Reproductive dynamics of female spotted seatrout (*Cynoscion nebulosus*) in South Carolina*

William A. Roumillat

Marine Resources Research Institute
South Carolina Department of Natural Resources
217 Ft. Johnson Rd.
Charleston, South Carolina 29412
E-mail address: roumillatb@mrd.dnr.state.sc.us

Myra C. Brouwer

South Atlantic Fishery Management Council
One Southpark Center; suite 306
Charleston, South Carolina 29407

Abstract—We describe reproductive dynamics of female spotted seatrout (*Cynoscion nebulosus*) in South Carolina (SC). Batch fecundity (BF), spawning frequency (SF), relative fecundity (RF), and annual fecundity (AF) for age classes 1–3 were estimated during the spawning seasons of 1998, 1999, and 2000. Based on histological evidence, spawning of spotted seatrout in SC was determined to take place from late April through early September. Size at first maturity was 248 mm total length (TL); 50% and 100% maturity occurred at 268 mm and 301 mm TL, respectively. Batch fecundity estimates from counts of oocytes in final maturation varied significantly among year classes. One-year-old spotted seatrout spawned an average of 145,452 oocytes per batch, whereas fish aged 2 and 3 had a mean BF of 291,123 and 529,976 oocytes, respectively. We determined monthly SF from the inverse of the proportion of ovaries with postovulatory follicles (POF) less than 24 hours old among mature and developing females. Overall, spotted seatrout spawned every 4.4 days, an average of 28 times during the season. A chronology of POF atresia for water temperature >25°C is presented. Length, weight (ovary-free), and age explained 67%, 65%, and 58% of the variability in BF, respectively. Neither RF (number of oocytes/g ovary-free weight) nor oocyte diameter varied significantly with age. However, RF was significantly greater and oocyte diameter was smaller at the end of the spawning season. Annual fecundity estimates were approximately 3.2, 9.5, and 17.6 million oocytes for each age class, respectively. Spotted seatrout ages 1–3 contributed an average of 29%, 39%, and 21% to the overall reproductive effort according to the relative abundance of each age class. Ages 4 and 5 contributed 7% and 4%, respectively, according to predicted AF values.

Manuscript submitted 13 May 2002
to Scientific Editor's Office.

Manuscript approved for publication
19 March 2004 by the Scientific Editor.

Fish. Bull. 102:473–487 (2004).

The spotted seatrout (*Cynoscion nebulosus*) is an estuarine-dependent member of the family Sciaenidae. Spotted seatrout are year-round residents of estuaries along the South Atlantic coast and spawning takes place inshore and in coastal areas (McMichael and Peters, 1989; Mercer[1]; Luczkovich et. al.[2]). As in many other sciaenids, spawning in this species occurs in the evening (Holt et al., 1985). Male spotted seatrout have the capacity to produce "drumming" sounds that are caused by the contraction of the swimbladder by specialized muscles that are seasonally hypertrophied from the abdominal hypaxialis muscle mass (Fish and Mowbray, 1970; Mok and Gilmore, 1983). Direct involvement of sound production with spawning has been shown for this and other sciaenids (Mok and Gilmore, 1983; Saucier et al., 1992; Saucier and Baltz, 1993; Luczkovich et al.[2]).

We have collected information on the spawning behavior of spotted seatrout in coastal South Carolina since 1990 (Saucier et al., 1992; Riekerk et al.[3]). Spawning aggregations were located by listening for drumming sounds from late afternoon until ~2300 h with passive hydrophone equipment. Spawning activity was subsequently verified through collections of newly spawned eggs and by the rearing of the larvae in the laboratory (Saucier et al., 1992).

Spotted seatrout are group-synchronous spawners with indeterminate fecundity and the protracted spawning season extends from April through September along the South Atlantic and Gulf of Mexico coasts (Overstreet, 1983; Brown-Peterson et al., 1988; McMichael and Peters, 1989; Saucier and Baltz, 1993; Brown-Peterson and Warren, 2001; Brown- Peterson et al., 2002; Nieland et al., 2002, Brown-

* Contribution 539 from the Marine Resources Research Institute of the South Carolina Department of Natural Resources, Charleston, SC 29422-2559.

[1] Mercer, L. P. 1984. A biological and fisheries profile of spotted seatrout, *Cynoscion nebulosus*. Special Scientific Report 40, 87 p. North Carolina Department of Natural Resources and Community Development, Division of Marine Fisheries, Morehead City, NC 28577.

[2] Luczkovich, J. J., H. J. Daniel III and M. W. Sprague. 1999. Characterization of critical spawning habitats of weakfish, spotted seatrout and red drum in Pamlico Sound using hydrophone surveys. Final report and annual performance report F-62-2 and F-62-2, p 65–68. North Carolina Department of Environment and Natural Resources, Division of Marine Fisheries, Morehead City, NC 28557.

[3] Riekerk, G. H. M., S. J. Tyree, and W. A. Roumillat. 1997. Spawning times and locations of spotted seatrout in the Charleston Harbor Estuarine System from acoustic surveys, 21 p. Final Report to Charleston Harbor Project, Bureau of Ocean and Coastal Resources Management, South Carolina Department of Health and Environmental Control, 1362 McMillan Ave., Charleston, SC 29405.

Peterson, 2003; Wenner et al.[4]). As in other indeterminate spawning fish, annual fecundity in this species is determined by the number of oocytes released during each spawning event (batch fecundity) and the number of spawning events occurring during the course of the spawning season (spawning frequency). Early efforts to estimate fecundity for spotted seatrout did not take into account the repetitive nature of spawning activities in this species (Pearson, 1929; Sundararaj and Suttkus, 1962; Overstreet, 1983) and only recently has an effort been made to coordinate batch fecundities with spawning frequencies (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002). This procedure is intuitively necessary to estimate the reproductive output for an entire spawning season and is made even more useful for fisheries management if separated by size class or age cohort within a population (Prager et al., 1987; Goodyear, 1993; Zhao and Wenner[5]).

An important component of assessment for management involves determining the spawning potential ratio (SPR), a measure of the effect of fishing on the reproductive potential of a stock (Goodyear, 1993). This value is usually calculated as the ratio of spawning stock biomass per recruit (SSBR) in the presence of fishing mortality (F) to the SSBR when F is equal to zero (Gabriel et al., 1989; Goodyear, 1993). Spawning potential ratio is currently used as a biological reference point for definition of recruitment overfishing (i.e., Vaughan et al., 1992). The calculation of SPR can be improved, however, by introducing egg production into the model. Fecundity is a much better predictor of reproductive potential than female biomass. Moreover, SPR calculations based on egg production may be more sensitive to the size-age composition of the spawning stock. However, accurate annual fecundity estimates for use in stock assessment do not exist for this or many other species in need of fisheries management. Therefore, our goal was to obtain batch fecundity (BF), spawning frequency (SF), and annual fecundity (AF) estimates for spotted seatrout by age class.

Materials and methods

Data to address the main objectives of this study were collected from late April through early September 1998–2000 as part of a long term monitoring effort (1991–present) to assess the relative abundance of age classes of recreationally important finfish in South Carolina estuaries. The study followed a monthly stratified random sampling design in three estuarine systems. The Cape Romain system comprised two strata; Romain Harbor and northern Bulls Bay. The Charleston Harbor system contained four strata: the Wando, Cooper, and Ashley Rivers, and Charleston Harbor. The Ashepoo-Combahee-Edisto (ACE) Basin system comprised a single stratum (Fig. 1). The number of sampling sites within each stratum ranged from 23 to 30. A subset of 12–14 sites was randomly selected each month. Sampling was conducted only during the daytime ebbing tide (0700–1800 h), primarily over mud and oyster shell substrates adjacent to the *Spartina alterniflora* marsh. At each site, we deployed a trammel net (182.8 m long by 2.4 m deep; outer walls: 17.8 cm square [35.6 cm stretch]; inner wall: 3.2 cm square [6.4 cm stretch]) from a rapidly moving shallow water boat in an arc against the shoreline at depths ranging from 0.5 to 2.0 m. We disturbed the water within the site in an effort to frighten fishes into the entrapment gear. We then hauled the trammel net back into the boat and removed the catch, which was kept alive in a 70-liter oxygenated holding tank. Spotted seatrout were measured for total length (TL) and standard length (SL) and a subsample of fish from each effort (5–10 individuals for each 20-mm size interval per month) were sacrificed, placed on ice, and transported to the laboratory for aging and reproductive data.

Specimens were processed in the laboratory 2–12 hours after capture. We recorded standard life-history parameters (TL, SL, fish weight, gonad weight, sex, and maturity) for each specimen. The following equation was used to convert lengths when necessary:

$$TL = 5.689 + 1.167(SL)\ (r^2=0.998) \qquad n=1191.$$

We removed sagittal otoliths for aging and preserved sections (<2% by weight) of each ovary in neutral buffered formalin for histological processing. The latter involved standard procedures for paraffin embedding and sectioning, and standard hematoxylin and eosin-y staining (Humason, 1972). Histological sections were viewed under a Nikon Labophot compound microscope equipped with a teaching head so that two readers could interpret sections simultaneously. Maturity estimation was modified from that of Wenner et al.[4] (Table 1).

