Fecundity and spawning season of striped mullet (*Mugil cephalus* L.) in South Carolina estuaries*

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Abstract—Fecundity in striped mullet (*Mugil cephalus*) from South Carolina correlated highly with length and weight, but not with age. Oocyte counts ranged from 4.47×10^5 to 2.52×10^6 in 1998 for fish ranging in size from 331 mm to 600 mm total length, 2.13×10^5 to 3.89×10^6 in 1999 for fish ranging in size from 332 mm to 588 mm total length, and 3.89×10^5 to 3.01×10^6 in 2000 for fish ranging in size from 325 mm to 592 mm total length. The striped mullet in this study had a high degree of variability in the size-at-age relationship; this variability was indicative of varied growth rates and compounded the errors in estimating fecundity at age. The stronger relationship of fecundity to fish size allowed a much better predictive model for potential fecundity in striped mullet. By comparing fecundity with other measures of reproductive activity, such as the gonadosomatic index, histological examination, and the measurement of mean oocyte diameters, we determined that none of these methods by themselves were adequate to determine the extent of reproductive development. Histological examinations and oocyte diameter measurements revealed that fecundity counts could be made once developing oocytes reached 0.400 μm or larger. Striped mullet are isochronal spawners; therefore fecundity estimates for this species are easier to determine because oocytes develop at approximately the same rate upon reaching 400 μm. This uniform development made oocytes that were to be spawned easier to count. When fecundity counts were used in conjunction with histological examination, oocyte diameter measurements, and gonadosomatic index, a more complete measure of reproductive potential and the timing of the spawning season was possible. In addition, it was determined that striped mullet that recruit into South Carolina estuaries spawn from October through April.

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The striped mullet (*Mugil cephalus* L.) is distributed circumglobally in tropical and semitropical waters between latitudes 42°N and 42°S (Thompson, 1963; Rossi et al., 1998). Even though considered a marine species, striped mullet are euryhaline and can be found year round throughout the full range of estuarine salinities in the southeastern United States (Jacot, 1920; Anderson, 1958). Striped mullet are a commercially important fish throughout the world sustaining both fisheries and aquaculture industries. In the southeastern United States (North Carolina and Florida) there are significant commercial fisheries for striped mullet, whereas in South Carolina and Georgia landings are more limited (NMFS[1]).

The primary fishery in most of these states is for "roe" mullet during the fall spawning migration. Throughout the rest of the year mullet are fished commercially for bait, if they are fished at all (Anderson, 1958). Striped mullet have a significant economic impact in the areas where they are more heavily fished by the commercial fisheries and the landings of this species from 1994 to 1998 yielded a landings (wholesale) value of 38.2 million dollars. Striped mullet are also one of the most important forage fishes that are found in the estuaries of the southeastern United States and represent a significant food source for upper-level piscivores (Wenner et al.[2]).

The biological features of striped mullet has been well documented (Jacot, 1920; Anderson, 1958; Thomson, 1963, 1966; Chubb et al., 1981), but much less information is available on the biological aspects of reproduction in the wild (Anderson, 1958; Stenger, 1959; Greeley et al., 1987; Render et al., 1995). There is a large body of work concerning striped mullet reproduction in aquaculture but many of these studies have used artificial manipulation of the reproductive cycle. Although the maturation process of oocytes may have been the same as that in wild striped mullet, the environment and conditions under which maturation occurs were artificial (Shehadeh et al., 1973a; Kuo et al., 1974; Pien and Liao, 1975, Kelly, 1990; Tamaru et al., 1994; Kuo, 1995). In addtion, despite the demonstrated ability to initiate reproduction (both in and out of season) for striped mullet, the majority of aquaculture studies have had to rely on wild fish for broodstock (Kuo et al., 1974; Pien and Liao, 1975; Kuo, 1995).

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[1] National Marine Fisheries Service. 2001. Personal commun. Statistics and Economic Division, 1315 East-West Highway, Silver Spring, Md. 20910. http://www.st.nmfs.gov/st1/index.html.

[2] Wenner, C. A., W. A. Roumillat, J. E. Moran, M. B. Maddox, L. B. Daniel, and J. W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina, part 1. Completion report, project F-37, p. 3–13 and project F-31, p. 6–35. South Carolina Marine Resources Research Institute, P.O. Box 12559, Charleston, SC 29422

In the southeastern United States the spawning season lasts from two to five months depending on the coastal area involved (Jacot, 1920; Broadhead, 1956; Anderson, 1958; Arnold and Thompson, 1958; Stenger, 1959; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). Striped mullet are considered isochronal spawning fishes (Greeley et al., 1987; Render et al., 1995), i.e. they have synchronous gamete development and individuals spawn all their reproductive material at once or in batches over a very short time period (days, as opposed to weeks). There have been limited observations of offshore spawning activity (Arnold and Thompson, 1958), and few examples of eggs and larvae collected offshore (Anderson, 1958; Finucane et al., 1978; Collins and Stender, 1989). Collins and Stender (1989) concluded that striped mullet spawn in and around the edge of the continental shelf off the coasts of North Carolina, South Carolina, Georgia, and the east coast of Florida (an area often referred to as the South Atlantic Bight) and have a protracted spawning season from October to April. This spawning season contrasts with that estimated by most other studies (Jacot, 1920; Broadhead,1956; Anderson, 1958; Arnold and Thompson, 1958; Stenger, 1959; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). These studies based their estimates on the reproductive condition of migrating adults and the subsequent recruitment of juvenile fish back into coastal estuaries—not on actual data on the offshore presence of striped mullet larvae. Female mullet have been shown to mature at two to three years of age at a size range from 230 mm to 350 mm standard length (Thomson, 1951, 1963; Greeley et al., 1987). Determination of spawning activity in mullet has been estimated by using gonadosomatic indices (Dindo and MacGregor, 1981; Render et al., 1995), examination of oocyte size and maturity stage (Stenger, 1959; Greeley et al., 1987), and by the presence of enlarged, developing ovaries in migrating fish (Jacot, 1920; Anderson, 1958).

The purpose of the present study was to develop size- or age-related estimates of fecundity in striped mullet from South Carolina estuaries. These fecundity estimates were determined in order to develop models for estimating potential fecundity from catch data, such as length and weight data. In addition, other indicators of reproductive activity such as gonadosomatic indices and oocyte size were examined to provide information on the duration of the spawning season. Potential fecundity estimates can give a barometer of reproductive potential based on morphological information from catch curve data and size-frequency distributions.

