Gonadal Maturation, Fecundity, and Strip-spawning of Female Spotted Seatrout[1]

Robert L. Colura, *Texas Parks and Wildlife Department, Perry R. Bass Marine Fisheries Research Station, Star Route Box 385, Palacios, TX 77465*

Anthony F. Maciorowski,[2] *Texas Parks and Wildlife Department, Perry R. Bass Marine Fisheries Research Station, Star Route Box 385, Palacios, TX 77465*

Anne Henderson-Arzapalo,[3] *Texas Parks and Wildlife Department, Perry R. Bass Marine Fisheries Research Station, Star Route Box 385, Palacios, TX 77465*

Abstract: Spotted seatrout (*Cynoscion nebulosus*) were collected from Matagorda Bay, Texas, from April 1984 through March 1986 and a gonosomatic index determined. Relative batch fecundity and ooycte maturation size were compared among females collected from April through October 1984, and randomly selected females were subjected to hormone-induced strip-spawning during June and July 1984. All females collected from April through August had yolked eggs and 4%-90% of fish collected in March, September, and October had yolked ova. No yolked ova were present in fish from November through February. Gonosomatic indices suggest greatest spawning activity occurred from April through May of each year. Median relative batch fecundity of females ($N = 169$) collected from April through October 1984 was 258 eggs/g body weight and was not significantly different from the median relative batch fecundity of 453 eggs/g body weight of hormone spawned fish ($N = 16$). Female spotted seatrout ($N = 16$) which were successfully spawned had mean $\pm$ SD vitellogenic ovum diameters (0.45 $\pm$ 0.12 mm) significantly larger than the mean diameter (0.37 $\pm$ 0.05 mm) of fish ($N = 15$) which did not ovulate.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 42:80–88

[1] This study was conducted with partial funding from the U.S. Department of the Interior, Fish and Wildlife Service, under DJ 15.605 (Project F-36-R).

[2] Present address: Battelle Columbus Division, 505 King Avenue, Columbus, OH 43210–2693.

[3] Present address: Fish Culture Research Laboratory, National Fisheries Center, P.O. Box 700, Kearneysville, WV 25430.

Spotted seatrout (*Cynoscion nebulosus*) have been spawned in captivity by photoperiod and temperature conditioning (Arnold et al. 1976) and following human chorionic gonadotropin (HGG) injections, have been strip-spawned (Colura 1974) or tank-spawned (Porter and Maciorowski 1984). These spawning techniques have provided eggs and fry for experimentation but have not been used to produce the fry required for hatchery scale production of fingerlings. Further, predictable large-scale spawning efforts have been hampered by difficulties in the identification and selection of wild-caught broodstock that can be consistently spawned by hormone therapy.

Spotted seatrout are fractional spawners (Arnold et al. 1976) which exhibit an extended spawning season (Pearson 1929, Stewart 1961, Hein and Shepherd 1979). Peak spawning has been reported in all months from April through August (Stewart 1961, Tabb 1961). Total fecundity estimates reported range from 0.4 million to 1.4 million eggs (Pearson 1929, Sundararaj and Suttkus 1962), whereas Overstreet (1983) found 4,283 and 7,340 eggs/g body weight (BW) respectively, for gravid and developing specimens. However, reported fecundity values are based on few fish and do not address estimation problems presented by fractional spawning (Morawska 1984).

The inability to consistently select highly fecund females in peak spawning condition is a factor limiting routine hatchery production of spotted seatrout. The present study attempts to refine broodstock selection. Our specific objectives were to: delineate the spawning season and peak spawning periods of spotted seatrout in Matagorda Bay, Texas, using a gonosomatic index (GSI); estimate relative batch fecundity (eggs/g BW); and characterize the oocyte maturation size.

Methods

Female spotted seatrout ($N = 743$) used for GSI determinations were collected at least twice monthly from Matagorda Bay, Texas, from April 1984 through March 1986. Fish were captured by hook and line or trammel and gill nets (Hegen and Matlock 1980, McEachron and Green 1985) and placed on ice at capture. Fish intended for spawning were captured on 12 and 18 June and 16 July 1984 by hook and line or trammel net (Hegen and Matlock 1980). Live fish were placed in a 140-liter live well supplied with compressed oxygen. All fish were then transported to the Perry R. Bass Marine Fisheries Research Station (MFRS), Palacios, Texas.