Size at first maturity was histologically derived by first evidence of cortical alveoli stage oocytes. To arrive at estimates of 50% and 100% maturity, data were subjected to PROBIT analysis.

Age determination

The left sagittae were marked with a soft lead pencil through the core and embedded in epoxide resin. A transverse section (~0.5-mm thick) was taken through the core by using a low-speed saw equipped with a pair

[4] Wenner, C. A., W. A. Roumillat, J. E. Moran Jr., M. B. Maddox, L. B. Daniel III, and J. W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: part 1. Final Report F-37, 177 p. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

[5] Zhao, B., and C. A. Wenner. 1995. Stock assessment and fishery management of the spotted seatrout, *Cynoscion nebulosus*, on the South Carolina coast, 90 p. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

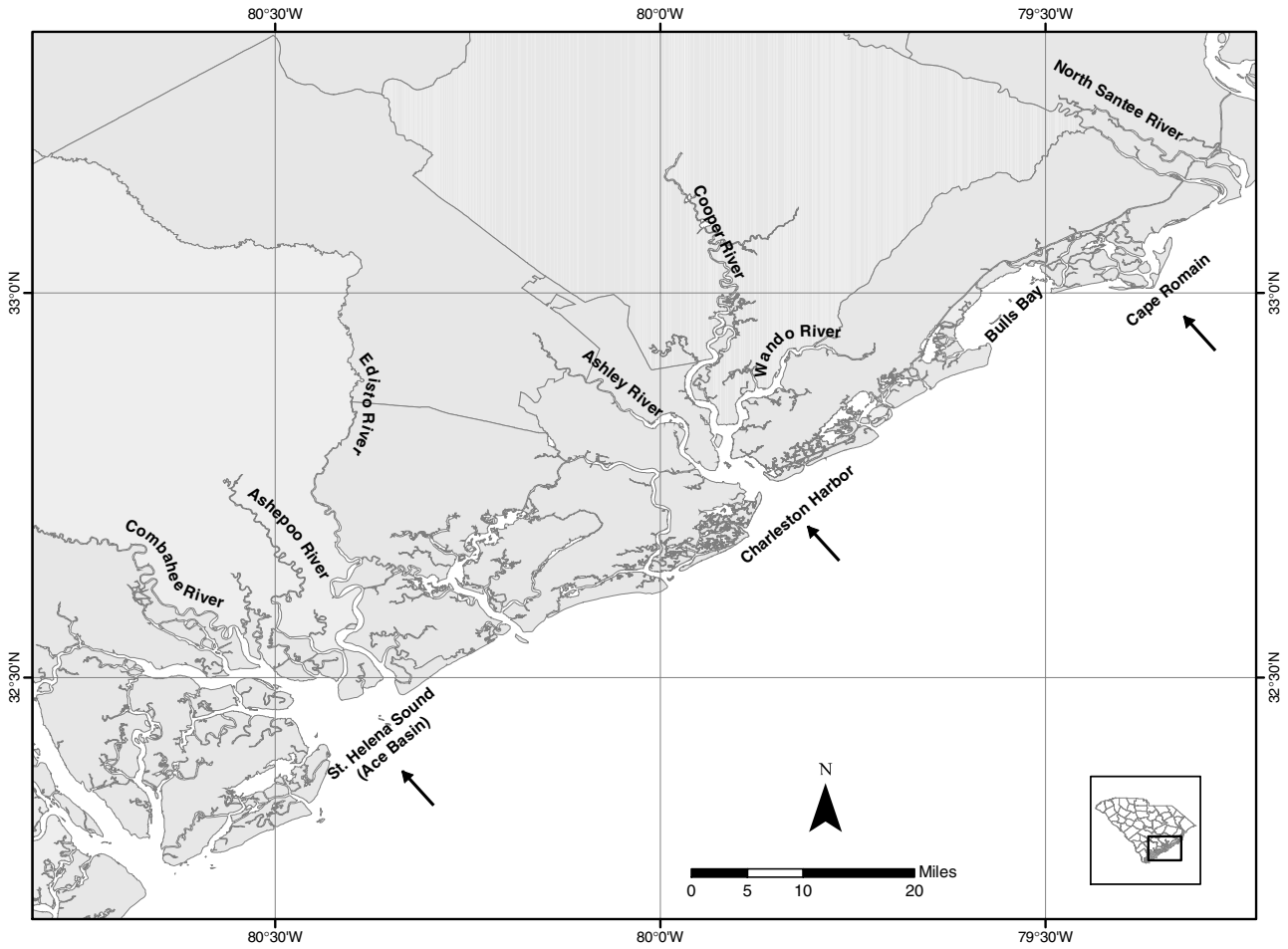

Figure 1

The three South Carolina estuarine systems (indicated by arrows) where *C. nebulosus* were collected.

Table 1

Criteria used for microscopic staging of *C. nebulosus* ovaries. FOM = final oocyte maturation; POF = postovulatory follicle.

Stage	Description
Immature	Ovary small in cross section. Early stage with only oogonia evident; later stage with small (<0.08 mm) primary oocytes, tightly packed. No evidence of early vitellogenesis.
Developing	First appearance of cortical alveoli stage oocytes through late vitellogenesis but no evidence of early FOM (lipid and yolk globule coalescence).
Ripe	Ovary containing oocytes demonstrating FOM (lipid and yolk coalescence through hydration).
Mature with day-0 POFs	Ovary exhibits POFs <24 h (see Table 2). Found at all water temperatures throughout the spawning season.
Mature with day-1 POFs	Ovary exhibits POFs >24 h consisting of closely packed granulosa cells (0.08–0.1 mm). Only identified in water < 25°C.
Spent	Ovary containing alpha- and beta-stage oocytic atresia.
Resting	Ovary containing small primary oocytes and oocytes with perinuclear nucleoli (<0.12 mm); usually some remnants of oocytic atresia.

of diamond wafering blades. The resulting section was mounted on a labeled microscope slide and examined with a Nikon SMZ-U microscope. A 1 January birth date was assumed for the process of aging. Spotted seatrout deposit an annulus in April or May (Murphy and Taylor, 1994; Wenner et al.[4]). Fish in the first three months of

Table 2

Criteria used for microscopic staging of *C. nebulosus* day-0 postovulatory follicles (POFs) in water temperatures above 25°C. Measurements represent longest axis of POFs.

POF chronology (in hours)	Description
0–4	Regular arrangement of granulosa-cell nuclei proximal to the basement membrane and obvious multiple layering as described by Hunter and Macewicz (1985). 200–300 μm (Fig. 4A).
5–8	Early signs of atresia, loss of the obvious layering, hypertrophy of granulosa cells, and a general compaction with an investment of blood vessels. 180–250 μm (Fig. 4B).
9–12	Well-defined lumen separating the internal granulosa cells from the outer wall of granulosa cells encompassed by theca. 150–200 μm (Fig. 4C).
13–24	Lumen reduced primarily by loss of granulosa tissue and proximity of peripheral layers. 130–175 μm (Fig. 4, D and E).

the year were aged by the addition of 1 year to the count of the number of annuli on the thin sections. In April or May, if the section had a large marginal increment, one was also added to the annular count. If the marginal increment was small or if the ring was detectable on the edge of the otolith section, age was equal to the number of annuli.

Seasonality

Spawning season for spotted seatrout in South Carolina was determined by using two techniques. The gonadosomatic index (GSI) was calculated as

$$(GW/OFWT) \times 100,$$

where GW = gonad weight (g); and
$OFWT$ = ovary-free weight (g).

For years prior to this study (1991–97), mean monthly GSI was obtained for all females by using data from the South Caroline Department of Natural Resources inshore fisheries archives (Wenner[6]). Reproductive seasonality among female spotted seatrout throughout the year was also examined by using histology (Table 1).

The first evidence of oocytes in final oocyte maturation (FOM) as evidenced by lipid and yolk coalescence; Brown-Peterson et al., 1988) or the occurrence of postovulatory follicles (POFs) defined the beginning of the spawning season. To determine the cessation of spawning, the percent occurrence of females in spawning condition (ripe and repeat spawners) and those in postspawning condition (spent and resting) were obtained for the months of August and September. To investigate the condition of females, we examined Fulton's condition factor (Ricker, 1975) over the spawning season using linear regression.

[6] Wenner, C. 2002. Unpubl. data. Marine Resources Research Institute, Marine Resources Division, South Carolina Department of Natural Resources, 217 Ft. Johnson Rd., Charleston, SC 29412.

