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Demographic characteristics of southern flounder, *Paralichthys lethostigma*, harvested by an estuarine gillnet fishery

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Abstract The North Carolina (NC) southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert), stock has experienced heavy exploitation during the past two decades. Recently, several management changes were initiated to lower harvest rates and restore stock biomass. Here, the age, growth and maturity of southern flounder harvested by a southeast NC estuarine gillnet fishery are characterised and compared with observations from previous studies and with statewide data on the stock to evaluate any regulatory effects and assess the potential for selective removal by the fishery. Despite regulatory changes, the estuarine gillnet fishery still harvested mainly age-0 and age-1 individuals that were mostly immature, meaning that the current fishing practices likely only allow a small portion of the harvestable stock the opportunity to reproduce. Relative to length-at-age patterns observed within the stock from statewide collections, fish captured by the gillnet fishery were above average length at each age; the legal size and the gear appeared to cause selective harvest of the fastest growers within each cohort. If the demographic characteristics of the catch observed in this study are broadly representative of gillnet fisheries in other estuarine nursery habitats throughout NC, the harvesting tactics in this sector of the fishery have the potential to cause population-level effects and negatively affect long-term fishery yield.

KEYWORDS: age structure, fishing practices, maturity, nursery, recruitment overfishing, selectivity.

Introduction

Southern flounder, *Paralichthys lethostigma* (Jordan and Gilbert), is distributed along the US South Atlantic coast from southern Virginia to central Florida and throughout the northern Gulf of Mexico with a gap in the range occurring in south Florida (Gilbert 1986; NCDMF 2005). Gonadosomatic indices indicate that offshore spawning by adult southern flounder occurs between November and March in the US South Atlantic (Safrit & Schwartz 1998). The oceanic larval stage is pelagic and lasts 30–60 days before metamorphosing individuals enter estuaries and migrate towards low-salinity head waters to settle (Burke *et al*. 1991; Walsh *et al*. 1999). Southern flounder is fast growing with early maturity and a moderately short life span. In the estuary, juveniles may grow as rapidly as 0.35–1.5 mm day^{-1} (Fitzhugh *et al*. 1996), and many females are thought to reach maturation lengths by the end of their second year (Monaghan & Armstrong 2000). A 9-year-old female is the oldest southern flounder on record from North Carolina (NC) waters (Takade-Heumaker & Batsavage 2009). Southern flounder is sexually dimorphic (Stokes 1977; Music & Pafford 1984; Wenner *et al*. 1990), with females reaching the largest sizes [maximum size ~800 mm total length (*TL*)] and most males rarely achieving sizes >400 mm *TL*. In NC, approximately 88% of the southern flounder that reach harvestable size and recruit to the fishery are females, so stock assessments focus solely on the female portion of the population (Takade-Heumaker & Batsavage 2009). Mature southern flounder leave estuaries and emigrate to offshore spawning grounds beginning in the autumn (Gilbert 1986). After spawning, fish return to coastal and estuarine waters in late spring. Commercial catches peak during late summer and autumn when emigrating fish are captured in stationary gillnets and pound nets. Nearly all commercial harvest occurs in estuarine waters, with harvest from gillnets occurring further inland relative to pound nets that are set closer to ocean

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inlets. Although tagging studies in the US South Atlantic suggest that spawning-related offshore migrations of most southern flounder are directed south (Monaghan 1992; Craig & Rice 2008), the extent of mixing among southern flounder stocks in NC and other states is unknown. As all landings occur in state waters, southern flounder stocks are managed separately by individual states. To date, assessments of NC southern flounder have assumed a unit stock.

The harvest of southern flounder has been a major component of the commercial fishing industry in the estuarine waters of NC, USA for nearly three decades (NC Division of Marine Fisheries, NCDMF 2005). Since 1994, southern flounder has become the most economically valuable inshore finfish resource in NC (NCDMF commercial harvest statistics 1994–2008), largely due to the increased contribution of gillnet-harvested fish to the commercial landings (Fig. 1). The most recent stock assessment suggests that high fishing mortality on younger age classes has characterised the NC southern flounder fishery since the early 1990s (Takade-Heumaker & Batsavage 2009). This is an indication that initial regulatory efforts did not effectively control fishing mortality or avoid inclusion of young fish in the catch. Until recently, management regulations were limited to minimum size limits and gear specifications related to mesh size and net dimensions (Mumford 1999). Regulatory tactics did not include commercial gear-use reductions or time and area closures, each of which can lower fishing mortality by reducing effort. The minimum size limit was established at 280 mm *TL* in 1979 and increased to 331 mm *TL* in 1988. Gear specifications established for pound nets, trawls and gillnets during this time period were designed mainly to facilitate the effectiveness of minimum size limits, primarily via mesh-size restrictions. Prior to 2005, the only management restrictions intended to reduce mortality of southern flounder directly were effected in other fisheries that captured juvenile southern flounder as bycatch (e.g. shrimp and crab trawl fisheries; Mumford 1999).