Dead fish were examined to determine sex, weight (± 10 g), and total length (TL) (± 1 mm). Intraovarian samples were collected from 22 fish in 1984 according to Hoff et al. (1972) and a portion preserved in Gilson's fluid (Simpson 1951). The mean diameter of fresh and preserved vitellogenic ova (as defined by Kuo et al. 1973) from each of the 22 fish was determined by measuring a random axis diameter of approximately 100 ova using an ocular micrometer and compound microscope at 100X. Means of fresh and preserved ovum diameters were computed and a ratio estimator ($\hat{R} \pm SE$) (Cochran 1977) was calculated to adjust for shrinkage of ova

in preserved samples. For GSI determinations, gonads were removed and the gonad free wet body weight (± 10 g) and the gonad wet weight (±0.01 g) determined. A GSI was subsequently calculated for each fish using methods of Overstreet (1983).

Ovaries of 169 stage IV and V females (Tabb 1961) collected from April to October 1984 for GSI determinations were retained for fecundity estimates. After removal and determination of gross ovarian weights, and approximately 0.5-cm cross section of ovary was removed, weighed (±0.01 g), and preserved in Gilson's fluid for at least 3 months. Eggs were separated by shaking the sample for approximately 30 seconds prior to counting. Preserved samples were diluted to 200–500 eggs/ml in a known volume, and 3 1-ml subsamples were withdrawn with a Hensen-Stemple pipette. Eggs were enumerated using a Ward plankton counting wheel and stereomicroscopy. The mean number of eggs/ml was calculated and used to estimate the number of eggs in the ovarian section which was subsequently used to estimate relative fecundity (Bagenal and Braum 1971). Approximately 50 eggs from each female were measured (random axis) to provide a size frequency distribution. The eggs from each female representing the largest most mature ova as determined by visual inspection were used to estimate the oocyte maturation size (Higham and Nicholson 1964). Eggs equal to or greater than the oocyte maturation size are thought to represent eggs to be released during the next spawning event and were used to estimate relative batch fecundity (Bagenal and Braum 1971). Scales were removed and analyzed to age all fish used for fecundity estimates. Scale collection, preparation, and age estimation procedures were described by Colura et al. (1984).

Live fish were transferred to a 370-liter fiberglass tank and anesthetized with Hypno,[R] (Jungle Laboratories Corp. Cibolo, TX), or Trance,[R] (Argent Chemical Laboratories, Redmond, WA) then examined to determine sex (Colura 1974), weighed (± 10 g), TL (± 1 mm) determined and females biopsied to obtain an intraovarian sample (Hoff et al. 1972). A portion of the ova sample was examined microscopically while fresh and developmental stage determined according to Colura (1974). The remainder of the sample was preserved and measured as previously described. After staging, 31 randomly-selected females (5 on 12 June, 5 on 18 June, and 21 on 16 July) and a similar number of males were selected for spawning, given an intramuscular injection of 50 mg oxytetracycline hydrochloride and transferred to an 1,850-liter recirculating seawater system equipped with a gravel, shell, and sand biofilter.

Females selected for spawning were intramuscularly injected with 1100 IU/kg human chorionic gonadotropin (HCG) the morning after capture and strip-spawned 26–32 hours later following the methods of Colura (1974). Spawned eggs from each female were placed in a beaker containing 1.0 liter of Matagorda Bay water. The total volume was determined, and 3 1-ml aliquots were removed with a Hensen-Stemple pipette. The number of eggs in each sample were counted and averaged to estimate the mean number of eggs/ml, then the total number of spawned eggs determined by volumetric estimation. Percent fertilization for each spawn was determined by examining 3 samples of approximately 100 eggs for mitotic division at least 2 hours post-fertilization.

Monthly mean GSI values within the 1984 and 1985 spawning seasons were compared by single classification analysis of variance (ANOVA) and the GT-2 method for testing contrasts among means (Sokal and Rohlf 1981). The spawning season was defined as those months in which all females collected contained yolked ova. Mean ovum diameters of strip-spawned fish producing fertile eggs and those producing infertile eggs were compared by ANOVA. Analysis of variance on ranked variables (Conover and Iman 1981) was used to compare means of total length among age groups of fish used to estimate fecundity. Medians of relative batch fecundity were compared among age groups and months using the Brown-Mood test (SAS 1985). The Brown-Mood test was also used to compare median relative batch fecundities of hormone spawned fish and wild fish used to make fecundity estimates. Statistical analyses were performed using the Statistical Analysis System (SAS 1985). All tests were considered significant at the $P = 0.05$ level.