Spawning frequency

We obtained samples for spawning frequency (SF) determination from 1 May through 31 August 1998, 1999 and 2000. Although samples were routinely collected throughout the year, only from early May through late August did we capture enough animals in the appropriate reproductive state for SF estimation. Spawning frequency was calculated as either the inverse of the proportion of ovaries with day-0 POFs (Hunter and Macewicz, 1985; Brown-Peterson et al., 1988) or with oocytes in FOM (Brown-Peterson et al., 1988; Lisovenko and Adrianov, 1991) among mature and developing females.

We designated two distinct morphological features of POFs based on time of specimen capture and water temperature. We interpreted the largest, least atrophied POFs to be <24 h old and termed them "day-0" POFs (Hunter and Macewicz, 1985). The presence of day-0 POFs in the ovary indicated that spawning had occurred the previous night. The second category comprised smaller POFs, which primarily consisted of closely packed granulosa cells determined to be >24 h old.

To complete the chronology of POF atresia we undertook round-the-clock sampling on 27–28 June and 26 July 2000. During these efforts, sampling continued beyond routine hours to encompass the period between dusk and dawn. The histological samples obtained allowed for the calibration of criteria used to age POFs (Table 2). To determine whether SF varied among months and age classes, Kruskal-Wallis tests were used. Because both factors (month and age) were fixed (model 1), it was not possible to test for their interaction by using a two-way parametric ANOVA without replication.

As a result of targeting fish for batch fecundity estimates (see below), we had available numerous specimens with oocytes in FOM with which to establish monthly SF. However, we knew that these specimens were disappearing from our shallow sampling sites into deeper spawning areas as the day progressed (Riekerk et al.[3]),

thus potentially adding bias to our SF estimates. Even though estimates of SF based on FOM were performed *a posteriori*, we chose to report them strictly for comparison to other studies with this method. Because sampling for females exhibiting FOM was accomplished in a directed fashion, statistical comparisons were not attempted.

Batch fecundity and relative fecundity

Observations taken over a decade of sampling the Charleston Harbor estuarine system showed that in females captured in shallow water (<1.5 m) during the spawning season, FOM began at about 1200 h (Wenner[6]). Similarly, Crabtree and Adams[7] reported FOM beginning in Florida spotted seatrout at about mid-day. Lowerre-Barbieri et al.[8] found hydrated females in shallow water in the vicinity of aggregations of drumming males in deeper water. We also speculated that from mid- to late afternoon hydrated females moved along the marsh edge toward deeper water spawning aggregations (8–25 m). Hydrophone surveys conducted in the Charleston Harbor area over several years (Riekerk et al.[3]; Wenner[6]) indicated that noise production typically began around 1800 h and ceased around 2200 h. Because this behavior has been associated with spawning in this and other sciaenids (Mok and Gilmore, 1983; Holt et al., 1985; Saucier et al., 1992; Saucier and Baltz, 1993), we assumed that spawning began at 1800 h and stopped at 2200 h. Thus we were able to target spotted seatrout in the mid- to late afternoon specifically to capture females with oocytes in the late stages of FOM for batch fecundity (BF) estimation. Because we have consistently identified recently spawned females in shallow areas, they apparently return to the marsh edge where they once again become available for capture with our sampling gear. Our stratified random sampling of estuarine areas along the coast (described previously) was designed to representatively sample these recently spawned females for SF estimation.

We conducted BF sampling during two consecutive afternoons fortnightly from the middle of April through the first week of September 1998, 1999, and 2000. We deployed a trammel net from a shallow water boat as described above at preselected sites in Charleston Harbor in depths ranging from 1.0 to 1.5 meters during the afternoon (1400–1800 h EDT) high tide.

Restricting our sampling to the hours immediately preceding the evening spawning event ensured that those females preparing to spawn were available for capture. Male spotted seatrout, identified by their drumming sounds, caught during this targeted effort were measured and released at the site of capture. We supplemented samples for BF estimation with specimens from local sportfishing tournaments held during summer months in the Charleston Harbor area.

We processed samples in the laboratory as previously described. If ovaries appeared by macroscopic examination to contain hydrated oocytes, they were fixed in 10% buffered seawater formalin for potential counts (Hunter et al., 1985). The appropriateness of these ovaries for BF counts was subsequently determined by examining the corresponding histological preparation.

To ensure that only those oocytes destined to be ovulated during the upcoming spawning event were counted, we chose to use only those oocytes undergoing FOM that could be easily separated by size from late vitellogenic oocytes (Nieland et al., 2002; Lowerre-Barbieri et al.[8]). If we observed numerous recent POFs in the histological sample, the corresponding whole ovary was not used for oocyte counts (because their presence indicated that ovulation had occurred). We reweighed ovaries (approximately 2 weeks after fixation) to the nearest 0.01 g and randomly extracted three 130–150 mg aliquots from eight potential locations in the ovary (each lobe was partitioned into quarters lengthwise). We stored subsamples in 50% isopropyl and counted oocytes under a Nikon SMZ-U dissecting microscope at 12× magnification. We counted each subsample twice by using a Bogorov tray and a hand-held counter and conducted a third count if the two initial counts were dissimilar by more than 10%. We used the mean number of oocytes in each subsample to calculate mean oocyte density (number of oocytes per gram preserved ovary weight) and total numbers of oocytes in the ovary. We compared mean oocyte densities among the four regions of each ovarian lobe and between the two lobes by using a two-way analysis of variance (ANOVA). Because our variances were heteroscedastic, we used nonparametric ANOVA (Kruskal-Wallis or ANOVA on ranks) for comparisons of mean BF among ages, months, and years. To investigate the relationships between BF and length, somatic weight (ovary-free body weight), and age, we used linear regression.

Relative fecundity (RF) was calculated as the number of oocytes per gram somatic weight (ovary-free). To select samples for inclusion in RF calculations, we looked for the presence of nuclear migration in histological preparations. We used this criterion to ensure that oocytes of similar morphological dynamics would be used, minimizing the potential for error. We used the Kruskal-Wallis test to investigate the effect of age on RF. Because sample sizes were quite uneven among months, we chose to compare RF between the beginning and end of the spawning season (May and August). This comparison was done by using a Mann-Whitney test. To corroborate any trends in RF, we also conducted diameter measurements on the preserved (10% buffered

[7] Crabtree R. E., and D. H. Adams. 1998. Spawning and fecundity of spotted seatrout, *Cynoscion nebulosus*, in the Indian River Lagoon, Florida. *In* Investigations into nearshore and estuarine gamefish abundance, ecology, and life history in Florida, p. 526–566. Tech. Rep. for Fed. Aid in Sport Fish Rest. Act Project F-59. Florida Marine Research Institute, Department of Environmental Protection, 100 Eighth Ave. SE, St. Petersburg, FL 33701.

[8] Lowerre-Barbieri, S. K., L. R. Barbieri, and J. J. Albers. 1999. Reproductive parameters needed to evaluate recruitment overfishing of spotted seatrout in the southeastern U.S. Final report to the Saltonstall-Kennedy (S-K) Grant Program (grant no. NA77FD0074), 23 p.

seawater formalin) oocytes. We used a video camera mounted on a Nikon SMZ-U dissecting microscope and coupled to a PC equipped with a frame-grabber and with OPTIMAS® Image Analysis software (version 6, Media Cybernetics, Bothell, WA). Two readers independently measured the diameter of approximately 30 preserved oocytes in each of three subsamples from 27 ovaries. To test for uniformity of size throughout the ovary, mean oocyte diameters were compared between ovarian lobes and among subsample locations within each lobe by using two-way ANOVA. We also compared mean oocyte diameters among months and ages by using two-way ANOVA.

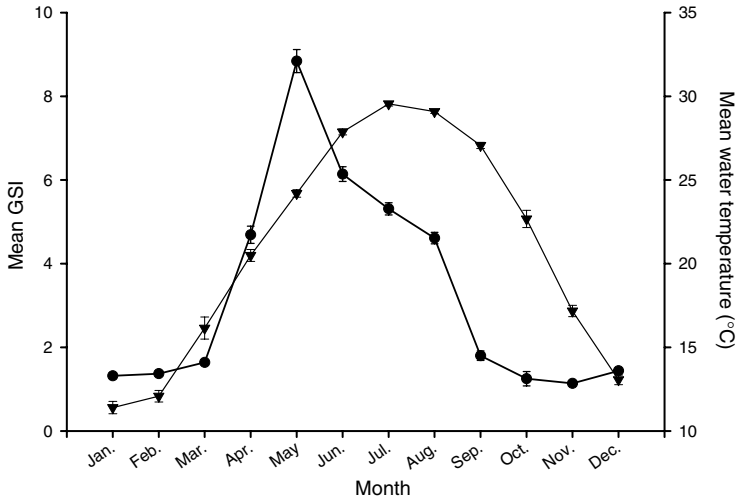

Figure 2

Mean monthly gonadosomatic index (GSI) for spotted seatrout in South Carolina for years 1991–2000 (circles). Mean water temperature for 1991–2000 (triangles). Error bars are standard errors. n = 1185.