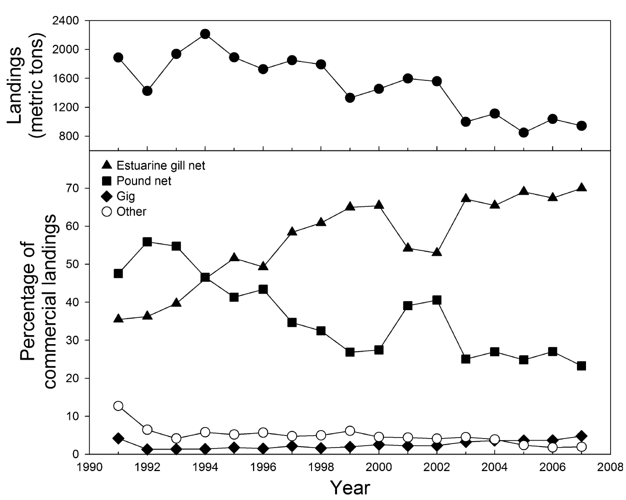

Figure 1. Landings trends and the proportion of total landings by gear type in the North Carolina commercial southern flounder fishery 1991-2007. Other gear includes shrimp trawls and crab trawls and pots, in which southern flounder are caught as bycatch.

Despite management efforts, recent assessment models estimated that harvest rates exceeded the current instantaneous fishing mortality rate target ($F = 0.48$, yielding a biomass target of 30% spawning potential ratio) every year between 1991 and 2007. During this period, estimated fishing mortality rates averaged 1.16, and age-0 individuals were documented in the catch every year (NCDMF 2005; Takade-Heumaker & Batsavage 2009). The most recent regulatory changes, initiated in 2005, included a 25-mm increase in the minimum size limit to 356 mm *TL*, a recreational bag limit of eight fish, a complete fishery closure during the month of December and several specific commercial gear limitations. However, the short-term fishing mortality target may not be achieved, as results from a recently completed tag-return study suggested that fishing mortality rates imposed by the gillnet fishery in one estuarine system likely still exceeded the target fishing mortality rate during the first 2 years after the new regulations (Smith *et al.* 2009). Indeed, the most recent stock assessment concluded that the stock was overfished and that overfishing was still occurring (Takade-Heumaker & Batsavage 2009).

Beyond the direct effects of harvest rate on stock biomass, the selective removal of immature or fast-growing individuals has been identified as a potential driver of lower long-term yield from a fish stock (Heino 1998; Conover & Munch 2002; Jørgensen *et al.* 2007). The gillnet sector of the NC commercial fishery for southern flounder operates extensively in low-salinity nursery areas within estuaries, habitats where the use of other commercial gears, such as gigs and trawls, is generally limited. The harvest in these habitats may be considerable as evidenced by the high rates of fishing mortality estimated recently within the nursery habitats of one southeastern NC estuary (Smith *et al.* 2009). Studies of other exploited marine species have found that when high rates of harvest are associated with the selective removal of fast-growing and immature individuals the fishery may induce changes to life history traits, such as age at maturity and individual growth rates, resulting in reduced long-term yield from the stock (Heino 1998; Conover & Munch 2002; Jørgensen *et al.* 2007).

Here, several demographic characteristics, including age structure, length distribution, size at age and maturity status are characterised for southern flounder captured in the commercial gillnet fishery operating in a NC estuary during two consecutive years. The ages and length-at-age patterns of harvested fish are compared with statewide data to illustrate the selective removal of younger, faster-growing individuals by the gillnet sector of the fishery. The maturity status of harvested fish is used to calculate the proportion of the catch consisting of immature individuals and to quantify the effects of harvest on present and future reproductive output. Catch demographics are interpreted collectively to document the potential effects of the fishing strategy practiced in the estuarine commercial gillnet sector of the fishery on spawning stock biomass and the long-term growth potential for the NC southern flounder stock.

Methods

Gillnet fishery samples

All southern flounder were captured during a large tag-return study conducted in the New River, NC (Fig. 2) between 2005 and 2007 (Smith *et al.* 2009). Commercial fishers captured fish that were tagged and released throughout the river using gillnets hung with 140-mm stretch mesh, the current minimum mesh size allowed for large-mesh gillnets in NC waters. All southern flounder caught during the tagging portion of the study were measured for total length, and then all legal-size (> 356 mm *TL*) fish were tagged prior to release. A small number ($n = 30$) of fish up to 25 mm *TL* less than the minimum size limit were tagged and later recovered, but the vast majority of fish less than legal size were not tagged and were only measured for total length when first captured. Thus, the age and maturity information presented is restricted primarily to the fish that were captured, tagged, released and subsequently recaptured, most of which were of legal size or greater. After release, tagged fish were recaptured by commercial and recreational fishers using gillnets, gigs or hook and line. Fishers who captured a tagged fish voluntarily reported their recovery using a phone number printed on each tag, and tag rewards were distributed to encourage reporting. Recovered fish were considered to represent a random subset of the tagged fish. Further, the pool of all tagged fish was assumed to represent a random sample of legal-size southern flounder captured in the New River commercial gillnet fishery; details on the specific components of the tagging experiment designed to meet these assumptions are presented in Smith *et al.* (2009).

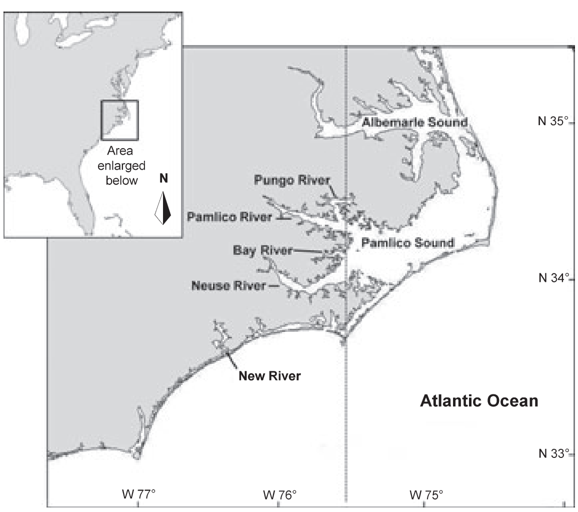

Figure 2. Map of the United States east coast (inset) and North Carolina coast (larger image). The vertical reference line, longitude W 76° 30′, demarcates the approximate division between the inshore areas of the larger sounds to the east and the smaller river systems to the west.