Materials and methods

Striped mullet were collected monthly from January 1998 through December 2000 by using a randomly stratified sampling regime within three different estuarine systems along the South Carolina coast: (Ashepoo-Combahee-Edisto (ACE) Basin, Charleston Harbor, and the Cape Romain estuary, Fig. 1). The female striped mullet used for fecundity estimates were collected from October through February each year because these were the only months when fecund females were present. Fecund female striped mullet were defined as specimens in the tertiary stage of vitellogenesis with oocyte diameters greater than 400 μm and that had gonadosomatic indices greater than 5.0. The vitellogenic stage for each specimen used in the fecundity estimates was confirmed histologically. Fish were captured during daylight ebbing tides with water levels ranging from 0.3 to 2.0 meters, and the majority (67.8%) were caught during late ebb. The fish were primarily caught with a 184-m trammel net with an outside stretch mesh of 350 mm and an inside stretch mesh of 63.5 mm, although a few of the 1999 samples (5) and the 2000 samples (3) were obtained by using a cast net 1.8 m in diameter and equipped with 10-mm mesh. Eight fecund females were also captured by using an electroshock boat in the freshwater and low-salinity areas of the Cooper River (one of the three rivers that make up the Charleston Harbor Estuary) in October 2000. The areas included in the present study have been sampled on a monthly basis since 1991 with trammel nets by the Inshore Fisheries Group of the South Carolina Department of Natural Resources as part of a gamefish monitoring program. During this period reproductively developing striped mullet of both sexes were generally observed from October through February and were presumably heading offshore to spawn (Jacot, 1920; Anderson, 1958; Arnold and Thompson, 1958). Male striped mullet that were reproductively developed were easy to discern because they were usually leaking milt and were not analyzed further. All other fish were brought back to the laboratory and eviscerated in order to determine sex and to collect ovaries for reproductive analysis. All of the samples were kept on ice and were generally examined within twenty-four hours of capture.

Standard morphometric measurements included total length (TL) in mm, fork length in mm (FL), and standard length in mm (SL), total weight (TW) in grams, ovary weight (OW) in grams, sex, and maturity. Body weight (BW) was calculated as total weight minus ovary weight ($BW=TW-OW$). Saggital otoliths were removed for aging. A small section of the posterior end of the ovaries, at the junction of the two lobes, was also removed for histological examination. The whole ovaries were fixed in 10% seawater-buffered formalin and the histological sample was fixed in 10% neutral-buffered formalin. Histological samples were processed by using standard procedures for paraffin embedding and sectioning (Humason, 1967). The sections were dried on slides and stained by using standard haematoxylin and eosin-Y staining techniques (Humason, 1967). Examination of the histological sections for maturity stage was done by using a compound microscope at 100× magnification. Each histological section was evaluated by two separate readers to determine agreement on maturity stage. If there was a discrepancy in maturity staging for any specimen, the discrepancy was either resolved by the two readers or that specimen was not used in the analysis. There were no discrepancies between readers on any of the reproductively developing females. Maturity was assessed according to a modified version of the schedule used by Wenner et al. (1986) adapted to work with isochronal

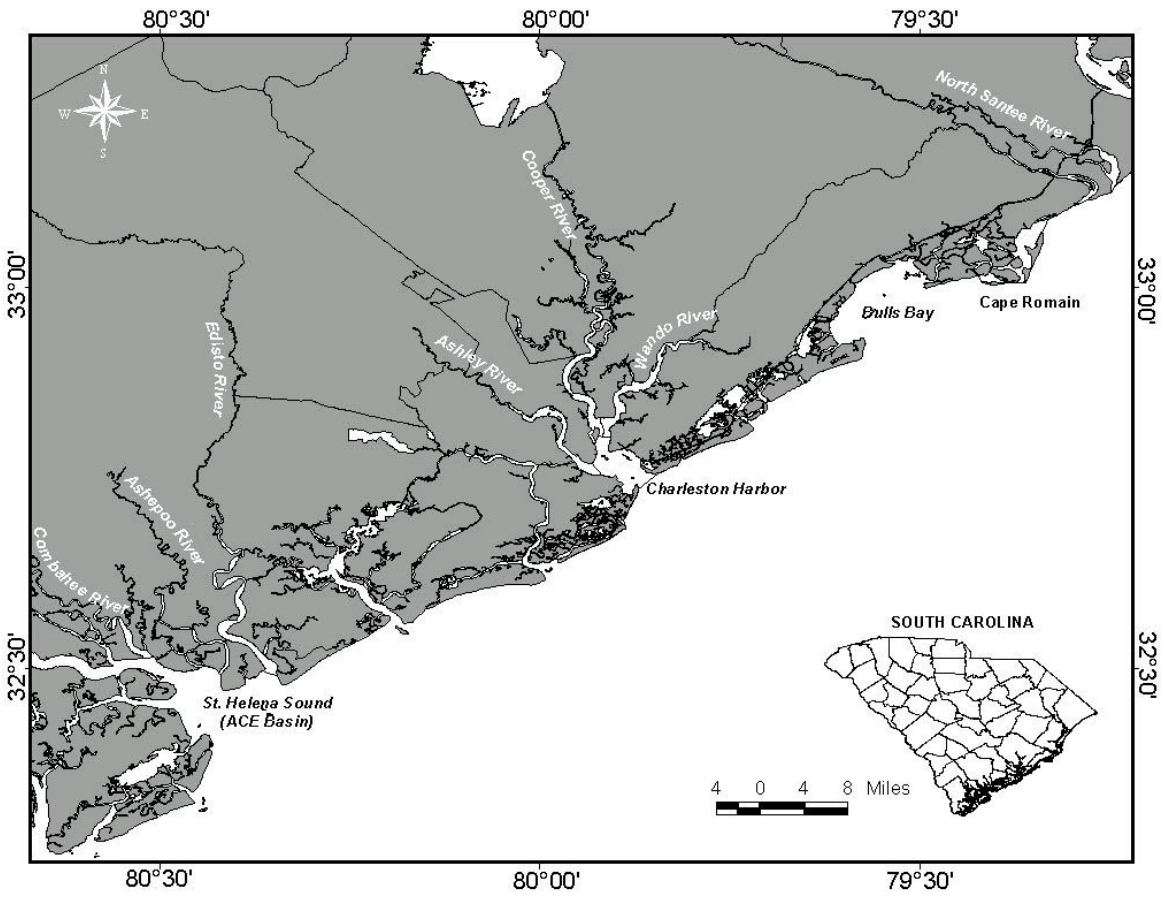

Figure 1

The South Carolina coast with the locations of estuaries and major river basins where striped mullet were collected from 1998 to 2000.

spawning fish, as well as previous models of reproductive development (Stenger, 1959; Wallace and Selman, 1981) (Table 1). This evaluation method was based on identification of morphological characteristics evident in histological sections. Each specimen was evaluated by two (in some cases three) readers and discrepancies between readers were either resolved or the specimen was excluded from the analysis.

A gonadosomatic index (GSI) was calculated for each specimen following the method of Render et al. (1995) where GSI was expressed as gonad weight (GW) divided by body weight (BW) such that

$$GSI = (GW/BW) \times 100.$$

The GSI values of the fecundity specimens were compared among the three sampling years, as well as with GSI values for female striped mullet collected during the rest of the year that were mature but not reproductively active.