Annual fecundity

Wiley (1996) demonstrated that spotted seatrout in South Carolina estuaries constitute a single population. Therefore, we felt justified in calculating monthly egg production (MEP) by multiplying the monthly SF (of specimens taken along the entire coast) by the mean monthly BF (of specimens from Charleston Harbor). Because not all age-1 female trout were mature at the beginning of the spawning season, the fraction of mature age-1 females obtained from previous work in South Carolina (Wenner[6]) was used to refine the MEP estimate. Because the latter was calculated by using SF obtained from data pooled across years, any comparison of MEP among years was deemed invalid. Kruskal-Wallis tests were used to determine whether MEP varied among months for each age class.

Monthly MEP estimates were summed to arrive at an annual fecundity (AF) estimate for each age class. Because the majority of individuals used in this study were aged 1–3, AF was estimated only for these age classes. We used linear regression to investigate the relationship between AF and age and thus predict AF for spotted seatrout aged 4 and 5. Using these predictions and the relative abundance of each age class in our samples, we estimated the contribution of each age class to the annual egg production.

All statistical analyses were conducted with the Statistical Package for the Social Sciences (version 9.0, SPSS Inc., Chicago, IL). The level of significance for all tests was 0.05.

Results

A total of 1038 spotted seatrout ranging in age from 1 to 5 was collected for this study. Because 97% of these belonged to age classes 1–3 we report reproductive parameters only for these ages. We examined a total of 941 mature and developing females, ranging in length from 248 mm to 542 mm TL, to determine spawning frequency (569, 285, and 87 for ages 1–3, respectively). Of these, 135 specimens (12 from sportfishing tournaments) were used to conduct oocyte counts (62, 52, and 21 for ages 1–3, respectively). These fish ranged in length from 268 to 530 mm TL. Minimum size at first maturity, as indicated by the presence of cortical alveoli stage oocytes in histological sections, was 248 mm TL. Size at 50% maturity was 268 mm, whereas 100% maturity was reached at 301 mm TL. Condition of females, as indicated by Fulton's condition factor, diminished over the course of the season (P<0.01, r^2=0.24).

Seasonality

Spawning in the Charleston Harbor area during the study period began in mid to late April as indicated by the presence of oocytes in late FOM or POFs in histological samples. During the study period, mean water temperatures ranged from 16° to 34°C. Highest temperatures were recorded during July and August for all three years of the study. The lowest documented water temperature when spawning began was 20°C. Cessation of spawning occurred when water temperature was 28°C. Mean monthly GSI for spotted seatrout captured along the South Carolina coast since 1991 (Fig. 2) showed a marked increase from 4.6 in April to 9.4 in May. Mean GSI in June declined to 6.3 and remained around 5.0 in July and August. A sharp decline was noted in September to 2.7, the lowest level for the season. Overall, mean gonadosomatic index (GSI) values followed the seasonal trend in water temperature (Fig. 2). Percent occurrence of females in spawning condition as evidenced from histological examination declined from approximately 87% in August to 12% in September. The percentage of

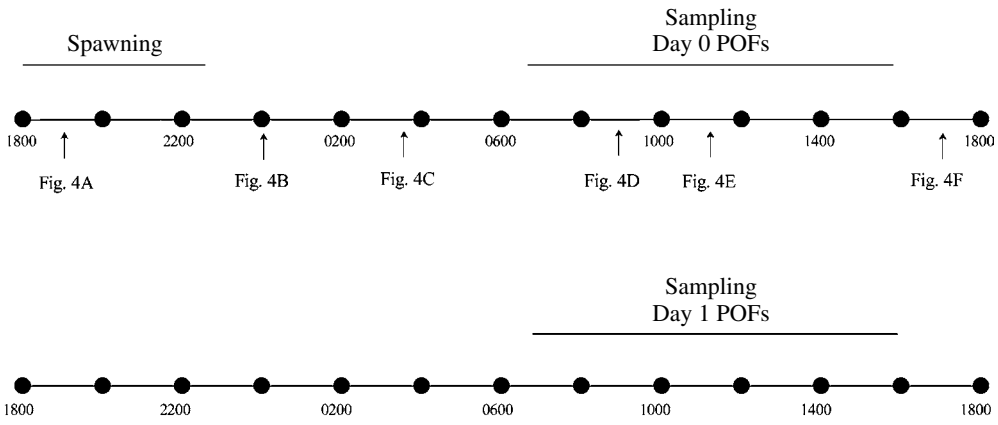

Figure 3

Forty-eight-hour chronological time line indicating *C. nebulosus* spawning time in Charleston Harbor, SC. Postovulatory follicles (POFs) observed at water temperatures >25°C were termed day-0 (<24 hours). Day-1 POFs (>24 hours) were observed only at water temperatures <25°C. Time of capture for specimens in Figure 4 are indicated.

post-spawning females increased from 9% in August to 91% in September. Thus, the spawning season for spotted seatrout in South Carolina extends from late April through early September.

Spawning frequency

Day-0 POFs were found through 1800 h of the day following a spawning event. Day-1 POFs were first observed in our routine samples when they were 36–37 hours old (the second day following a spawning event) only when water temperatures were below 25°C. Day-1 POFs were excluded from our analysis of SF because they did not provide evidence of a previous night's spawning event.

Figure 3 illustrates the time line for POF atrophy in spotted seatrout from 1–42 h after the onset of spawning at 1800 h. Because evidence of spawning for the first 12 h was documented only during a period when water temperatures were greater than 25°C, all of the examples shown are indicative of atrophy in warmer temperatures (Fig. 4). As indicated in Table 2, there was a time-dependent deterioration of POFs such that only those <24 h were detectable at water temperatures >25°C.

Small sample sizes prevented calculation of monthly SF for each age class by year. Therefore, we pooled data for all three years of this study to obtain a single monthly SF estimate by age class (Tables 3 and 4). The interaction between month and age on SF could not be statistically tested; however, age-3 fish spawned more frequently than younger fish (Kruskal-Wallis, $P<0.05$) and all seatrout spawned more frequently in June (Kruskal-Wallis, $P<0.05$). Peaks in SF observed for fish ages 2 and 3 in July and August, respectively (Tables 3 and 4), were not statistically significant.

Monthly SF values based on the occurrence of ovaries containing oocytes in FOM are also presented in Table 3. However, statistical comparisons were not feasible because of the nonrandom collection of specimens. Overall SF was estimated to be once every 4.4 days and once every 5.1 days with the POF and FOM methods, respectively.

Table 3

Spawning frequency (SF) expressed as the number of spawnings per month for *C. nebulosus* ages 1–3 for the spawning seasons of 1998–2000. Numbers in parentheses represent days between spawnings. *n* = number of fish in a sample. FOM = final oocyte maturation; POF = postovulatory follicle.

Age (yr)	Month	*n*	SF FOM method	SF POF method
1	May	89	4.53 (6.85)	4.18 (7.42)
	June	166	4.53 (6.62)	9.40 (3.13)
	July	185	4.68 (6.62)	6.54 (4.74)
	August	129	6.26 (4.95)	4.57 (6.79)
	Total	569	19.9 (6.18)	26.34 (4.67)
2	May	114	11.5 (2.78)	6.80 (4.56)
	June	79	5.70 (5.26)	7.60 (3.95)
	July	48	0.65 (47.62)	9.04 (3.43)
	August	44	9.87 (3.14)	6.34 (4.89)
	Total	285	30.67 (4.01)	29.36 (4.19)
3	May	46	10.10 (3.07)	7.42 (4.18)
	June	23	5.22 (5.75)	9.12 (3.29)
	July	10	3.10 (10.00)	3.10 (10.00)
	August	8	11.61 (2.67)	11.61 (2.67)
	Total	87	32.54 (2.67)	31.14 (3.95)
Overall		941	24.3 (5.06)	27.7 (4.44)