Size and age

A length frequency distribution was generated using all fish initially captured during tagging to determine the length range of southern flounder targeted in the commercial gillnet fishery and the percentage of the catch consisting of sub-legal fish. Although all sub-legal fish captured were counted and measured, no age data were collected for these fish.

Recaptured southern flounder were retrieved from fishers and returned to the laboratory for processing. Total length was again recorded at recovery; and the left sagittal otolith was removed, sectioned, mounted and aged according to methods established for southern flounder in the Gulf of Mexico (Gulf States Marine Fisheries Commission 2003). Although the use of whole otoliths is regarded as an acceptable method for ageing southern flounder, the use of sectioned otoliths is considered to be the least error-prone approach. Marginal increment analysis of southern flounder in Louisiana indicated that opaque increment (annulus) formation occurred between January and May for all fish > 330 mm *TL* (Fischer & Thompson 2004); thus, all age-1 and older fish in this study should have deposited at least one annulus by the time of their capture, as all fish were collected after May each year. As southern flounder exhibit sexually dimorphic growth patterns and females comprise the majority of the catch, only female fish were included in the age analyses.

The relative frequency of harvested southern flounder ages provided an assessment of the harvest pressure among age classes within the New River gillnet fishery during this study. Catch-at-age patterns for other NC southern flounder commercial gillnet fisheries, estimated during 2000–2006 by combining length-specific regional landings data with annual age-length keys, were also evaluated. The statewide data were grouped separately for the river-based fisheries located further inland and for the larger, coastal sound-based fisheries and then examined during two separate time periods (2000–2004 and 2005–2006) representing harvest prior to and after the 2005 management changes. For tagged fish recovered by fishers, both age and length at recovery were positively biased by time at large (i.e. all fish had the opportunity to age and grow during the time at large between tagging and recovery). Therefore, the total length at tagging and the back-calculated age at tagging, calculated as age at recovery minus time at large, were used in the analysis of age structure and length at age.

To estimate the length at age of the catch, age was assigned based on the otolith-estimated age in years for all recoveries of previously tagged fish. Length-at-age estimates for the commercially harvested fish (ages 0–3) in this study were compared with lengths at age for southern flounder collected statewide during 2005 and 2006 from both fishery-dependent and fishery-independent sources (provided by NCDMF). The statewide data included both legal and sub-legal fish captured from both inshore and offshore waters using several gear types, including gillnets, trammel nets, trawls, pound nets, gigs, spears and hook and line, and were interpreted to represent length at age patterns for a comprehensive sample of southern flounder from throughout the state. Comparison of lengths at age in the New River with the statewide data provided an assessment of the potential for selective harvest (i.e. removal of fish large or small for their age) within the New River gillnet fishery.

Maturity

Only tagged fish harvested during the months of October and November, just prior to the spawning season, were used to analyse the maturity status of the catch (Hunter & Macewicz 2003). Fish in pre-spawning condition were first encountered in October; prior to that time, mature and immature individuals were indistinguishable based on gross examination. The fishery is closed in December; and, due to very low commercial landings in the estuary during the winter (Fig. 3), no fish were recovered during January or February. Because most mature southern flounder emigrate to deep, offshore waters and are inaccessible

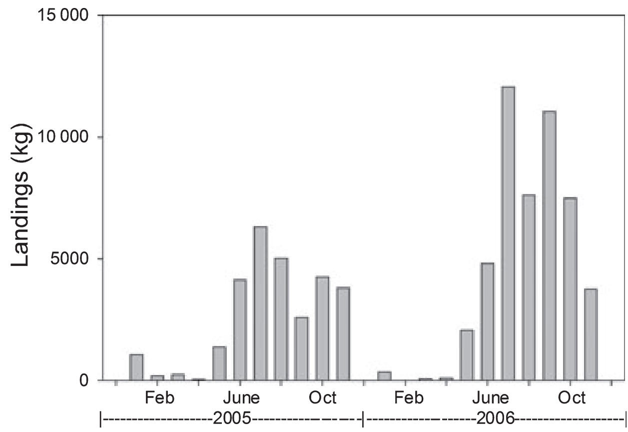

Figure 3. New River commercial landings of southern flounder by month for the 2005 and 2006 harvest seasons.

during the actual spawning season, maturity schedules were constructed using southern flounder captured during October and November before they had emigrated from the estuary. The results of reproductive analyses from this study were then compared with the lengths and ages at maturity reported in previous regional studies that also analysed fish just prior to emigration.

Only 2% of tagged fish recovered (n = 627) were males. Further, among 180 fish captured during the months of October and November and subsequently examined for maturity, only one male was discovered; therefore, only females were considered in the maturity analyses. Females were classified as mature or immature using a combination of macroscopic and histological examination. Females were initially assigned to one of five discrete macroscopic ovarian classifications, modified from a previous staging guide for southern flounder (Wenner *et al.* 1990). The developing stage described by Wenner *et al.* (1990) was further divided into early and late developing stages (stages II and III; Table 1). Histological examination of prepared tissues is considered to be the most accurate ovarian staging method and is commonly used to validate macroscopic staging techniques (West 1990). The ovaries from a subsample of 31 females (17.2% of the total number of fish used in the maturity analysis) were examined histologically. Ovaries possessing cortical alveolar oocytes were considered mature (Murua & Saborido-Rey 2003). Samples were taken from fish assigned to macroscopic stages II–IV (no stage V individuals were observed) for histological examination, and the proportion of histologically mature ovaries within each macroscopic stage was estimated.