Fecundity determinations were made from a total of 129 advanced-stage developing ovaries: 50 from 1998, 37 from 1999, and 42 from 2000. All of the ovaries were determined by histological examinations and criteria outlined in Table 1 to be actively vitellogenic, having tertiary yolk-stage oocytes. All oocytes counted for fecundity were 400 μm or larger and in the tertiary yolk stage (Pien and Liao, 1975) (Fig. 2). This 400-μm threshold has also been used in other studies of oocyte development in striped mullet for determining the point at which the oocytes that would be spawned during that year were identifiable (Shehadeh et al., 1973a). Unlike other species (particularly batch spawners), where fecundity counts should ideally be conducted by using hydrated oocytes only, the striped mullet oocytes used for fecundity counts were still presumably several weeks from hydration and spawning. However, because mullet are synchronous spawners, it is relatively easy to distinguish the developing oocytes from the undeveloped ones because of the drastic difference in size between the two, as well as by the uniformity in size of the developing oocytes once they reach 400 μm.

Fecundity was estimated by using a modified gravimetric method. The fixed whole ovary was patted dry and reweighed. The ovarian lobes were divided into four discrete regions along each lobe's longitudinal axis and three subsamples (chosen at random) were taken between the two lobes and preserved in 50% isopropyl until oocyte counts could be conducted. The subsamples ranged in weight from 0.025 g to 0.033 g. The subsamples from each specimen

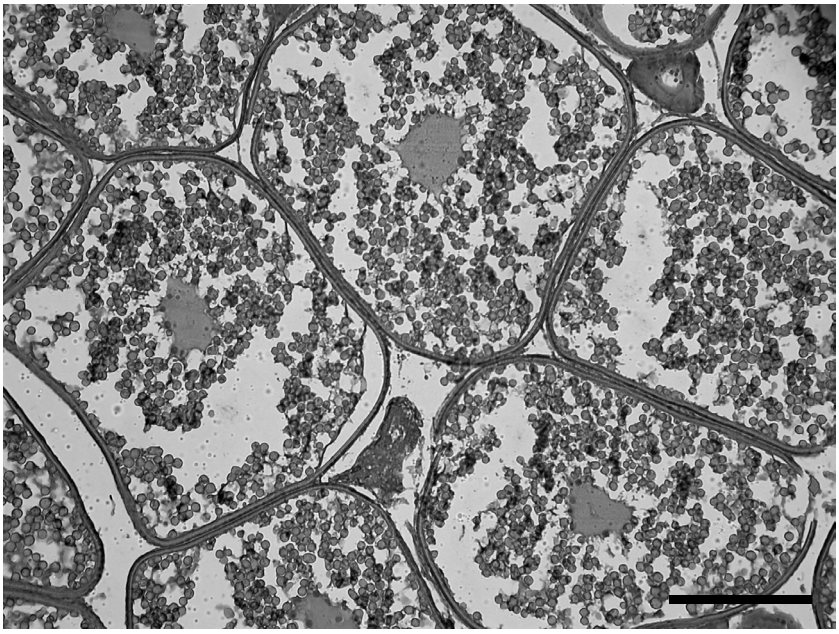

Figure 2
A striped mullet oocyte in the tertiary yolk stage of vitellogenesis. Scale bar = 250 μm.

Table 1
Histological criteria used to determine reproductive stage in female striped mullet (*Mugil cephalus*) once sexual differentiation has occurred (Wenner et al.: see footnote 2 in the general text).

Reproductive stage	Description
1. Immature	Inactive ovary with previtellogenic oocytes and no evidence of atresia. Oocytes are < 80 μm, lamellae still contain somatic and connective tissue bundles. Ovary wall is very thin (one or two cell layers).
2. Developing	Developing ovary have enlarged oocytes generally greater than 120 μm in size. Cortical alveoli are present and actual vitellogenesis occurs after oocytes reach 180 μm in size and continue to increase in size. Abundant yolk globules with oocytes reaching a size of >600 μm.
3. Running ripe	Completion of yolk coalescence and hydration in most oocytes.
4. Atretic or Spent	More than 30% of developed oocytes undergoing the atretic process.
5. Inactive or Resting	Previtellogenic oocytes only but traces of atresia possible. In comparison to immature females, most oocytes are >80 μm, lamellae have some muscle and connective tissue bundles. Lamellae are larger, have moore oocytes, and are elongated. A thicker ovarian wall with blood vessels, muscle, and nerve tissue.

were then teased apart. After separation, the oocytes were spread out on a Bogorov tray and counts of oocytes, greater than 400 μm, were made by using a dissecting microscope at 12× magnification. Each subsample was counted twice and counts were averaged. A third count was performed if the first two counts differed by more than 10%. Oocyte density was calculated by dividing the mean number of oocytes by the mean weight of all three subsamples for each specimen. The oocyte density was then used to calculate the total oocyte number for each ovary, or individual fecundity, by multiplying mean oocyte density by whole ovary weight.

In order to determine mean oocyte diameter for each specimen, 20–30 oocytes were removed from each counted subsample and grouped together in a petri dish. Each oocyte was then measured along the longest axis by using Optimas™ Image Analysis software (version 6, Media Cybernetics, Bothell,WA). Mean oocyte diameter was calculated as the average of all measurements for each

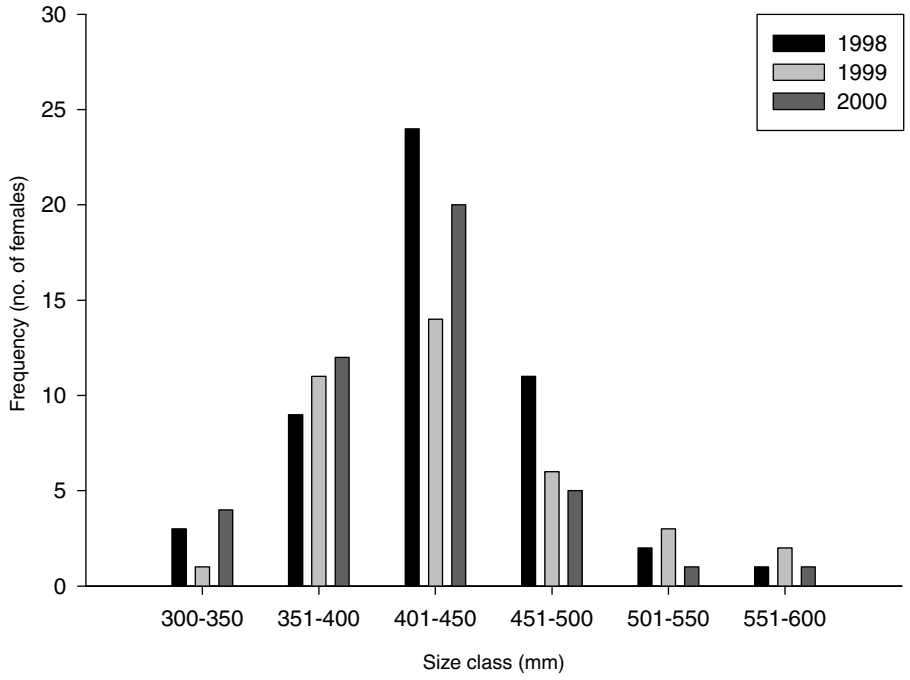

Figure 3

Size-frequency distribution of female striped mullet used in fecundity determinations from South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 50 fish for 1998, n = 37 for 1999, and n = 42 for 2000.

subsample. The overall mean oocyte diameter for each specimen resulted from the calculated average of the means of the three subsamples. Measurements were not made on fresh oocytes but shrinkage was estimated from the amount of whole ovary shrinkage because fresh ovary weight and preserved (in 10% formalin) ovary weight were known. The estimated unpreserved oocyte diameter was determined by multiplying the preserved oocyte diameter by 1 and adding the percentage of ovary shrinkage. The difference in the preserved oocyte diameter and the estimated fresh oocyte diameter was then compared by using a paired t-test to determine if there was a significant difference between preserved and unpreserved oocyte diameters.