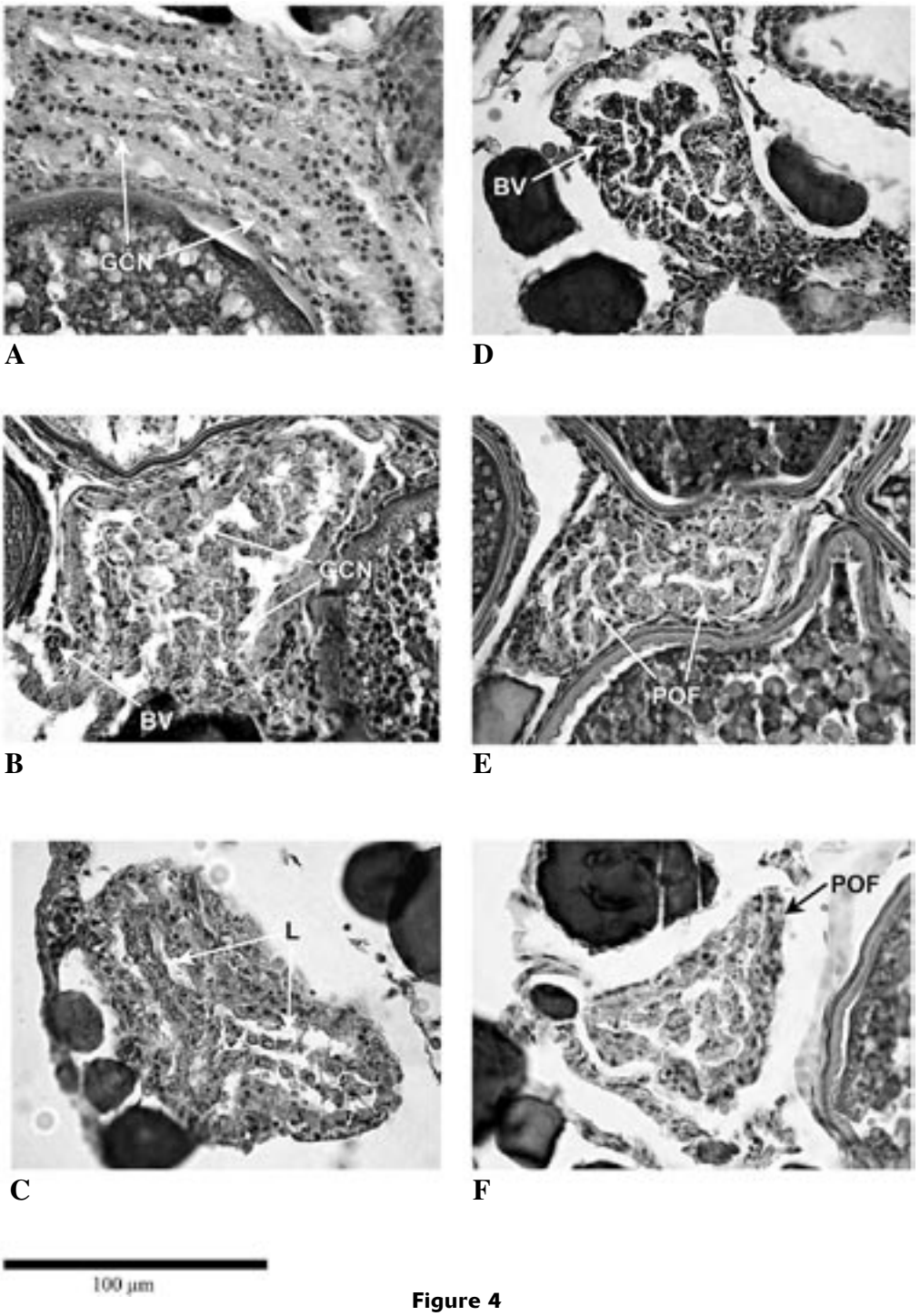

Figure 4

Photomicrographs of *C. nebulosus* postovulatory follicles (POFs) showing chronology of atresia at water temperatures >25°C. (**A**) 0–4 hours after spawning, (**B**) 5–8 hours after spawning, (**C**) 9–12 hours after spawning, (**D, E, F**) 13–24 hours after spawning.

Batch fecundity

As expected, we found a significant difference in mean BF among age classes (ANOVA on ranks, $P<0.05$). Age-1 spotted seatrout produced an average of 145,452 oocytes per batch spawned. Fish aged 2 and 3 spawned an average of 291,123 and 529,976 oocytes per batch, respectively. Therefore, mean BF was compared among months

Table 4

Fecundity parameters for *C. nebulosus* ages 1–3 from South Carolina estuaries. BF = batch fecundity in numbers of oocytes; SF = spawning frequency based on the postovulatory follicle (POF) method and expressed as the number of spawnings per month; MEP = monthly egg production = $(BF \times SF)\%mature$. Annual fecundity is the sum of mean monthly MEP values for each year class and represents the total number of oocytes produced by any given female from 1 May to 31 August. Numbers in parentheses indicate sample sizes.

Age (yr)	Month	Mean BF	SF	% mature	Mean MEP
1	May	117,760 (12)	4.18 (89)	78.6	386,897
	June	135,403 (16)	9.40 (166)	94.0	1,196,418
	July	141,237 (16)	6.54 (185)	97.0	895,978
	August	176,594 (18)	4.57 (129)	100	807,035
	Annual fecundity=3,286,328 oocytes				
2	May	280,724 (34)	6.80 (114)	100	1,908,926
	June	307,322 (10)	7.60 (79)	100	2,335,650
	July	370,170 (1)	9.04 (48)	100	3,346,337
	August	307,195 (7)	6.34 (44)	100	1,947,620
	Annual fecundity=9,538,533 oocytes				
3	May	487,475 (13)	7.42 (46)	100	3,617,061
	June	519,630 (4)	9.12 (23)	100	4,739,027
	July	765,911 (2)	3.1 (10)	100	2,374,325
	August	590,994 (2)	11.61 (8)	100	6,861,439
	Annual fecundity=17,591,852 oocytes				

Table 5

Monthly relative fecundity (number of oocytes /grams ovary-free weight) for *C. nebulosus* ages 1–3 for the spawning seasons 1998–2000. SD=standard deviation.

Month	Mean	Minimum	Maximum	SD	*n*
May	518.6	223.9	976.1	146.2	46
June	603.2	205.7	1306.1	241.8	20
July	820.9	662.2	1314.4	279.0	5
August	693.6	397.3	1021.8	207.9	12

and years for each age class separately (Table 4). There were no significant interannual or monthly variations in mean BF for any of the age classes (age-1: $P=0.59$, $n=62$; age-2: $P=0.17$, $n=52$; age-3: $P=0.07$, $n=21$). However, BF analysis for age-2 fish excluded the month of July because only one two-year-old specimen was captured that month during the study period. We investigated the relationship between BF and total length by using linear regression analysis. After pooling data across years, we found that total length explained 67% of the variability in spotted seatrout BF (Fig. 5A). Batch fecundity showed a similarly strong relationship to female somatic (ovary-free) weight (Fig. 5B) but did not relate to age as strongly (Fig. 5C). The equations below describe these relationships:

$BF = 2179.65(TL) - 520597$ $(r^2=0.67)$ $P<0.001$
$BF = 530.60(OFWT) + 18537.77$ $(r^2=0.65)$ $P<0.001$
$BF = 169398.21(Age) - 30956.33$ $(r^2=0.58)$ $P<0.001$.

Mean MEP was significantly different among months for age-1 spotted seatrout (Kruskal-Wallis, $P<0.05$). Age-1 fish spawned the least number of oocytes in May and most in June (Table 4). Statistical comparisons among months for ages 2 and 3 were inconclusive.

Relative fecundity

Relative fecundity among 83 spotted seatrout ages 1–3 ranged from 224 oocytes to 1314 oocytes/g OFWT (Table 5). Age did not have an effect on relative fecundity (Kruskal-Wallis, $P=0.75$). We found that spotted seatrout in South Carolina produced significantly more oocytes per gram ovary-free weight at the end than at the beginning of the spawning season (Mann Whitney, $P<0.05$). Mean oocyte diameters did not vary significantly between ovarian lobes or among locations within each lobe (ANOVA, $P=0.28$). A comparison among