Table 1. Macroscopic ovarian classification criteria used for southern flounder. These criteria were modified from Wenner *et al.* (1990) to include an additional stage (stage III) representing a late phase of developing ovaries

Macroscopic stage	Description of ovaries
I. Immature	Very small and thin; transparent or little colour
II. Early developing	Still small, but beginning to show yellow-orange colour
III. Late developing	Larger, rotund and turgid; deeper yellow-orange colour
IV. Fully developed	Very large; yellow-orange colour; often highly vascularised with oocytes visible
V. Running ripe	Large and soft with many large, free-flowing (with slight pressure) hydrated oocytes

For histological preparation, ovaries were first preserved in 10% buffered formalin, then rinsed in a series of ethanol and toluene solutions, and finally infiltrated with paraffin. The paraffin-infiltrated tissues were embedded in paraffin blocks from which 4-μm-thick sections were extracted. Sections were mounted on glass microscope slides and stained with eosin-Y and hematoxylin. Histological staging of these tissues followed West (1990) (Table 2).

A length-based maturation ogive relating the probability of maturity (p) to total length (i) was generated by fitting the following logistic model using the R glm procedure and the option family = binomial (link = 'logit') (R Development Core Team 2005):

$$p_i = e^{(a+b\times i)} \Big/ (1 + e^{(a+b\times i)})$$

where a and b are estimated parameters that can then be used to calculate the length at which 50% of the fish are mature ($L_{50} = -a/b$). In addition to analysing length at maturity for the southern flounder captured during this study, data from previous regional studies that estimated maturity schedules for southern flounder captured in both NC (Monaghan & Armstrong 2000) and South Carolina (Wenner *et al.*1990) waters were compiled for comparison. Wenner *et al.* (1990) did not generate a maturation ogive or an estimate of L_{50} for their data, but the number of fish captured and the percentage mature in 10-mm length bins were reported. These data were used to estimate the total number of immature and mature fish that were caught at each length by multiplying the total number caught in each length bin by the percentage mature in that bin. The length for all fish in each length bin was assumed to be equal to the midpoint of the length bin. The logistic regression model presented above was then used to generate a maturation-at-length ogive and an L_{50} estimate for South Carolina southern flounder.

Table 2. Microscopic ovarian stage criteria used for southern flounder (based on West 1990)

Histological stage	Description of ovaries
I. Early perinucleolar	One or few large nucleoli, basophilic ooplasm, no cytoplasmic inclusions
II. Late perinucleolar	Less basophilic ooplasm, a cortical alveolus and/or a lipid droplet
III. Cortical alveolar	Many small lipid droplets and cortical alveoli dispersed in cytoplasm
IV. Late lipidogenic	Lipid droplets fill ooplasm and coalesce centrally, cortical alveoli at follicular envelope
V. Vitellogenic	Yolk protein globules present
VI. Final maturation	Germinal vesicle breakdown, lipid and yolk coalesced, may be hydrating
VII. Post-spawning	Post-ovulatory follicles, atretic oocytes

The probability of maturity at each age (p_a) was estimated as the proportion mature at age. To evaluate previous reproductive contributions from the catch, the fraction of the gillnet catch that may have spawned during the prior reproductive period (PS_{t-1}) was also estimated, using catch-at-age data and the maturity schedule. The product of the proportional catch at each age (c_a) and the age-specific maturation probability during the prior year's spawning season (p_{a-1}) was calculated for each represented age class (0 to age$_{max}$), and then summed across age classes:

$$PS_{t-1} = \sum_{0}^{age_{max}} p_{a-1} c_a$$

Results

In total, 3203 southern flounder were captured in New River commercial gillnets during tagging trips in 2005 and 2006. The average length of these fish was 362 mm *TL*, and 95% of all fish caught were 244–459 mm *TL*. More than one-third (35.7%) of the fish captured during this study were sub-legal (Fig. 4) and, thus, discarded without being tagged. A total of 1970 legal-sized southern flounder in good condition were tagged and released, of which 627 were recovered and used to estimate the demographic characteristics of the catch. Among the recovered fish, 93.4% were captured in the commercial gillnet fishery, with a combined 6.6% captured in the gig and recreational fisheries. The majority (87.7%) of fish captured in the gillnet fishery during 2005–2006 were estimated to be age 1; 7.5% were age 0, 4.6% were age 2 and only 0.2% were age 3

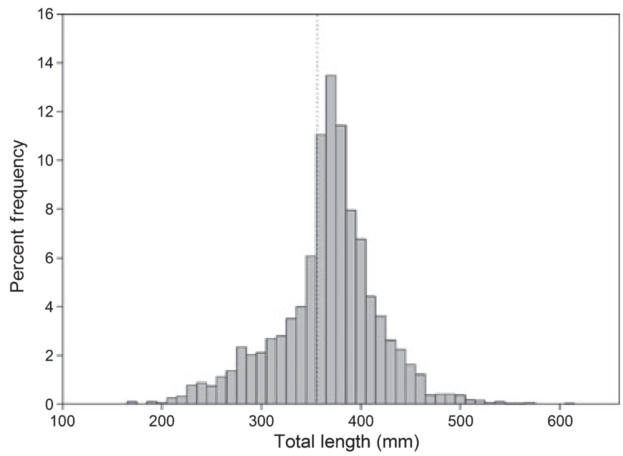

Figure 4. Length distribution of all southern flounder captured in the New River using 140-mm stretch mesh gill nets during this study. The vertical reference line marks the minimum legal size limit, 356 mm TL.