Age was determined by using the left sagittal otolith, which was embedded in epoxy resin. A 0.5-mm transverse section encompassing the otolith core was cut with an Isomet low-speed saw with diamond wafering blades. The thin section of the otolith was embedded in epoxy and observed with a dissection microscope at the magnification appropriate for the otolith's size. Age was recorded as the number of rings (annular bands) present. The otoliths were initially aged by one reader. A second reader then evaluated a subsample of specimens from 1998 and 2000 and all the otoliths from 1999. Ages were validated by the percentage of agreement between the two age determinations, an analysis of variance (ANOVA) between the two groups of ages, and a paired t-test comparing the means and variances of the two groups (Campana et al., 1995).

Results

Fecund female striped mullet (again, defined as those females with ovaries containing oocytes >400 μm in the tertiary stage of vitellogenesis) were collected from late October through February; most of the specimens were caught in November and December for all three years. Size-frequency distributions did not vary over the three years of the study (Fig. 3). Fewer fish (n=37) were taken in 1999 versus 1998 (n=50) and 2000 (n=42). The most abundant size class for each year was that from 401 to 450 mm. In the overall size-frequency distributions, fecund females made up greater than 44% of those fish larger than 400 mm in 1998 and comprised all of the specimens over 500 mm. In 1999 the fecund females made up 12.5% of fish in the 401–450 mm size range, 39% of the fish in the 451–500 mm size range, and 100% of the fish in the size classes over 500 mm. In 2000, fecund females made up 17.7% of the 401–450 mm size class, 35.7% in the 451–500 mm size range and, like 1998 and 1999, all of the specimens over 500 mm.

The majority of females used in our fecundity study were 3 or 4 years old (Fig. 4), accounting for 80.0% of the specimens in 1998 and 73.3% in 1999. However, the age distribution in 2000 showed that the frequency of 2-, 3-, and 4-year-olds was the same and that these three ages made up 82.0% of the fecund fish sampled that year. Three-year-old fish made up the largest single group in 1998 and 1999. The age determined from the otoliths was validated as part

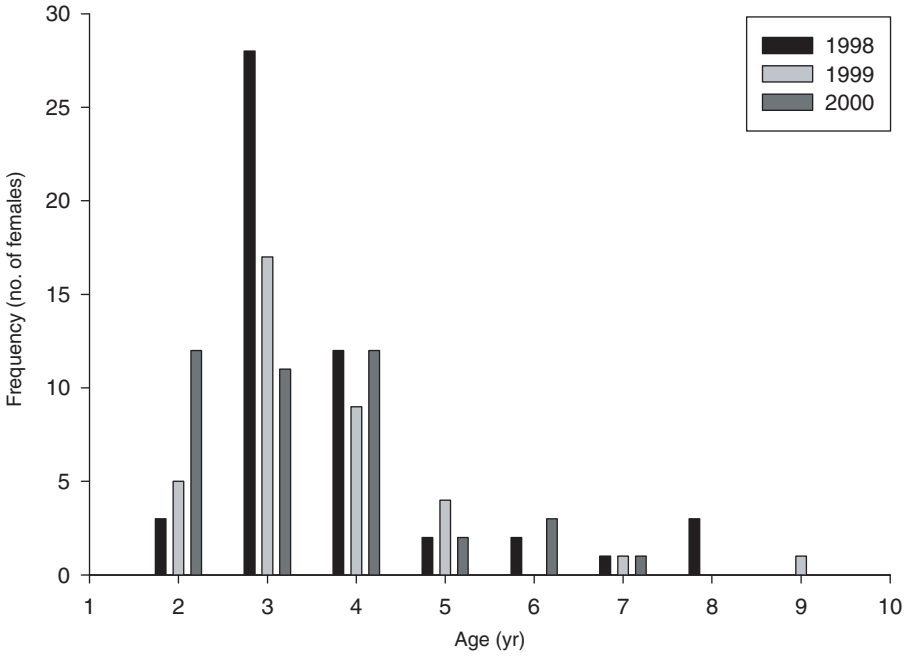

Figure 4

Age-frequency distribution of fecund striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 50 fish for 1998, n = 37 for 1999, and n = 42 for 2000.

of another study where size and age structure of striped mullet in South Carolina was examined (Wenner and McDonough, unpubl. data[3]). A comparison of multiple readings of the same group of otoliths assessed aging precision. One year (1999) was chosen at random and all of the otoliths (n=1234) from that year were aged by a second reader. The ages of the two independent determinations were then compared by using a one-way ANOVA and a t-test. The variance statistic was 2.78 for the original ages and 2.81 for the second age reads, which were not significantly different (P=0.001) and both had almost identical normalized residuals. Overall, there was an 83.4% agreement on ages between readers. The results from the ANOVA (F=1555.0, df=10, P=0.000) and the t-test (t=2.898, df=1233, significance [2-tailed]=0.004) both confirmed that there was no significant difference between the separate age determinations. Therefore, the age recorded by the first reader for all specimens was used in the analysis.

The length-weight relationship for fecund female striped mullet was compared by using a linear regression of (natural) log-transformed body weight against total length to see if there were any differences between years. The regression coefficients from each year were compared by using a test of significance between more than two slopes (Zar, 1984). The weight measurement used was total body weight minus gonad weight (TW–OW=BW) because ovary weight had a considerable influence on total body weight in the fecund specimens (GSI values of 7.7 to 27.7). There was no significant difference in the total length to Ln body weight regressions between different years (F=9.22, P=0.001, df=129). Because there was little difference in the regressions between years and in order to increase sample size, data from all three years were combined to obtain the overall total-length to Ln-body-weight relationship of Ln $BW = -11.1 + 2.92\ (TL)$ (Fig. 5). In contrast, the length and body-weight-at-age relationships were highly variable; a wide range of sizes occurred in the 2-, 3-, and 4-year age classes (Fig. 6). The high degree of variability was also exacerbated by the smaller number of fish age 5 or older.

The gonadosomatic index (GSI) for fecund mullet ranged from 7.7 to 27.5 in 1998, 9.3 to 27.7 in 1999, and 9.5 to 26.6 in 2000. In contrast, the GSI for mature females that were not undergoing any reproductive development ranged from almost zero to 4 for all three years of the study. The relationship of GSI to size (TL or BW) was not very strong in any year. However, GSI was positively correlated (P=0.01) with oocyte diameter and negatively correlated with oocyte density (Table 2) because of the inverse relationship of oocyte density and oocyte diameter. The correlation coefficient for GSI and age were very close to zero and slightly negative (Table 2).