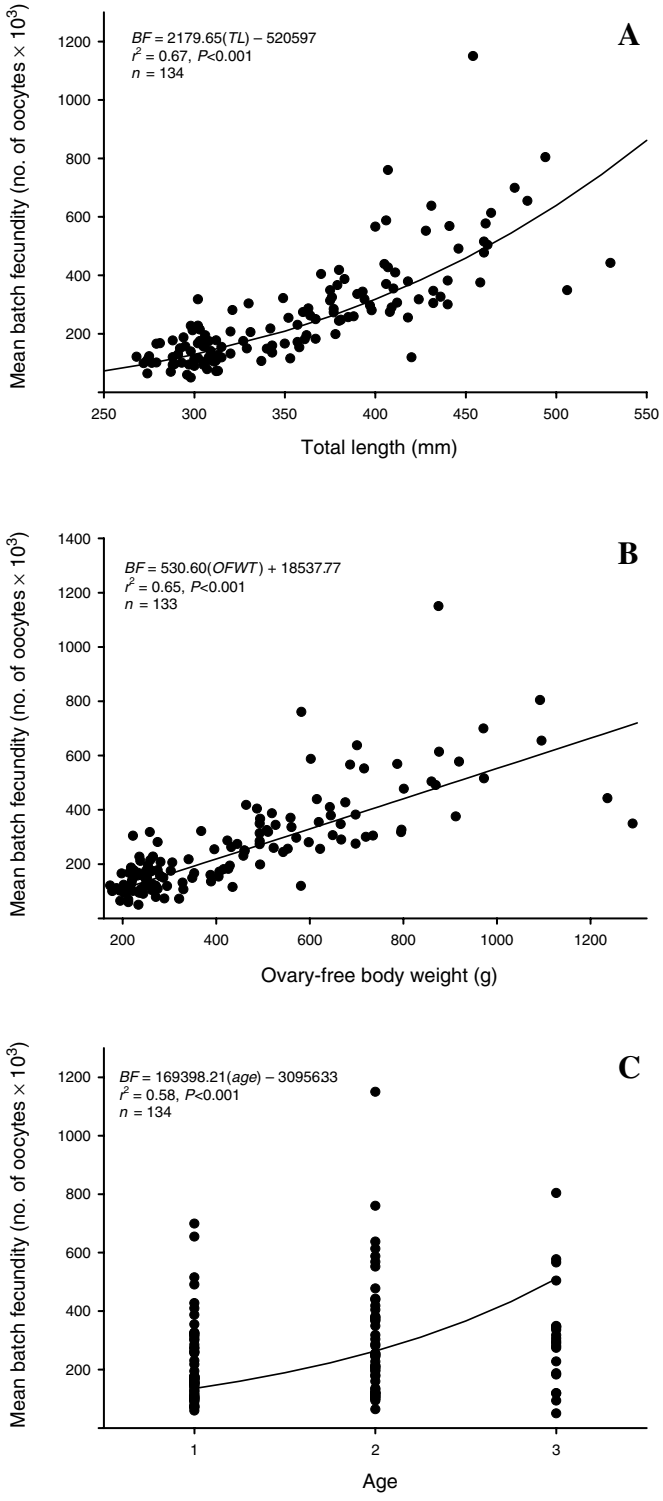

Figure 5

Relationship between batch fecundity (BF) and total length (TL) (**A**), between BF and ovary-free weight (OFWT) (**B**), and between BF and age (**C**) for *C. nebulosus* ages 1–3. Linear regression on data pooled for spawning seasons 1998–2000.

months and ages revealed that age had no effect on oocyte diameter (ANOVA, P=0.82). However, the effect of month corroborated the pattern of increasing RF as the spawning season progressed: oocytes were significantly smaller at the end of the season (ANOVA, P<0.05).

Annual fecundity

Annual fecundity estimates (summation of MEP) were approximately 3.2 million, 9.5 million, and 17.6 million oocytes for each age class, respectively (Table 4). The equation below describes the relationship between AF and age:

$$AF = 7152762(Age) - 4166620 \ (r^2=0.99) \ P<0.05.$$

From this relationship, the predicted AF for ages 4 and 5 were 24,444,430 and 31,597,190 oocytes, respectively. We expanded AF in relation to the abundance of each age class in our standard random samples for the three years of the study. We estimated that the overall average contribution from age-1 fish to the reproductive output for the season was approximately 29%, whereas fish aged 2 and 3 contributed 39% and 21% of oocytes, respectively. Ages 4–5 comprised less than 3% of specimens sampled and contributed 7% and 4% based on predicted AF values.

Discussion

Studies on the reproductive biology of *Cynoscion nebulosus* have established group-synchrony and indeterminate fecundity for this species throughout its range (i.e. Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002; Mercer[1] and references therein). Fish with these features release gametes in several batches over a protracted spawning season and annual fecundity is not fixed prior to the onset of spawning (Wallace and Selman, 1981).

Based on mtDNA variation among spotted seatrout, the existence of two populations, one in the Gulf of Mexico and one in the South Atlantic, was established by Gold et al. (1999). However, variations in reproductive parameters have been suggested among geographic locations within the Gulf of Mexico (Brown-Peterson et al., 2002). Wiley (1996) suggested that spotted seatrout comprise a single stock in South Carolina; therefore reproductive parameters presented in the present study should be applicable only to the spotted seatrout population inhabiting coastal waters of this state. Further studies should be conducted to evaluate the applicability of these parameters to the entire southeast coast.

Other investigators (Brown-Peterson et al., 1988; Wieting, 1989; Brown-Peterson and War-

ren, 2001; Nieland et al., 2002; Lowerre-Barbieri et al.[8]) have used the gonadosomatic index (GSI) to delineate the spawning season in spotted seatrout. Even though the GSI provided a good approximation of the spawning season, histological data alone provided more precise evidence. Spotted seatrout in South Carolina began spawning near the end of April of each year and ceased by early September. Similarly, Lowerre-Barbieri et al.[8] reported that the spawning season for spotted seatrout in Georgia extended from late April to mid-September. We found histological evidence of initial spawning in specimens captured in 20°C water, although approximately 75% of spawning occurred when ambient water temperatures were greater than 25°C. In laboratory experiments, Brown-Peterson et al. (1988) found no successful spawning in water below 23°C but pointed out that others (McMichael and Peters, 1989) found eggs and larvae in 20.4°C water.

We found that females became mature approximately one full year after their birth. A female born in May of one year would be reproductively active in May of the following year. Females born later in the season would not be mature as the same successive season began; therefore, not all one-year-old females were mature when the spawning season began in May, but became mature before that season ended. This maturity schedule has also been reported for spotted seatrout in Louisiana (Nieland et al., 2002). However, Lowerre-Barbieri et al.[8] found that all one-year-old females were mature in coastal Georgia. A limited sample size or habitat segregation of mature and immature trout (Lowerre-Barbieri et al.[8]) may have contributed to their result.

The size at first maturity for spotted seatrout in this study was 248 mm TL. This size is comparable to what others have reported in other areas of the species' range (Brown-Peterson et al., 1988; Brown Peterson and Warren, 2001; Nieland et al., 2002; Mercer[1] and references therein; Lowerre-Barbieri et al.[8]). Our estimate of size at 50% maturity (268 mm TL) was larger than what Nieland et al. (2002) reported for 100% mature trout in Louisiana (250 mm TL). However, Nieland et al.'s (2002) statement that animals are 100% mature at 250 mm TL, does not agree with the growth equation they report for female trout when age = 1. Because we found size at 100% maturity among female spotted seatrout in South Carolina to be about 300 mm TL, we wonder whether Nieland et al.'s (2002) growth equation for female TL was meant to represent SL. Were this the case, they might have offered a different rationale for size at maturity among trout in Louisiana.

Brown-Peterson et al. (1988) and Brown-Peterson and Warren (2001) reported size at 100% maturity of 356 mm and 309 mm TL (using the SL-TL conversion found in our "Methods: section) for spotted seatrout in Texas and Mississippi, respectively. Brown-Peterson et al. (1988), however, chose a combination of gears that may not have sampled the trout population in Texas representatively for size-at-maturity estimation. In Mississippi, Brown-Peterson and Warren (2001) used a more appropriate gear for capture of late juvenile and early adult fish. Our estimate of size at 100% maturity was quite similar to theirs.

Spawning frequency

Determining the number of multiple spawning events during a single season for individual fish has been problematic. Initially, there was little understanding of the reproductive dynamics of spotted seatrout, and BF estimates were reported to represent the output for a whole season (Pearson, 1929; Sundararaj and Suttkus, 1962; Overstreet, 1983). Hunter et al. (1985) and Hunter and Macewicz (1985) developed techniques to overcome these limitations by providing protocols for the use of hydrated oocytes in determining BF and SF among group-synchronous species.

To use the techniques of Hunter (1985) and Hunter and Macewicz (1985) appropriately, it is critical to obtain a representative sample of the spawning population. DeMartini and Fountain (1981) and Lisovenko and Adrianov (1991) maintained that the relative occurrence of hydrated oocytes (as determined macroscopically) was an effective measurement of SF when the spawning population was sampled representatively. However, when sampling a species that spawns in aggregations at specific geographic locations, as do many of the sciaenids, it is inherently impossible to obtain a statistically representative sample of the spawning population for SF estimation based on FOM. Because the window of opportunity is temporally and spatially constrained, obtaining a sample that includes all sizes and ages involved is not feasible; the only choice in this situation is to sample in a directed fashion. This was the sampling strategy used to target females for BF counts; the majority of the animals captured whose oocytes evidenced FOM were obtained in a nonrandom fashion. Additionally, we assumed that fishes demonstrating FOM were moving toward deeper water spawning aggregations and away from our capture gear. For these reasons, we felt that our SF estimates based on the proportion of females with oocytes in FOM were biased and we excluded them from AF estimation. This is an important matter to keep in mind when comparing frequencies of spawning based on different methods.

Because obtaining representative numbers of animals with late-maturing oocytes is not often feasible, researchers have relied on the relative abundance of postovulatory follicles (POFs) to calculate SF (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Nieland et al., 2002; Lowerre-Barbieri et al.[8]). The POF method lacks the limitations (described above) of the FOM method. Because the method we chose allowed us to sample all sizes and ages of fish in the estuary, obtaining representative numbers of animals with POFs was accomplished effectively. Therefore, we felt that our estimates of SF based on the POF method were more precise and we chose to use them in deriving AF.