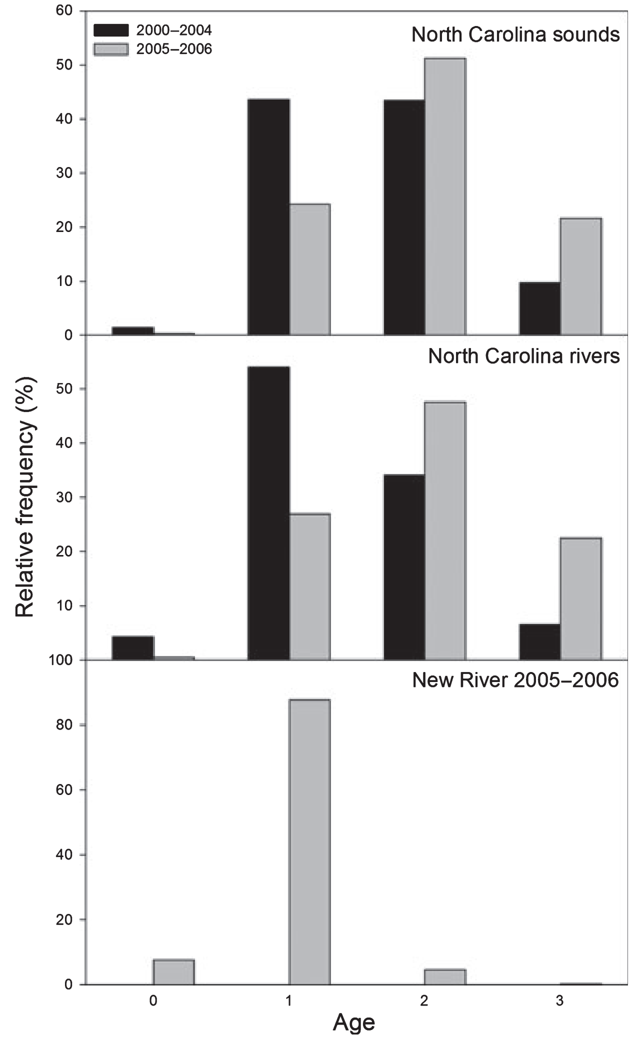

Figure 5. Catch at age of southern flounder in the commercial gill net fishery before (2000-2004) and after (2005-2006) recent management changes, including a 25-mm increase in the size limit and increased minimum mesh size for large-mesh gillnets. North Carolina sounds includes data from Pamlico and Core Sounds. NC rivers includes data from western Albemarle Sound and the Pamlico, Pungo, Bay and Neuse Rivers, as well as all river systems in the southern region of the state (including the New River). The data collected in the New River during this study are presented separately for comparison. Statewide commercial catch-at-age data were collected and provided by the NC Division of Marine Fisheries.

(Fig. 5). Catch-at-age data compiled from southern flounder commercial gillnet fisheries in different regions of the state also indicated that the harvest consisted primarily of younger (age 1 and 2) age classes. Although all fisheries observed an increase in the harvest of older age classes after the 2005 management changes, age-1 individuals remained a large fraction of the catch. Prior to the 2005 regulatory changes, the fisheries in the larger sounds harvested proportionally fewer age-0 and age-1 flounder than the river fisheries. In 2005–2006, the proportional contribution of different age classes between sound and river fisheries was more uniform. The fish harvested commercially in the sounds are captured mostly in pound nets (roughly 60% of the catch) and, to a lesser extent (roughly 30% of the catch), in gillnets. By contrast, the river fisheries are executed mostly with gillnets, and $>80\%$ of commercial landings in river fisheries during 2005–2007 were harvested by gillnet (NCDMF harvest statistics). During 2005–2006, 1.6–2.2% of all commercial landings of southern flounder in NC were reported from the New River (Takade-Heumaker & Batsavage 2009). The data collected during this study indicated the continued harvest of mainly age 1 flounder by the New River fishery during the 2 years following management changes.

The average length-at-age for flounder ages 0–2 captured in the New River gillnet fishery during this study was larger than the average length at age for fish collected throughout the state during 2005–2006 (Fig. 6). As they were pooled across multiple data sources and gear types, the statewide samples were assumed to represent the full range of lengths at age present in the NC stock during this time period, whereas the current data reflected just the legally sized fish captured in the New River commercial gillnet fishery. The larger lengths at age observed in the New River fishery imply that the fishery may selectively harvest the largest, and likely fastest growing, members within each age class as it passes through the fishery. The New River length-at-age statistics in Figure 6 do not include sub-legal fish, which represented a considerable fraction (35.7%) of the total catch. Most