Mean GSI by month for males and females (Fig. 7) increased from October through April, peaking in November–

[3] Wenner, C. A., and C. J. McDonough. 2001. Cooperative research on the biology and assessment of nearshore and estuarine fishes along the southeast coast of the U.S. Part IV: Striped mullet, *Mugil cephalus*. Final rep, Grant no. NA77FF0550, 82 p. Marine Resources Research Institute, South Carolina Dept. of Natural Resources, P.O. Box 12559 Charleston, S.C. 29422-2559.

December. The duration of the reproductive season, as evidenced by advanced reproductive condition determined by the GSI, was also confirmed from histological assessments of maturity stages for all gonads collected during this time period (not just those used for the fecundity study) which indicated that reproductively developing males were present August through February, whereas reproductively developing females were present August through April (Fig. 8).

There was no significant difference in oocyte density among subsamples taken from different areas of the ovary lobe. This result was obtained by using an ANOVA of oocyte densities between the four divided areas of the ovary lobes where subsamples were taken (F=0.421, df=3). This analysis allowed us to accept the assumption that oocytes were equally distributed throughout the ovary lobes, which provided validation for the random sampling of oocytes from different areas of the lobe in order to determine individual fecundity.

The regression of individual fecundity with total length (TL) was not a linear relationship, whereas the regression of fecundity on body weight (BW) was linear. Therefore, the comparisons of individual fecundity to total length (TL) and body weight (BW) were made by using both the raw data and the data with natural log transformations. The range of specimen total lengths was 291 to 600 mm in 1998, 332 to 588 mm in 1999, and 325 to 592 mm in 2000 and for body weight 242 to 2149 g in 1998, 335 to 2008 g in 1999, and 284 to 2144 g in 2000. Mean fecundity, compared between years with a two sample t-test, was significantly different between 1999 and 2000 (t=0.019, df=78, P=0.985) but was not significantly different between 1998 and 1999 (t=0.974, df=86, P=0.336)

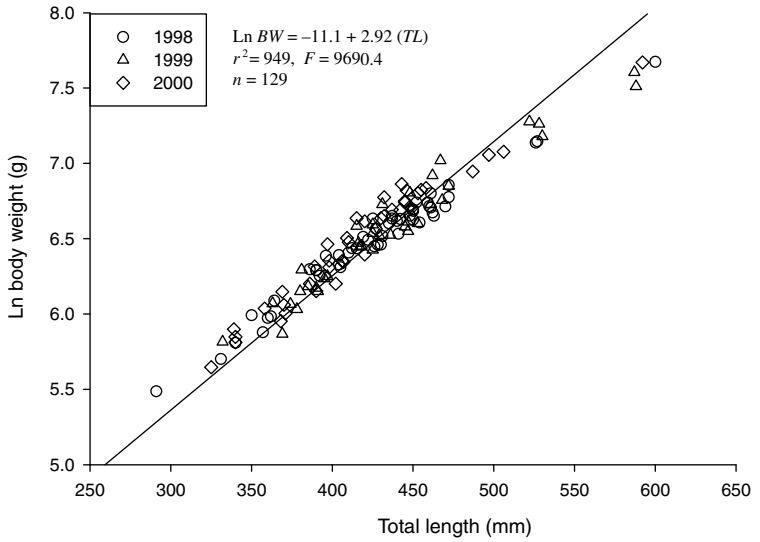

Figure 5

Regression analysis of log-transformed (Ln) body weight on total length for fecund striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129.

Table 2

Pearson correlation coefficients, with significance values, for the morphological variables and fecundity of striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129. TL = total length, BW = body weight, ODM = oocyte diameter, ODN = oocyte density, GSI = gonadosomatic index, FEC = fecundity.

		Age	TL	BW	ODM	ODN	GSI	FEC
Age	Pearson correlation	1.000						
	significance (2-tailed)							
TL	Pearson correlation	0.117	1.000					
	significance (2-tailed)	0.186						
BW	Pearson correlation	0.164	0.951**	1.000				
	significance (2-tailed)	0.062	0.000					
ODM	Pearson correlation	0.004	0.101	0.029	1.000			
	significance (2-tailed)	0.964	0.254	0.741				
ODN	Pearson correlation		–0.050	–0.008	0.095	–0.628**	1.000	
	significance (2-tailed)	0.575	0.927	0.282	0.000			
GSI	Pearson correlation	–0.029	0.128	–0.006	0.543**	–0.645**	1.000	
	significance (2-tailed)	0.746	0.147	0.947	0.000	0.000		
FEC	Pearson correlation	0.113	0.892**	0.888**	0.059	0.142	0.260**	1.000
	significance (2-tailed)	0.200	0.000	0.000	0.504	0.105	0.003	

** Correlation is significant at the 0.01 level (2-tailed).

and between 1998 and 2000 (t=1.368, df=92, P=0.179). However, given that the mean fecundity was 1.18 million oocytes in 1998, 1.16 million oocytes in 1999, and 1.09 million oocytes in 2000, the difference in mean fecundity between 1999 and 2000 was probably not biologically significant. It was determined that data could be pooled across years for several reasons. The coefficients of determination for each year indicated that there was a similarly strong relationship of fecundity to total length and body weight in all three years and the coefficients of variation for each year (0.408 for 1998, 0.594 for 1999, and 0.457 for 2000 at P=0.001) were not significantly different. By pooling the data from all three years we were able to determine two models of potential fecundity based on total length (TL) and body weight (BW) (Fig. 9):

$$\text{Ln } \textit{Fecundity} = -6.86 + 3.42(\text{Ln } \textit{Total Length}) \quad [r^2=0.803, F=527.2, \text{df}=129]$$

$$\text{Ln } \textit{Fecundity} = 6.95 + 1.05(\text{Ln } \textit{Body Weight}) \quad [r^2=0.804, F=530.6, \text{df}=129].$$

The r^2 values for untransformed data were very close to the values obtained with transformed data (r^2=0.795, F=502.9 for fecundity on total length [TL] and r^2=0.787, F=479.4 for fecundity on body weight [BW]). The high r^2 values, as well as the high correlation coefficients between fecundity and total length and body weight (Table 2) indicated that potential fecundity was size dependent.

Unlike fecundity, oocyte density did not change with size (TL or BW) in 1998 and 1999 and increased with size in 2000. The increase in density in 2000 was due to a group of fish captured in freshwater in October having relatively low GSIs and high densities of oocytes that also happened to be some of the largest fish captured that year. Oocyte density was negatively correlated with GSI (Table 2), and thus indicated that increasing GSI resulted in lower oocyte densities.