Results

All female spotted seatrout collected from April to August contained yolked ova, and this period was defined as the spawning season. No yolked ova were observed from November through February. Females began developing in March with 10% and 47% of the females displaying yolked ova in 1985 and 1986, respectively. Ovarian regression was observed in September and October with yolked ova present in 4%-90% of fish collected.

In general, mean GSI values followed a pattern corresponding to the occurrence of yolked ova. Lowest values were observed from October to March followed by sharp increases in April and May (Fig. 1). GSI values decreased somewhat during the summer then declined sharply in September or October. During the 1984 spawning season, the May mean GSI value (4.65) was significantly greater than the

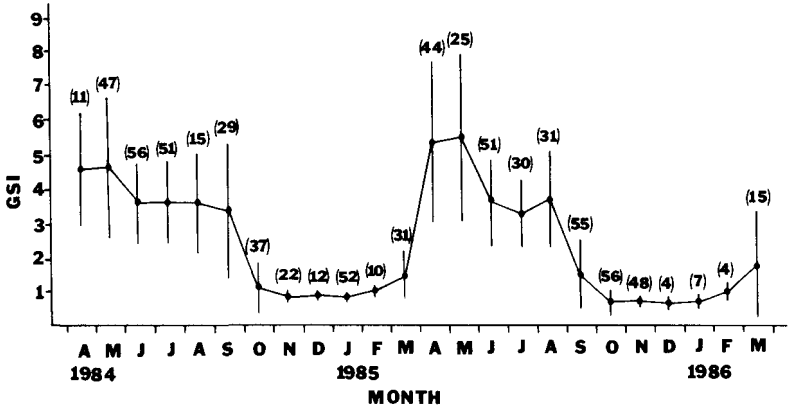

Figure 1. Mean ± SD gonosomatic index (GSI) of female spotted seatrout from Matagorda Bay, Texas, April 1984-March 1986. Numbers in parenthesis represent sample size.

June and July means (3.57 and 3.63). April and May 1985 mean GSI values (5.26 and 5.43) were significantly greater than the June through August means (3.23, 3.63, and 3.70).

The percentage of female spotted seatrout spawning at any given time during the extended spawning season was difficult to determine. At capture, ova of 10 fish collected for GSI determination exhibited lipid coalescence or hydration suggesting spawning was imminent. The mean ± SD GSI value of these 10 preovulatory fish was 5.91 ± 2.00 and ranged 3.41–10.07. Fish with GSI values ≥5.91 comprised 29% of the April and May 1984 and 1985 collections, but only 4% of June through August 1984 and 1985 collections.

Ovum diameter (adjusted for shrinkage using $\hat{R} = 0.69 \pm 0.039$) frequency distributions of preserved ovarian cross sections from fish used for fecundity estimates typically displayed a bimodal pattern. The first mode consisted of primary oocytes approximately 0.04–0.15 mm in diameter, and the second mode contained yolked eggs measuring approximately 0.45–0.64 mm in diameter. Therefore, 0.45 mm was selected as the oocyte maturation size. Counts of ova ≥0.45 mm varied greatly among individual fish (0–6727 eggs/g BW) and resulted in standard deviations greater than the mean (Table 1). Accordingly, the median was selected as the more appropriate measure of central tendency (Sokal and Rohlf 1981). The median relative batch fecundity of the 169 fish was 258 eggs/g BW (mean ± SD = 467 ± 778 eggs/g BW). Fecundity estimates were made for 7 of the 10 fish found to be preovulatory. Median relative batch fecundity of these fish was 254

Table 1. Means ± SD of total length, weight, relative batch fecundity, and median relative batch fecundity estimates by month and age (years) of spotted seatrout collected April-October 1984, Matagorda Bay, Texas.