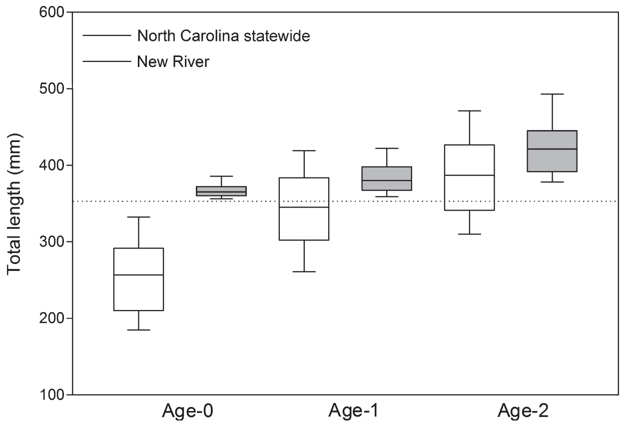

Figure 6. Boxplots of the length at age of southern flounder captured in the New River commercial gill net fishery compared with the length at age of southern flounder collected statewide in 2005–2006 as part of both fishery-dependent and -independent North Carolina Division of Marine Fisheries sampling programs. The statewide data are assumed to be an accurate representation of length at age of southern flounder within the North Carolina stock during the study years. The horizontal reference line indicates the minimum size limit, 356 mm TL.

sub-legal fish were probably age 0 and age 1, so the length-at-age statistics presented for the New River fishery are likely to be biased high for these ages.

All ovaries were macroscopically classified as stages I–IV (Table 1); no stage V ovaries were observed. Histological examination revealed that the assigned macroscopic stage was a reasonably good estimate of maturation. Most of the ovaries that were assigned to macroscopic stage II were found to be immature upon histological examination, possessing only perinucleolar oocytes, while those ovaries assigned to macroscopic stage III or IV were mostly mature, possessing either cortical alveolar or lipidogenic oocytes (Table 3). Based on the histological results, macroscopic stages III and IV were assumed to represent mature female ovaries for southern flounder. When these criteria were applied to all macroscopically staged fish, 87 ovaries out of the 179 examined were estimated to be immature, resulting in only 92 mature individuals. Thus, 48.6% of all southern flounder captured by the New River gillnet fishery during October and November of 2005–2006 were estimated to be immature.

The logistic model parameters estimating length at maturity of New River female southern flounder were $a = -30.2$ (SE = 4.58) and $b = 0.0784$ (SE = 0.0119), and generated an L_{50} estimate of 385 mm TL. The logistic model parameters estimated for South Carolina female southern flounder based on the data originally presented in Wenner *et al.* (1990) were $a = -24.9$ (SE = 3.71) and $b = 0.0718$ (SE = 0.0108), and generated an L_{50} estimate of 347 mm TL. The estimate of L_{50} for NC southern flounder generated by Monaghan and Armstrong (2000) was 345 mm TL.

All age-0 southern flounder captured during this study were immature. Based on macroscopic and histological staging, an estimated 56.6% of age-1 fish and 88.9% of age-2 fish were mature (Table 4). Based on the observed catch at age and the percent mature at age estimated in this study, only 2.8% of the catch may have had the opportunity to spawn during the reproductive season prior to the year of their capture. Using the age-specific maturity schedule for NC southern flounder generated by Monaghan and Armstrong (2000), an estimated 19.5% of the catch during this study may have been mature during the prior reproductive season (Table 4).

Discussion

The quantitative information on the age, growth and maturity of southern flounder harvested in the New River commercial gillnet fishery presented here sheds light on the potential population-level effects of the fishing strategy employed. The large proportion of young (age-0 and age-1) and immature fish included in the commercial catch indicates a high potential for

Table 3. Maturity of southern flounder ovaries determined by macroscopic and histological examination. The majority of samples examined histologically from macroscopic stages III and IV appeared to be mature (contained cortical alveolar or more advanced oocytes)

	Histology			
Macroscopic stage	Number examined macroscopically	Number examined histologically	Frequency with cortical alveolar or more advanced oocytes	Proportion mature based on histology
I. Immature	0	0		
II. Early developing	87	13	2	0.15
III. Late developing	41	8	6	0.75
IV. Fully developed	51	10	9	0.90
V. Running ripe	0	0		

Table 4. Age structure of southern flounder caught in commercial gillnets in New River, North Carolina (% catch), the percentage mature at age during the prior year (% mature) measured in this study and by Monaghan and Armstrong (2000), and the percentage of the catch spawning in the prior year (% spawning). The use of each maturity schedule indicated that a low percentage of females in the catch were mature and may have spawned during the prior spawning season

	New River estuary 2005 and 2006			Monaghan and Armstrong (2000)	
Age	% catch	% mature	% spawning	% mature	% spawning
0	7.5				
1	87.7	0	0	18.1	15.9
2	4.6	56.6	2.6	73.5	3.4
3	0.2	88.9	0.2	90.7	0.2
Total	100.0		2.8		19.5

Table 5. Relative contribution of sub-legal fish to southern flounder catches in gillnets with three different mesh sizes. Data were collected as part of fishery-independent sampling conducted by North Carolina Division of Marine Fisheries during 2001–2003 (NCDMF 2005)

Stretched-mesh size (mm)	Number of fish captured	% catch below minimum size limit (< 356 mm *TL*)
140	3121	21
146	2621	15
152	4005	9.3

recruitment overfishing in the gillnet sector of the NC southern flounder fishery. Based on a maturity schedule and catch at age, only 2.8–19.5% of the fish harvested during this study might have reproduced prior to capture. This interpretation was appropriate because most southern flounder in NC were harvested during late summer and autumn and prior to the winter spawning season. When considering the consequences of fishing practices on the spawning potential of the NC southern flounder stock, it is important to determine the fraction of the catch that may have had the opportunity to spawn during the previous winter. Estimates of the maturity status of the catch indicated that approximately half of all legal-sized female southern flounder captured in the New River in the months leading up to the spawning season were immature. If fishing practices continue as presently executed, few fish in the catch during any given year will have spawned during the prior reproductive season, and only half of the fish targeted by the fishery will be likely to spawn during the subsequent winter, presuming they escape the fishery. The findings demonstrate that the commercial gillnet fishery in this estuary is harvesting a largely immature fraction of the population, compromising the contribution of New River flounder to the NC southern flounder spawning stock.

