large aggregations of adults have never been observed in these habitats. Our sampling during the prespawning period was aligned with previous studies of southern flounder reproductive biology (Wenner et al. 1990; Monaghan and Armstrong 2000) and presents both advantages and potential drawbacks. Collecting fish immediately before the main spawning period is advantageous because it reduces the likelihood of encountering fish in postspawning condition that lack any reliable maturity indicators and could be misinterpreted as immature (Hunter and Macewicz 2003; Lowerre-Barbieri et al. 2011b). Alternatively, the time required for an individual female to progress from possessing only primary oocytes to possessing the advanced vitellogenic oocyte stages necessary to participate in the upcoming spawning season is unknown for southern flounder, as for many fishes. This means that certain individuals assigned as immature early in our sampling period (e.g., October) could have matured during later months and emigrated to spawn. Although the temporal distribution of advanced oocyte stages and the monthly patterns in GSI each support October–December as an appropriate period to evaluate maturity in southern flounder, we cannot completely rule out potential temporal biases.

Macroscopic and Histological Agreement

We propose that the >60 mm increase in southern flounder L_{50} from previous estimates can be explained mostly by maturity classification error. Past studies (Wenner et al. 1990; Monaghan and Armstrong 2000) used primarily macroscopic staging with some level of histological validation, the details of which are not reported. If a considerable fraction of immature fish were incorrectly assigned mature status based only on macroscopic staging criteria during those studies, the resulting maturity ogives would be shifted to smaller sizes. Using our data from 2009 to 2010, a maturity ogive for southern flounder estimated using only macroscopic maturity assignments had an L_{50} of 375 mm TL, demonstrating the plausibility of significant bias related to macroscopic classification. Although macroscopic staging techniques are widely used to evaluate reproductive biology as part of contemporary fish sampling programs, the lack of validation of their predictions has been a growing concern (Tomkiewicz et al. 2003; Vitale et al. 2006; Ferreri et al. 2009). The inclusion of subjective descriptors such as "small," "rotund," and "yellowish-orange" in the macroscopic staging guides for southern flounder mimic those for other species and make objective classification difficult. This problem was most acute for the developing macroscopic stage of southern flounder in which only 61% of fish were found to possess the advanced oocyte stages necessary to be considered mature.

We do not contend that the temporal shift in maturity scheduling that we observed was necessarily fishery related. Typically,

an increase in exploitation reduces population density, freeing up resources to accelerate growth that often results in a decrease in size and age at maturity (Hutchings 2002). In addition, fisheries targeting larger, older individuals can represent a selective force against late age at maturity (i.e., fisheries-induced evolution of life history traits) and also produce a shift in maturity to smaller, younger fish. Shifts in reproductive timing have been documented for a number of heavily exploited marine fish stocks (Bowering and Brodie 1991; Rijnsdorp 1993; Trippel et al. 1997). However, fisheries-induced evolution is unlikely to produce large changes in life history traits within relatively short time periods (Hilborn and Minte-Vera 2008; Andersen and Brander 2009). More importantly, both ecological and evolutionary processes would be expected to favor individuals maturing at smaller sizes and younger ages—the opposite pattern to our observations for southern flounder.

Because the life history of southern flounder includes migration and offshore spawning, any prespawn sampling that takes place in estuarine waters will necessarily include mainly fish in earlier stages of maturation (e.g., running-ripe southern flounder are only rarely encountered close to shore). The time at which secondary oocyte development has just initiated is when gross morphological characters of the gonad are often the most difficult to interpret. In the absence of histological analysis, annual maturity schedules for southern flounder will depend upon correct macroscopic classification of early developing individuals. Clearly, given our lack of agreement for fish in developing stages, improved macroscopic classification techniques need to be developed unless histological analysis can be completed routinely. While histological analysis provides the highest level of confidence in estimating maturity schedules, we recognize that large-scale, multiyear histological investigations will not be economically feasible for most fishery management agencies. Therefore, it is even more important to periodically evaluate the performance of macroscopic classification guides, allowing for the evaluation of existing macroscopic staging criteria and possible development of new criteria.

Management Implications

Errors in maturity assignments frequently manifest in biological reference points and impact the effectiveness of management strategies. One common reference point used in fisheries management is F_{SPR}, defined as the instantaneous fishing mortality rate estimated to maintain SPR at some predetermined level. Although no single F_{SPR} is ideal for all species, previous studies suggest that SPR levels of 35–40% should result in high yield with minimal risk of collapse and contribute to rebuilding of overfished stocks (Overholtz et al. 1986; Clark 1993; Goodyear 1993). For southern flounder, NCDMF has set the management threshold at $F_{30\%} = 0.4880$ and the target at $F_{35\%} = 0.4081$, compared with the present $F = 0.7534$ (Takade-Heumacher and Batsavage 2009; NCDMF 2010). Using the histologically validated maturity schedule from this study and combining the current length at entry with $F = 0.4081$ yielded an SPR estimate of 42.3%, slightly higher than the 35% predicted by the NCDMF age-structured model.

The continuation of elevated harvest rates in the North Carolina southern flounder fishery (Takade-Heumacher and Batsavage 2009) despite the implementation of several management restrictions in 2005 necessitated an evaluation of additional strategies to restrict harvest. Several management alternatives, including catch quotas, limited entry programs, trip and creel limitations, and more severe gear limitations, were not favored for multiple socioeconomic and biological reasons, while increasing minimum size limits and temporal (i.e., seasonal) closures were strongly considered (NCDMF 2010). Ultimately, no strategies specific to southern flounder were implemented because it was projected that harvest rates would be sufficiently reduced by time-at-sea restrictions (i.e., the amount of time gill nets could be fished) imposed to limit interactions with protected species. However, our findings have important implications for any future implementation of changes to the minimum harvestable size of southern flounder. The differences between the SPR isopleths for currently assumed and histologically validated maturity schedules become greater at larger lengths at entry. For example, at the present length at entry (356 mm TL), $F_{40\%}$ is estimated to be 0.65 and 0.44 for the currently assumed and histologically validated maturity schedules, respectively; this means more than a 30% reduction in F when using the histologically generated maturity schedule. However, if length at entry were increased 50 mm (2 in) to 406 mm TL, the difference in the $F_{40\%}$ estimates due only to the maturity schedule would exceed 50%. If F is not controlled and the condition of the stock degrades to lower levels of SPR, the effect of the maturity schedule on estimates of F needed to rebuild SPR is even further magnified.

Ultimately, the goal of any fisheries management plan should be to minimize the loss of both current and future yields. Increasing length at entry affords more fish the opportunity to spawn and reduces the likelihood of recruitment overfishing. However, our findings highlight the need to understand the interaction between the timing of entry into the fishery and the timing of maturity. Finer control of harvest rates will probably be required when the timing of these events closely coincide. Lost yield from the harvest of immature individuals is a threat to many fish stocks. Heino (1998) demonstrated this phenomenon using an age-structured population model for Atlantic cod in the northeast Arctic, whereas Conover and Munch (2002) used empirical results from a controlled laboratory experiment to demonstrate lost yield in Atlantic silverside *Menidia menidia* caused by selective removals. Additionally, fisheries-induced evolution requires only two conditions: unequal mortalities across phenotypes and heritability of characteristics that have disparate vulnerabilities to fishing (Hutchings and Reynolds 2004). The nearly two decades of elevated harvest rates suggest that some degree of lost yield or evolution of life history traits may be occurring within the North Carolina southern flounder stock. The recognition of older ages and larger sizes at maturity than

originally thought, along with adequate control of harvest rates, should contribute to restoring long-term yield in this fishery.

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