The POF method depends on the ability to assess the disappearance of these structures. Hunter and Macewicz (1985) systematically sampled captive spawning anchovies to develop histological criteria for POF atrophy in

19°C water. Their criteria have been used by others to estimate rates of POF atrophy in other species and thereby determine the percentage of a population undergoing spawning over a discrete time period (Brown-Peterson et al., 1988; Fitzhugh et al., 1993; Taylor et al., 1998; Macchi and Acha, 2000; Brown-Peterson and Warren, 2001; Nieland et al., 2002). However, even though it has been demonstrated that the rate of POF atresia depends largely on ambient water temperature (Fitzhugh and Hettler, 1995), few (Brown-Peterson et al., 1988; Macchi and Acha, 2000; Nieland et al., 2002) have taken this into account when establishing the age of POFs for SF estimations. Our diurnal sampling of reproductively active spotted seatrout during warm water conditions enabled us to establish criteria to accurately estimate the age of POFs throughout the spawning season. Furthermore, we verified our assessments by sampling around the clock on two occasions to collect fish over the time period immediately following a spawning event.

Spotted seatrout ages 1–3 in SC spawned less frequently than those from the Indian River Lagoon, Florida (Crabtree and Adams[7]) but both studies showed that older fish spawned more frequently than younger animals. Our estimates for spotted seatrout aged 1–3 were 4.7, 4.2, and 4 days, respectively. Trout in these age classes in Florida were reported to spawn once every 4, 2.8, and 2.5 days, respectively. These differences probably not only reflect the distinct biological environments of each region but also indicate potential discrepancies in aging methods. No age-specific estimates of SF are available for other areas in the species' range. Brown-Peterson and Warren (2001) found SF among spotted seatrout in Biloxi Bay, MS, to be significantly lower than that of fish inhabiting the other two areas included in their study. They suggested that Biloxi Bay was a less conducive spawning habitat because of several factors, including shoreline development and a reduced amount of aquatic vegetation. However, because we found that SF varied significantly among age classes (age-3 fish spawned more frequently), the relative age composition of fish sampled by Brown-Peterson and Warren (2001) in the three estuaries might also have played a critical role in the determination of SF.

Batch fecundity

The best approach for estimating BF is to use only oocytes in FOM (Hunter et al., 1985; Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002; Nieland et al., 2002; Lowerre-Barbieri et al.[8]). When it is not possible to obtain these, BF estimations can and have been carried out in some species by using the largest vitellogenic oocytes (Overstreet, 1983; Hunter et al., 1985; Wieting 1989). These efforts have the potential of being less accurate because isolating those oocytes destined to be spawned is difficult if the latter have not yet reached final maturation (Nieland et al., 2002). Inevitably this scenario would result in a nonmeasurable overestimation of female reproductive output. Brown-Peterson et al. (1988) and Brown-Peterson and Warren (2001) used a modification of this approach to estimate BF of spotted seatrout in Texas and Mississippi, respectively. However, even though the potential existed for overestimating BF, their estimates fell well below those presented in the present study, as did those presented by Nieland et al. (2002) for spotted seatrout ages 2–4 in Barataria Bay, Louisiana. Mean BF for ages 1–3 (170 thousand, 226 thousand, and 274 thousand oocytes, respectively) spotted seatrout in Indian River Lagoon, Florida (Crabtree and Adams[7]), also differed from those reported here. Our estimate took into account that not all age-1 females were mature at the beginning of the season. Crabtree and Adams,[7] however, did not adjust their estimate to reflect this discrepancy. Moreover, due to differences in aging methods, their age-1 and 2 cohorts possibly included ages 2 and 3, respectively. In addition, in the Florida study as well as in ours, relatively few numbers of older specimens were examined.

The relationships between BF and length, weight, and age in the present study were significant and predictive. Of these, TL exhibited the most predictive relationship. This fact may explain why age-1 and age-2 spotted seatrout in Georgia had mean BFs considerably higher than ours (175 thousand and 407 thousand, respectively; Lowerre-Barbieri et al.[8]): the size ranges for age-1 and age-2 in the Georgia study were greater than ours. Total length seems to be the most reliable predictor of BF among spotted seatrout in Georgia and SC (Lowerre-Barbieri et al.,[8] this study) and in Louisiana (Nieland et al., 2002). However, Crabtree and Adams[7] found that BF related best to ovary-free weight among spotted seatrout in Florida. We found ovary-free weight to be the second best predictor of BF. Overall, it appeared that TL and ovary-free weight were better predictors of BF than age for this species (Brown-Peterson, 2003).

As with SF, monthly egg production (MEP) estimates for SC spotted seatrout varied throughout the season. Because BF was not significantly different among months for any of our age classes, the variation in MEP resulted directly from the frequency of spawning. Monthly egg production estimates for age-1 fish were lowest in May and highest in June because SF was lowest in May and highest in June. Spawning frequency is a critical reproductive parameter because it seems to dictate annual reproductive output (DeMartini and Fountain, 1981; Brown-Peterson and Warren, 2001; Crabtree and Adams[7]); therefore, SF should be carefully considered, particularly for managed species.

Relative fecundity

We found that relative fecundity (RF), the number of oocytes per gram of somatic weight, did not show a significant relationship with female size. This finding was expected because dividing fecundity by ovary-free weight standardizes the values independently of size. However, this finding was in contrast to that of Brown-Peterson and Warren (2001). They collected specimens during the morning only, whereas we sampled ours throughout the day. This procedure allowed us to examine ovaries

over the entire range of maturation and to select only those clearly showing nuclear migration (based on histological observations) ensuring that only oocytes in the same phase of FOM (Brown-Peterson et al., 1988) were included in RF calculations. If sampling is conducted during a time period that is not close to active spawning (i.e., when oocytes are in different phases of FOM), then the number of oocytes per gram may be miscalculated.

As with BF, our RF estimates were higher than those reported for seatrout in the Gulf of Mexico (Brown-Peterson et al., 1988; Brown-Peterson and Warren, 2001), although spotted seatrout reproductive parameters appeared to vary considerably even within the Gulf of Mexico (Brown-Peterson et al., 2002). This was attributed to differential environmental conditions or food availability (or to both) (Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002). The significant seasonal increase in RF that we observed for spotted seatrout in South Carolina, however, has not been reported elsewhere. Brown-Peterson et al. (1988) found no differences in mean monthly RF among spotted seatrout in Texas. Brown-Peterson and Warren (2001) found significantly higher RF values in June than in August. In both instances, however, a small sample size may have biased their results.

Comparisons of mean oocyte diameters among months related the increase in RF to a general decrease in oocyte size over the course of the season. This phenomenon is widespread among marine pelagic spawners, and scientists have put forth several explanations to account for it (see Chambers, 1997). Bagenal (1971) suggested that egg size decreased over the spawning season owing to concurrent increased food availability for larvae. Others have suggested an inverse relationship between temperature and egg size (Ware, 1975; Wootton, 1994; Miller et al., 1995) or a seasonal decrease in egg size that is correlated to the condition of spawning females (DeMartini and Fountain, 1981; Chambers and Waiwood, 1996). The latter seems to apply to spotted seatrout in this study because a diminishing trend through the spawning season was observed in the condition factor of females.

Annual fecundity

Brown-Peterson (2003) presented AF estimates for spotted seatrout throughout their range. Our estimates were substantially below those for spotted seatrout in Indian River Lagoon (Crabtree and Adams[7]) but approximated those of Lowerre-Barbieri et al.[8] for trout in Georgia. A possible reason for the higher values in Florida was the more protacted spawning season in that area (50 days longer). No comparisons of AF estimates presented in this study and those of spotted seatrout in the Gulf of Mexico (Brown-Peterson, 2003) were made because they were not specific to age classes.

The main impetus behind the present study was to establish annual fecundity (AF) estimates by age class. We found that age-1 through age-3 spotted seatrout occurred abundantly in SC estuaries and that each of these age cohorts showed unique fecundity dynamics. The AF for an average age-1 fish was one-third that of age-2 (~3.28 million vs. 9.5 million). One year-old fish, however, constituted the majority of fish in our samples; their abundance was twice that of 2-year-olds and seven times that of 3-year-old fish. Even though the average age-3 trout produced almost twice as many oocytes during the season (17.5 million) as the average age-2 fish, their reduced abundance in our estuaries made their overall contribution only half that of 2 year-olds. Ages 4 and 5 were estimated to produce approximately 24.4 million and 31.6 million oocytes per female, respectively; however, the oocyte production by the predominant age groups overshadowed theirs. When analyzed in relation to the occurrence of the other age classes in our estuaries, age-2 fish contributed the greatest number of fertilizable oocytes to the environment (39%).

Reliable fecundities based on age and on length are optimal for stock assessment models (Williams[9]). This study provided AF estimates for three age classes that can be used in age-based models for the spotted seatrout population in South Carolina. Annual fecundity estimates based on length, however, have not been attempted even though length appears to be the best predictor of fecundity in spotted seatrout (see references in Brown-Peterson, 2003). Further analyses to investigate the relationship between egg production and fish length for each month of the spawning season would allow for more precise management efforts based on individual length-based estimates of AF.