The fish harvested in the New River during this study were mainly captured in upper-estuary habitats displaying low to moderate salinities (5–20 g L^{-1}) and shallow water depths (< 2 m). Young southern flounder, which display a greater tendency to occupy habitats located further inland relative to larger, older individuals, are believed to use these upper-estuary regions as nurseries (Wenner *et al.* 1990; NCDMF 2005). Fishery-independent length frequency data collected for southern flounder in several NC estuaries provide empirical support for this assertion (NCDMF, unpublished data). Therefore, the demographics of harvested southern flounder among different estuarine habitats will likely reflect differences in size and age composition related to age-specific habitat segregation. Indeed, even prior to the 2005 management changes, commercial southern flounder catches in the sounds adjacent to ocean inlets included proportionally more older (age-2 and age-3) fish compared with those in the river-based estuarine fisheries executed further inland (Fig. 5; black bars). Since 2005, a shift to harvest greater proportions of age-2 and age-3 fish was observed statewide (Fig. 5; grey bars). However, the presence of a strong year class in 2003 (represented as age-2 fish in 2005 and age-3 fish in 2006) may have been largely responsible for the observed shift in harvest demographics (Takade-Heumaker & Batsavage 2009). The potential effects on the size- and age structure of the catch due to the 2005 management changes and the strong 2003 year class are confounded and difficult to separate. Regardless, the impacts of these events were not evident in the New River fishery that still included mostly age-1 fish during both 2005 and 2006. This pattern was interpreted as an indication that the New River fishery may be representative of the fishery sector operating in shallow, estuarine nursery habitats located along the western edge of the sounds and the upper reaches of the coastal rivers. The harvest of fewer age-2 and age-3 fish in the New River gillnet fishery compared with other larger river systems in the state may be a function of the habitat, where the New River fishery is executed, which consists of a relatively narrow riverbed and primarily shallow (< 2 m) waters at distances up to 30 km from the ocean inlet. Comparatively, the other river systems for which commercial catch data are available include large expanses of open, deep water (see larger rivers in Figure 2) that may serve as habitat for larger flounder. Because the catch data are compiled for entire systems, the demographics in the shallower nursery habitats of these larger river systems may be diluted by the inclusion of larger, older fish from deeper habitats. Although southern flounder landings in NC have historically been higher in the sounds located closer to ocean inlets, the proportion of landings from the western regions of the sounds and from the river systems has increased in recent years (NCDMF 2005), most likely related to the increased use of gillnets to target flounder throughout the state (see Fig. 1). The present findings suggest that the trend towards increased harvests in estuarine nursery habitats is likely to result in the continued inclusion of young fish in the commercial catch, which could serve to counteract the objectives of the recent changes to southern flounder management.

The length at 50% maturity estimated for female southern flounder in this study (385 mm *TL*) was greater than previous estimates for southern flounder in NC (345 mm *TL*; Monaghan & Armstrong 2000) and SC (347 mm *TL*; based on data contained in Wenner *et al.* 1990). Furthermore, lower proportions of mature fish at age 1 and age 2 were predicted by this study than previously (Monaghan & Armstrong 2000). Logistic regression models can be biased by data that are unbalanced between mature and immature observations or by poor contrast in length. However, the maturity data presented here were roughly balanced between observations of mature and immature fish, and the logistic model appeared to fit the observed data closely based on visual inspection of residuals. Although sub-legal flounder were generally discarded without being examined for maturity, some sub-legal fish were tagged, and 16 of these were recaptured by the commercial fishery and returned during the months prior to the spawning season. All these fish were examined for maturity status (representing 9% of the total 179 fish examined for maturity). In addition, 16% of all observations used in the logistic regression model were immature fish that were smaller than the smallest mature fish. Thus, although it is possible that some female southern flounder mature at smaller body sizes than examined in this study, the evidence presented here indicates a low probability that female southern flounder in the New River matures at sizes below the legal harvest limit.

Length- and age-based maturity estimates can be biased by several sources of error, particularly sampling gear selectivity, variation in growth and mortality rates (Dieckmann & Heino 2007; Wang *et al.* 2009) and staging-classification error, all of which may have played a role in the patterns observed for southern flounder in the New River. All of the fish examined were harvested with gillnets, which are highly size-selective, and this study demonstrated that the flounder harvested in the New River commercial gillnet fishery had realised faster growth than the average southern flounder from throughout NC. Recent estimates of the harvest rates in the New River (Smith *et al.* 2009) and statewide (Takade-Heumaker & Batsavage 2009) indicate that fishing mortality in the New River was greater than the statewide average during 2005–2006. Finally, histological validation demonstrated that assuming macroscopic stages III and IV to represent mature ovaries was 75–90% accurate, but the error in maturity assignment based on macroscopic staging could have generated some additional bias in the New River maturity estimates. Sampling and staging biases may also have influenced earlier studies of this species to an unknown extent. The two previous regional studies (Wenner *et al.* 1990; Monaghan & Armstrong 2000) were also restricted to assessing maturity of southern flounder captured in the estuary during the autumn prior to offshore emigration, and the present observations suggest that earlier studies that relied only on macroscopic assessment of maturity may have misclassified some immature ovaries without attempting to correct for staging error. This study modified the macroscopic classification criteria originally described by Wenner *et al.* 1990 to distinguish between early developing (stage II) and late developing (stage III) ovaries. While most stage III ovaries were confirmed to be mature when examined histologically, most stage II ovaries were immature. The length-based maturation ogive estimated here would have been closer to the previous regional estimates if these stages were combined and considered mature.