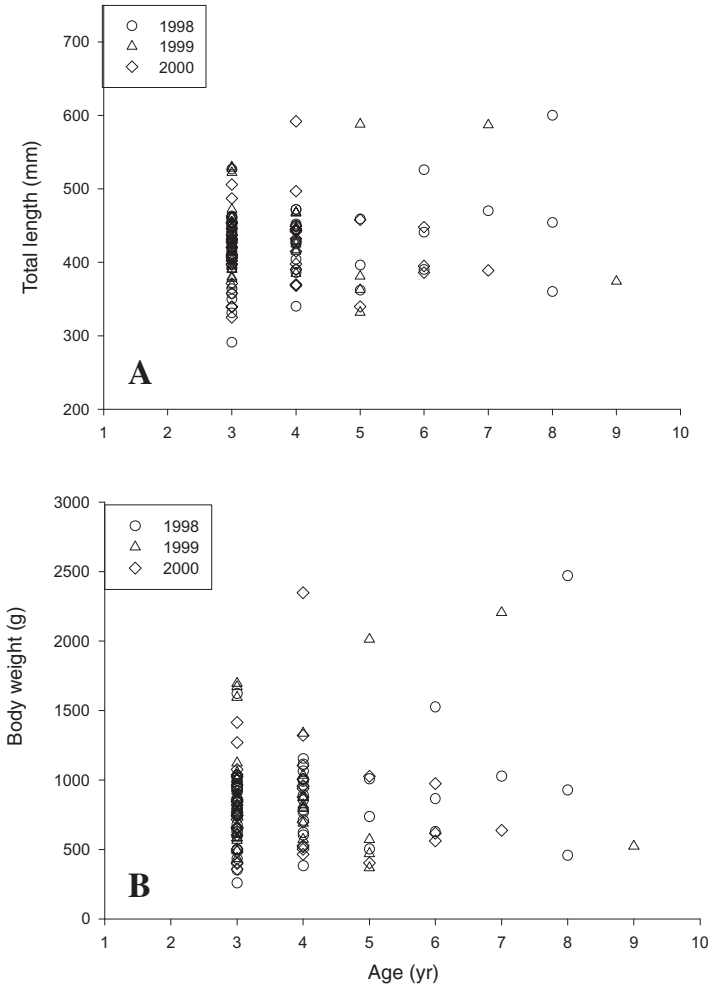

Figure 6

Relationship of total length (**A**) and body weight (**B**) to age for fecund striped mullet in South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 129.

There was not a high degree of variability in oocyte diameter over the entire size range for the three years of the study. Oocyte diameter did appear to increase with age in 2000 and remained stable for 1998 and 1999. However, the increase in oocyte diameter in 2000 was not statistically significant.

In a comparison of mean oocyte diameter in each size class (total length) by month of capture, the data for all three years were pooled in order to obtain adequate representation in each month. Oocyte size ranged from 463 to 682 μm and the mean size was 596 μm. The largest mean oocyte diameters were found in specimens captured in January and February. Specimens were captured during the months of November and December for all size classes and there was an increase in oocyte diameter with each progressive month. In particular, females in the 400–500 mm size range (which represented the largest number of specimens) were examined and there was a progression of increasing oocyte diameter with month of capture through the reproductive season.

The increase in oocyte size, as the reproductive season progressed, was more apparent when mean oocyte diameter by month for each year separately was examined. Specimens were collected from October through February in 1998, November through January in 1999, and October through December in 2000. Equal effort was made during all of these months of each year to capture specimens, but they were not always available for capture. There was an increase in mean oocyte size per month as the spawning season progressed in all three years (Fig. 10). Even though the largest oocyte size measured was 682 μm, this measurement was that of a preserved oocyte. If we factor in a mean shrinkage of 4%, maximum oocyte size becomes 709 μm.

The paired t-test showed no significant difference between the preserved oocytes and the predicted size of fresh oocytes (t=–26.2, df=128, P=0.000).

Discussion

Fecundity in striped mullet from South Carolina correlated highly with length and weight, but not with age. Oocyte counts ranged from 4.47×10^5 to 2.52×10^6 in 1998 for fish ranging in total length from 331 mm to 600 mm, 2.13×10^5 to 3.89×10^6 in 1999 for fish ranging in total length from 332 mm to 588 mm, and 3.89×10^5 to 3.01×10^6 in 2000 for fish ranging in total length from 325 mm to 592 mm. These fecundity levels correspond with general fecundity levels (2.0×10^5 to 14.0×10^6) found in striped mullet in northeast Florida (Greeley et al., 1987), the Gulf Coast (Render et al., 1995) as well as studies in Europe and Asia (review by Alvarez-Lajonchere, 1982). One marked difference in fecundity between the present study and some in the literature was the difference in oocyte density. Render et al. (1995) found densities ranging from 798 to 2616 oocytes/g ovary weight, whereas densities in the present study ranged from 1710 to 14,817 oocytes/g ovary weight. However, although fecundity increased with both total length and body weight in 1998 and 1999, densities did not. The lower oocyte densities in the larger fish were most likely indicative of larger oocytes. This feature is common in both synchronous and asynchronous spawning fishes (Greeley et al., 1987; Render et al., 1995; Fox and Crivelli, 1998; DeMartini and Lau, 1999). Because total length and body weight were more highly correlated with increased fecundity, the larger specimens would have made a greater individual reproductive contribution during any given spawning season (Korhola et al., 1996; Kaunda-Arara and Ntiba, 1997; DeMartini and Lau, 1999). Making estimates of potential fecundity from age alone was difficult because of the variability in the size-at-age relationship; however, fecundity estimates from total length or body weight appeared more reliable and reflected values closer to the fecundity levels observed in the present study.

Previous studies have reported that female mullet become reproductively mature at three years of age (Thomson, 1951; 1963; Stenger, 1959; Chubb et al., 1981; Render et al., 1995). Greely et al. (1987) suggested that fecundity specimens collected in northeastern Florida were as young as two years at maturity. But, Greely et al. (1987) did not age the striped mullet used in their study and inferred age from a size-at-age growth schedule (Thomson, 1966). In the present study, two-year-olds made up only a small percentage of the fecund fish in 1998 and 1999. However, the results were different for the 2000 specimens in that there was an almost equal distribution in the age frequency of two-, three-, and four-year- olds. However, three- and four-year-olds made up the greatest percentage of females with advanced ovaries. Maturing fish were those undergoing active vitellogenic development and were generally captured in and around inlets or estuaries. Our study would suggest that female striped mullet reach 50% maturity at age 2 and 100% maturity at age 3. Three- and four-year-olds made up the majority of reproductively advanced fish in all years, whereas less abundant older fish made less of a contribution towards total reproductive effort.

There are several possible explanations accounting for the wide age distribution in maturity stages; the most likely is that fecundity is size related despite the highly variable growth rates and the widely ranging size at age in adult striped mullet. Size at maturity has been found to range widely from 230 mm standard length (Thomson, 1963; Greeley et al., 1987; Tamaru et al., 1994) up to 410 mm standard length (Thomson, 1963; 1966; Chubb et al., 1981) for two- and three-year-old fish. The lower end of this size range agrees quite readily with the lower size range (291 mm TL=239 mm SL) found in our study. In a concurrent study of maturity schedules related to size and age in South Carolina striped mullet (McDonough, unpubl. data), male striped mullet were found to mature at two years of age and as small as 250 mm total length (190 mm standard length). Other species of mullet have been shown to mature over a wide range of sizes. The Pacific mullet (Mugil so-iuy) becomes mature upon reaching approximately 430 mm total length (Okumus and Bascinar, 1997).