Acknowledgments

We thank members of the Inshore Fisheries Section of the South Carolina Department of Natural Resources for assisting in field data collection throughout this study (C. Wenner, J. Archambault, H. von Kolnitz, W. Hegler, E. Levesque, L. Goss, C. McDonough, C. Johnson, A. Palmer). C. Wenner, H. von Kolnitz, and E. Levesque conducted age assessments. Histological processing was provided by C. McDonough, R. Evitt, A. Palmer, and W. Hegler. Assistance with oocyte counts was provided by C. McDonough, T. Piper, K. Maynard, and R. Evitt. Data management was coordinated by J. Archambault, C. Wenner, E. Levesque, and three anonymous reviewers provided helpful suggestions on the manuscript. Funding for this study was provided by the National Marine Fisheries Service under MARFIN grant no. NA77FF0550.

Literature cited

Bagenal, T. B.
1971. The interrelation of the size of fish eggs, the date of spawning, and the production cycle. J. Fish. Biol. 3:207–219.

[9] Williams, E. 2003. Personal commun. National Marine Fisheries Service, 101 Pivers Island, Rd., Beaufort, NC 28516.

Brown-Peterson, N. J.
2003. The reproductive biology of spotted seatrout. *In* Biology of the spotted seatrout (S. A. Bortone, ed.) p. 99–133. CRC Press, Boca Raton, FL.
Brown-Peterson, N. J., P. Thomas, and C. Arnold.
1988. Reproductive biology of the spotted seatrout, *Cynoscion nebulosus*, in South Texas. Fish. Bull. 86:3 73–387.
Brown-Peterson, N. J., M. S. Peterson, D. L. Nieland, M. D. Murphy, R. G. Taylor, and J. R. Warren.
2001. Reproductive biology of female spotted seatrout, *Cynoscion nebulosus*, in the Gulf of Mexico: differences among estuaries? Environ. Biol. Fish. 63:405–415.
Brown-Peterson, N. J., and J. R. Warren.
2002. The reproductive biology of spotted seatrout, *Cynoscion nebulosus*, along the Mississippi Gulf coast. Gulf. Mex. Sci. 19:61–73.
Chambers, R. C.
1997. Environmental influences on egg and propagule size in marine fishes. *In* Early life history and recruitment in fish populations (R. C. Chambers and A. E. Trippel, eds.), p. 93–102. Chapman and Hall, London.
Chambers, R. C., and K. G. Waiwood.
1996. Maternal and seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. 53:1986–2003.
DeMartini, E. E., and R. K. Fountain.
1981. Ovarian cycling frequency and batch fecundity in the queenfish, *Seriphus politus*: attributes representative of spawning fish. Fish. Bull. 79:547–560.
Fish, M. P., and W. H. Mowbray.
1970. Sounds of western North Atlantic fishes. A reference file of biological underwater sounds. The Johns Hopkins Press, Baltimore and London.
Fitzhugh, G. R., and W. F. Hettler.
1995. Temperature influence on postovulatory follicle degeneration in Atlantic menhaden, *Brevoortia tyrannus*. Fish. Bull. 93:568–572.
Fitzhugh, G. R., B. A. Thompson, and T. G. Snider.
1993. Ovarian development, fecundity and spawning frequency of black drum, *Pogonias cromis,* In Louisiana. Fish. Bull. 91:244–253.
Gabriel, W. L., M. P. Sissenwine, and W. J. Overholtz.
1989. Analysis of spawning stock biomass per recruit: an example for Georges Bank haddock. N. Am. J. Fish. Manag. 9:383–391.
Gold, J. R., L. R. Richardson, and C. Furman.
1999. Mitochondrial DNA diversity and population structure of spotted seatrout (*Cynoscion nebulosus*) in coastal waters of the Southeastern United States. Gulf Mex. Sci. 1:40–50.
Goodyear, C. P.
1993. Spawning stock biomass per recruit in fisheries management: foundation and current use. *In* Risk evaluation and biological reference points for fisheries management (S. J. Smith, J. J. Hunt, and D. Rivard, eds.), p. 67–81. Can. Spec. Publ. Fish. Aquat. Sci. 120.
Holt, G. J., S. A. Holt, and C. R. Arnold.
1985. Diel periodicity of spawning in sciaenids. Mar. Ecol. Prog. Ser. 27:1–7.
Humason, G. L.
1972. Animal tissue techniques. W. H. Freeman and Co. San Francisco and London.
Hunter, J. B., N. C. H. Lo, and R. J. H. Leong.
1985. Batch fecundity in multiple spawning fishes. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy *Engraulis mordax* (R. Lasker, ed.), p. 67–77. NOAA Tech. Rep. NMFS 36.
Hunter, J. R., and B. J. Macewicz.
1985. Measurement of spawning frequency in multiple spawning fishes. *In* An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy *Engraulis mordax* (R. Lasker, ed.), p. 79–94. NOAA Tech. Rep. NMFS 36.
Lisovenko, L. A., and D. P. Andrianov.
1991. Determination of absolute fecundity of intermittently spawning fishes. Voprosy ikhtiologii 31:631–641.
Macchi, G. J., and E. M. Acha.
2000. Spawning frequency and batch fecundity of Brazilian menhaden, *Brevoortia aurea*, in the Rio de la Plata estuary off Argentina and Uruguay. Fish. Bull. 98:283–289.
McMichael, R. H., and K. M. Peters.
1989. Early life-history of spotted seatrout, *Cynoscion nebulosus* (Pisces: Sciaenidae), in Tampa Bay, Florida. Estuaries 12:98–110.
Miller, T. J., T. Herra, and W. C. Leggett .
1995. An individual-based analysis of the variability of eggs and their newly hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. Can. J. Fish. Aquat. Sci. 52:1083–1093.
Mok, H. K., and R. G. Gilmore.
1983. Analysis of sound production in estuarine aggregations of *Pogonias cromis, Bairdiella chrysoura,* and *Cynoscion nebulosus* (Sciaenidae). Bull. Inst. Zool., Acad. Sinica (Taipei) 22:157–186.
Murphy, M. D., and R. G. Taylor.
1994. Age, growth and mortality of spotted seatrout in Florida waters. Trans. Am. Fish. Soc. 123:482–497.
Nieland, D. L., R. G. Thomas, and C. A. Wilson.
2002. Age, growth and reproduction of spotted seatrout in Barataria Bay, Louisiana. Trans. Am. Fish. Soc. 131:245–259.
Overstreet, R. M.
1983. Aspects of the biology of the spotted seatrout, *Cynoscion nebulosus*, in Mississippi. Gulf Res. Rep. Suppl. 1:1–43.
Pearson, J. C.
1929. Natural history and conservation of the redfish and other commercial sciaenids on the Texas coast. Bull. U.S. Bur. Fish. 44:129–214.
Prager, M. H., J. F. O'Brien, and S. B. Saila.
1987. Using lifetime fecundity to compare management strategies: a case history for striped bass. Am. J. Fish. Manag. 7:403–409.
Ricker, W. E.
1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191:1–382.
Saucier, M. H., and D. M. Baltz.
1993. Spawning site selection by spotted seatrout, *Cynoscion nebulosus*, and black drum, *Pogonias cromis*, in Louisiana. Environ. Biol. Fish. 36:257–272.
Saucier, M. H., D. M. Baltz, and W. A. Roumillat.
1992. Hydrophone identification of spawning sites of spotted seatrout *Cynoscion nebulosus* (Osteichthys: Sciaenidae) near Charleston, South Carolina. N. Gulf Sci.12:141–145.
Sundararaj, B. I., and R. D. Suttkus.
1962. Fecundity of the spotted seatrout, *Cynoscion nebulo-*

sus (Cuvier), from Lake Borgne Area, Louisiana. Trans. Am. Fish. Soc. 91:84–88.

Taylor, R. G., H. J. Grier, and J. A. Whittington.
1998. Spawning rhythms of common snook in Florida. J. Fish Biol. 53:502–520.

Vaughan, D. S., G. R. Huntsman, C. S. Manooch III, F. C. Rohde, and G. F. Ulrich.
1992. Population characteristics of the red porgy, *Pagrus pagrus*, stock off the Carolinas. Bull. Mar. Sci. 50: 1–20.

Wallace, R. A., and K. Selman.
1981. Cellular and dynamic aspects of oocyte growth in teleosts. Am. Zool. 21:325–343.

Ware, D. M.
1975. Relation between egg size, growth, and natural mortality of larval fish. J. Fish. Res. Board Can. 32: 2503–512.

Wieting, D. S.
1989. Age, growth and fecundity of spotted seatrout (*Cynoscion nebulosus*) in Louisiana. M.S. thesis, 94 p. Louisiana State Univ., Baton Rouge, LA.

Wiley, B. A.
1996. Use of polymorphic microsatellites to detect population structure in spotted seatrout, *Cynoscion nebulosus* (Cuvier). M.S. thesis, 42 p. Univ. Charleston, Charleston, SC.

Wootton, R. J.
1994. Life histories as sampling devices: optimum egg size in pelagic fishes. J. Fish Biol. 45:1067–1077.