The difficulties in comparing previously estimated length-based maturation ogives to the estimates generated here for the New River gillnet catch highlight several potential biases that can affect length- and age-based maturity estimates. The use of probabilistic maturation reaction norms as an index of maturation has been suggested to avoid these specific types of biases (Dieckmann & Heino 2007); however, poor contrast in macroscopic maturity staging and low sample sizes for age-0 and age-2 flounder in the New River precluded the use of probabilistic reaction norms in this study. The use of probabilistic maturation reaction norms should be considered in future studies of southern flounder reproductive biology that can include a broader range of age classes with histological analysis for a greater proportion of individuals.

Recently, the potential for the harvest of natural populations to cause changes in yield-determining population demographic characteristics, such as age at maturity and individual growth rates, has become more widely appreciated. The results of theoretical modelling (Heino 1998), controlled laboratory experiments (Conover & Munch 2002) and empirical data analysis (Heino *et al.* 2002) each have concluded that the selective removal of fast-growing or immature fish can lead to reduced growth potential within a harvested stock. For instance, Heino (1998) used age-structured population models to reveal that selection favoured earlier maturation when the harvest included both immature and mature fish. The model illustrated how a population of early maturing fish, with a gene pool altered by fishing, could produce a lower annual yield than a population of late maturing fish. Heino (1998) concluded that the harvest of immature fish could cause genetic changes that would lower sustainable yields. Similar outcomes were predicted when the fastest growing members of a cohort were removed by selective harvesting practices, with the evolution of slower individual growth rates and lower yield potential for the remaining individuals (Conover & Munch 2002).

The length-at-age patterns of legal fish observed here revealed that the New River gillnet fishery harvested mostly age-0 and age-1 fish that were above average size for their age. If the statewide length-at-age distribution accurately represents southern flounder growth patterns across the state (including the New River), the lengths at age of fish harvested in the New River indicate that many of the river-based gillnet fisheries operating in the nursery habitats may be selectively removing faster-growing fish. The current examination of length at age did not include the sublegal, discarded fish, but this does not detract from the finding that most legal-sized age-0 and age-1 southern flounder were well above the average length at age observed statewide. When combined with the observed catch at age, the maturity schedule predicted that most of the southern flounder harvested in the New River during this study were unlikely to have spawned the prior winter. The high rate of harvest mortality in the southern flounder fishery (Smith *et al.* 2009; Takade-Heumaker & Batsavage 2009), coupled with the low numbers of mature fish in the catch, suggest low rates of escapement and a diminished future contribution to the stock by these fast-growing fish.

Management tactics with the potential to minimise the harvest of immature fish include further technical measures related to gear restrictions and the introduction of spatial closures to remove the gillnet fishery for southern flounder from specific estuarine nursery habitats. Technical measures that alter gear selectivity by increasing gillnet mesh sizes have been shown to reduce the bycatch of small fish in many other fisheries (Psuty-Lipska *et al.* 2006; Revill *et al.* 2007; Sabrana *et al.* 2007). Indeed, data from fishery-independent studies included in the most recent fishery management plan for southern flounder (NCDMF 2005) demonstrated that an increase in the stretch-mesh size from 140 mm (the current minimum mesh size) to 152 mm considerably reduced the bycatch of smaller fish (Table 5). The evidence presented here implies that the most recent management changes have not eliminated age-0 fish from the catch, and that age-1 fish still account for a large fraction of the harvest statewide. It is also likely that the New River fishery, as well as other fisheries based in upper-estuary nursery habitats, may harvest a younger segment of the southern flounder population than is typically observed in the gillnet fisheries executed in the larger sounds in waters closer to ocean inlets. Increasing the minimum size limit and changing the selectivity pattern of gillnets by increasing the minimum stretched-mesh size would represent a minimal measure to reduce the catch, including bycatch and discarding, of juvenile southern flounder.

Given the potential negative impacts of harvesting fast-growing individuals before they have contributed to reproduction, the current findings should be interpreted with caution by southern flounder fishery managers. Although the time scale may be debatable, it is likely that the harvesting practices common in the estuarine gillnet fishery are slowly eroding the yield-generating capacity of the NC southern flounder stock. If the gillnet fishery were instead to target older age classes, allowing for sufficient escapement of mature fishes, most potentially detrimental effects might be alleviated. Increasing the minimum size limit to the average length at age 2 (406 mm *TL*; NCDMF 2005), in addition to mesh size increases in the gillnet fishery to target larger fish, would reduce the catch of immature southern flounder by gillnets. Based on the maturity schedules estimated in this study and previously (Monaghan & Armstrong 2000), targeting age-2 fish would allow between 57 and 74% of females the chance to spawn in the reproductive season prior to becoming subject to the fishery, a considerable improvement over current practices. Long-term monitoring of variation in growth rates, as well as age and length at maturity might be prudent in the NC fishery for southern flounder, as changes in these population demographic characteristics should help to forecast potential detrimental effects of any harvest strategy on the long-term yield of the stock.

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