Monthly GSI levels clearly showed that the time period of reproductive activity is from October through April. Female striped mullet in all reproductive developmental stages were observed during the course of our sampling, with the exception of stage-3 (hydrated oocytes) females and females with

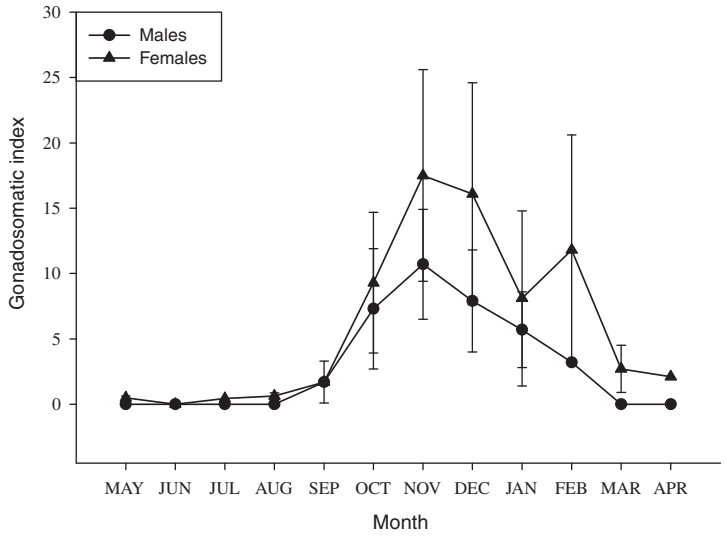

Figure 7

Mean gonadosomatic index value by month for male and female striped mullet from South Carolina estuaries from 1998 to 2000. n (number of fish in sample) = 455.

recently spawned ovaries (characterized by the presence of postovulatory follicles). Atretic ovaries were observed from December through May. There were no postovulatory follicles observed, indicating that any atretic ovaries were not from recently spawned fish. The fish with atretic ovaries were characteristically emaciated for their size (TL and BW) and were most common from January through March. The presence of females with atretic ovaries starting in December is strong evidence that spawning occurred in November, if not earlier, and females with atretic ovaries caught as late as May demonstrate that spawning may still occur as late as April. Additional evidence for the October through April spawning period has also been shown in backcalculated birth dates for juvenile striped mullet by daily growth increments (McDonough and Wenner, 2003). This evidence supports the concept of offshore spawning in striped mullet and a yet undetermined time period required for moving from the estuaries to the spawning areas and for returning again to the estuaries. Other authors have come to the same conclusion from similar evidence in estuaries throughout the southeast (Jacot, 1920; Broadhead, 1956; Anderson, 1958; Stenger, 1959; Shireman, 1975; Dindo and MacGregor, 1981; Greeley et al., 1987; Render et al., 1995; Hettler et al., 1997). All of the fecundity specimens were caught from October through February when the mean monthly GSI was highest. Pien and Liao (1975) found that mullet oocytes reached a hydrated size of 900 to 1000 μm. The size of oocytes used for fecundity counts in the present study ranged from 463 to 682 μm. The maximum size of oocytes in the tertiary stage of vitellogenesis from our study was 600 μm or greater. This result agrees with those of previous studies where the maximum size of oocytes prior to either hydration or atresia (if spawning did not occur) ranged from 600 to 700 μm (Shehadeh et al., 1973b; Kuo et al., 1974). There was no evidence of prespawning atresia in any of the specimens used for fecundity estimates.

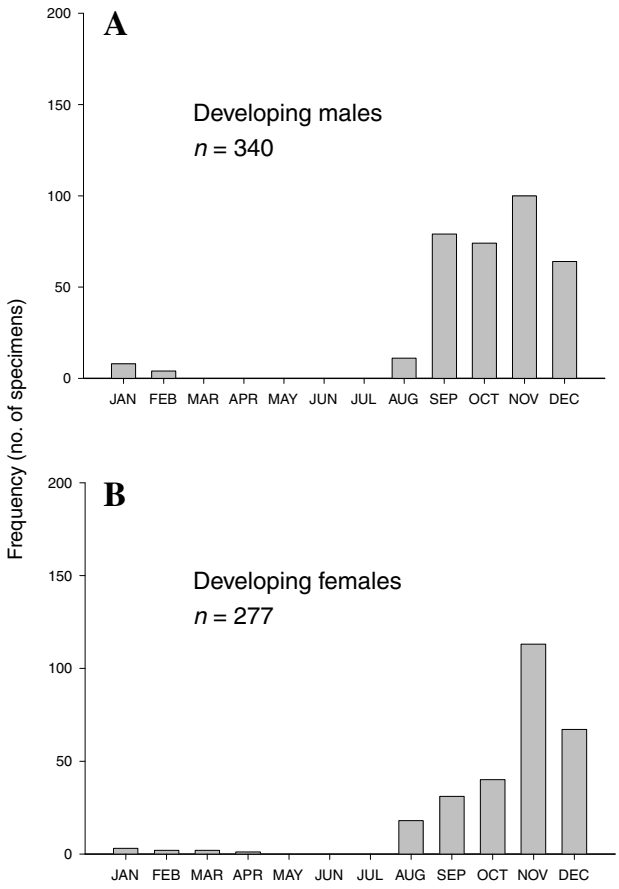

Figure 8

Frequency distribution of reproductively developing male (**A**) and female (**B**) striped mullet by month in South Carolina estuaries from 1998 to 2000.

The appropriateness of using a GSI alone to determine the level of reproductive development has been questioned, particularly for serial or asynchronous spawning fishes (De Vlaming et al., 1980; Hunter and Macewicz, 1985). Striped mullet can have a wide range of GSI values that range from practically zero to over thirty (Render et al., 1995). The GSI range for females in our study ranged from almost zero to 27.7. Because of the high variability in GSI with size, it does not appear appropriate to use GSI alone in order to assess reproductive development in striped mullet. When used in conjunction with histological analysis and mean oocyte diameter of tertiary-stage oocytes, GSI does provide excellent supporting evidence of reproductive schedules and spawning season duration. GSI is probably more appropriately used for isochronal spawning fishes than for serial spawning fishes because of the uniform development of oocytes in the former. However, it is still difficult to meet all the basic assumptions of the GSI index as given by De Vlaming et al. (1980) because of the high variability of GSI with size. Another technique that has been used in aquaculture situations to assess maturity and sex involves the use of a cannula to remove oocytes from the ovaries of live fish which are then evaluated (Shehadeh, et al., 1973a; Kuo et al., 1974). This technique, although useful for determining sex and the extent or stage of reproductive development, would be inappropriate for estimating potential fecundity.

Historically, reproductively developing mullet have been found in southeast United States waters from November through December (Jacot, 1920; Anderson, 1958; Stenger, 1959). During our study, reproductively developing mullet were caught in the Charleston Harbor Estuary from October through February. Gonad development of these fish was discernible through gross morphological observation, and histological sections showed that vitellogenesis was well underway. Other studies have shown that previtellogenic oocytes were usually less than 160 μm and that the onset of vitellogenesis began when the oocytes reached a size of 180 µm (Dindo and MacGregor, 1981; Greeley et al., 1987; Tamaru et al., 1994; Render et al., 1995). Developing individuals caught during our study that were not used for fecundity counts had less-developed ovaries, GSI values less than 7, and mean oocyte diameters less than

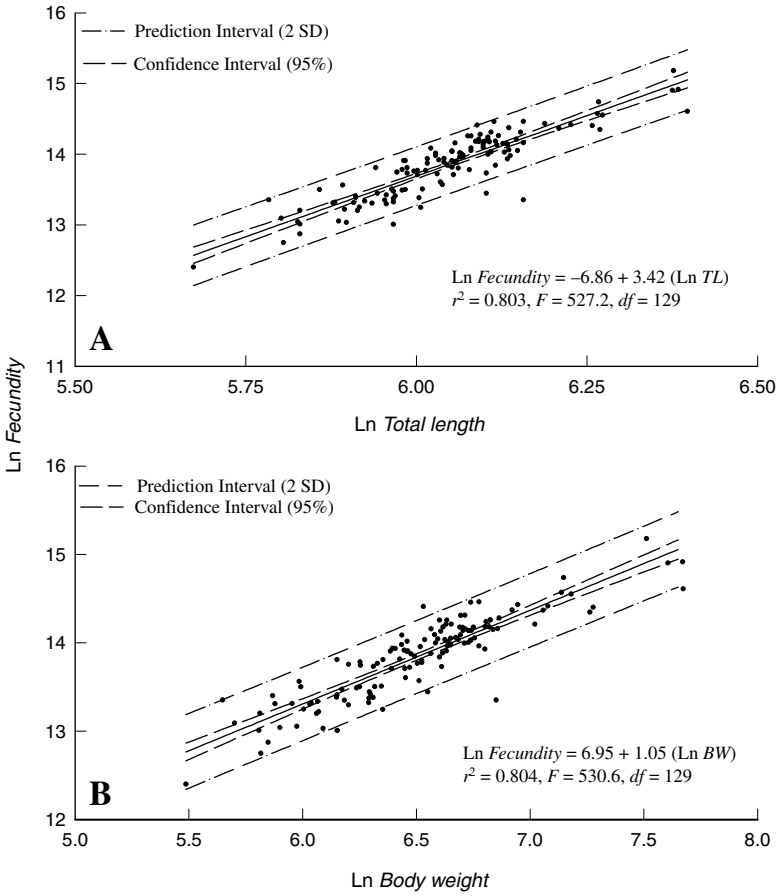

Figure 9

Regression of the natural log (Ln) transformation of individual fecundity on total length (**A**) and body weight (**B**) for striped mullet combined data, 1998 to 2000, from South Carolina estuaries. *n* (number of fish in sample) = 129.

350 μm. The vitellogenic activity in these ovaries was still in the primary and secondary stages. These specimens could not be used for fecundity counts because not all of the oocytes destined for that year's spawning batch had developed enough to be separable from the smaller oocytes that would not develop. Once the developing oocytes reached a size of 400 μm or larger, they became more uniform in size and in appearance and it was obvious which oocytes would constitute that year's spawn. At that point, fecundity could be determined more accurately because all the oocytes to be counted were significantly and equally larger. This point in particular is important in that it makes fecundity estimates from nonhydrated oocytes more accurate for isochronal spawning fishes, such as striped mullet. Fecundity estimates made in fishes that are batch-spawners should only be made from hydrated oocytes because of the presence of multiple developmental stages (Hunter and Macewicz, 1985). The presence of different vitellogenic stages in the ovary of a repeat-spawning fish makes it necessary to determine individual batch fecundity and spawning frequency before any estimate of annual fecundity can be made. In isochronal spawning fishes, such as striped mullet, this process is made simpler by the fact that oocytes mature at a similar rate (Greeley et al., 1987). During early vitellogenesis (180 μm to 350 μm), there is a higher degree of variability in the rate of development and a range of developmental stages would be present from the presence of precortical alveoli through secondary and tertiary vitellogenesis (Render et al., 1995). Estimating numbers of oocytes (uniform in an individual but varying in size because of season) during this stage would naturally make oocyte density and oocyte-size relationships inconsistent. This could possibly be corrected by using some timing factor such as month during the spawning season. The present study did demonstrate an inverse relationship between oocyte density and oocyte diameter. When month of capture was taken into consideration, oocyte density decreased with increasing oocyte size as the spawning season progressed.

In conclusion there were several biological aspects of striped mullet reproduction demonstrated in this study. Fecundity levels in striped mullet increased with total

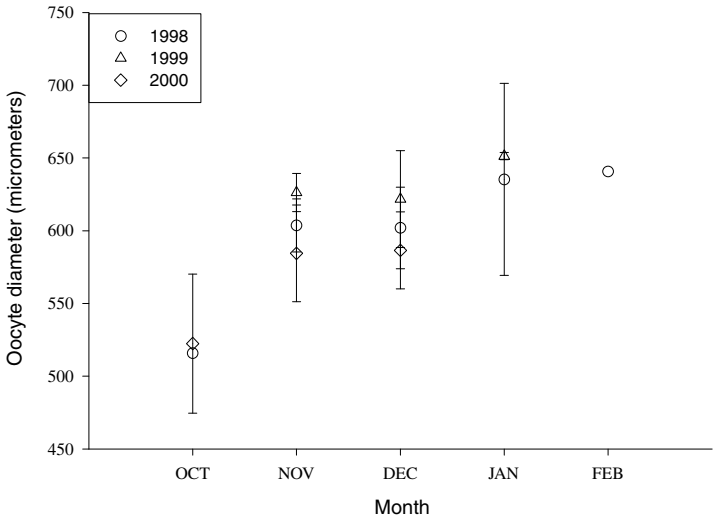

Figure 10

Mean oocyte diameter per month during the spawning season for striped mullet in South Carolina estuaries 1998 to 2000. Error bars represent standard deviation.

length (TL) and body weight ($BW=TW–OW$). Oocyte density remained relatively stable with size in the fecund fish and this allowed reasonable estimates of potential fecundity based on total length and body weight. Age-specific fecundity was highly variable and there appeared to be no consistent relationship. The reproductive season for striped mullet in South Carolina extends from October through April as determined by mean monthly GSI levels and histological confirmation of reproductive state. Potential spawning periods would also occur within this period as evidenced by the elevated GSI levels and the presence of atretic (or possible postspawning) ovaries from December through May. The gonadosomatic index itself is more useful to evaluate reproductive potential when used in conjunction with other techniques, such as histological analysis and oocyte diameter. The models of potential fecundity as they relate to size (total length and body weight) could be useful when applied to catch statistics of length and weight in populations with known size- and age- frequency distributions. This application would allow reasonable estimates of potential fecundity for these populations.

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