	N	Total length (mm)	Weight (g)	Mean eggs/g body wt	Median eggs/g body wt
Month					
Apr	9	457 ± 57	999 ± 379	329 ± 291	215
May	42	418 ± 93	833 ± 534	726 ± 1,382	279
Jun	40	420 ± 94	809 ± 510	293 ± 246	246
Jul	38	406 ± 71	645 ± 317	457 ± 488	344
Aug	14	422 ± 89	793 ± 488	313 ± 380	172
Sep	22	406 ± 63	642 ± 342	386 ± 422	225
Oct	4	428 ± 47	745 ± 271	738 ± 643	717
Age					
1	24	321 ± 28	330 ± 78	382 ± 420	268
2	29	353 ± 35	426 ± 113	278 ± 356	137
3	44	414 ± 39	693 ± 178	394 ± 429	301
4	36	466 ± 63	990 ± 350	586 ± 1,144	259
5	14	514 ± 31	1,280 ± 463	652 ± 463	570
6	7	526 ± 43	1,370 ± 326	181 ± 230	103
7	3	699 ± 53	2,080 ± 382	2,249 ± 3,438	335

eggs/g BW (mean ± SD = 325 ± 235 eggs/g BW) with a range of 124–792 eggs/g BW. Median monthly relative batch fecundities were not significantly different among months.

Fish collected for fecundity estimates represented ages 1–7 years (Table 1). Twenty-four fish were age-1 and most of these fish (21) had some ova ≥0.45 mm with 3 specimens exhibiting GSI values >5.91. Mean TL among age groups was significantly different but median relative batch fecundity among age groups was not significantly different, indicating relative batch fecundity does not increase with age or length.

Of the 31 female spotted seatrout (range 300–1,100 g BW) selected for spawning, only 16 ovulated and produced fertile eggs (mean ± SD fertilization rate = 62 ± 19%) following injection with HCG. Fish that did not ovulate (N = 15) had a mean ± SD ovum diameter of 0.37 ± 0.05 mm, (range 0.27 – 0.48 mm) whereas fish that produced fertile eggs exhibited a mean ± SD ovum diameter of 0.45 ± 0.12 mm (range 0.36–0.56 mm). Mean ovum diameter of successfully spawned fish was significantly greater than that of fish which did not ovulate. The median relative batch fecundity of hormone spawned fish was 453 eggs/g BW (mean ± SD = 497 ± 239 eggs/g BW) but was not significantly different from the median relative batch fecundity (258 eggs/g BW) of fish collected for fecundity estimates.

Discussion

Gonosomatic index values observed in this study confirmed an extended, although somewhat shorter, spawning season for Matagorda Bay Spotted seatrout than the March to November spawning season previously reported for the mid-Texas coast (Pearson 1929). Absence of vitellogenic ova in some Texas spotted seatrout in March, September, and October has not been previously reported and suggested spawning by all females occurred only from April through August. Investigators in Florida (Stewart 1961, Tabb 1961) and Louisiana (Sundararaj and Suttkus 1962) have suggested spotted seatrout exhibit 2 peak periods of spawning activity over the spawning season. However, no evidence for a peak spawning period other than April and May was observed for Matagorda Bay fish which agrees with previous findings from middle Texas coastal bays (Pearson 1929).

Great variability of spotted seatrout relative batch fecundity estimates resulted in finding no significant differences in the estimates due to time or size or age of fish. It is not known if the observed variability was caused by asynchronous development of ova to be released at the next spawning event or if the relative batch fecundity of individual fish varies from spawn to spawn. The latter would appear probable since relative batch fecundity estimates of the preovulatory fish ranged 124–792 eggs/g BW.

Published information regarding age at which spotted seatrout females first spawn is limited. Sundararaj and Suttkus (1962) indicated spotted seatrout showed signs of gonadal maturation at age-1, but doubted age-1 fish actually spawned.

Brown et al. (1984) stated 30% of spotted seatrout in Aransas Bay, Texas, reached sexual maturity at age-1. Based on GSI values and the mean diameter of yolked ova, some age-1 spotted seatrout from Matagorda Bay were presumably capable of spawning.

The percentage of spotted seatrout spawning at any given time in Matagorda Bay cannot be directly determined from available data. However, GSI values ≥ 5.91 indicated 29% of female spotted seatrout were in spawning condition during April and May, whereas only 4% exhibited comparable gonadal development from June through August. Similarly, few generalizations can be made about the spawning frequency of individual fish. Tucker and Faulkner (1987) reported a single captive female maintained in an outdoor tank spawned 6 times from 2 June through 29 August 1986. Laboratory maintained spotted seatrout (8 females, 7 males) were reported to have spawned 82 times over 13 months (Arnold et al. 1976, Lasswell et al. 1977).

Previous studies on oocyte maturation size and spawning eligibility of spotted seatrout are limited. Colura (1974) successfully spawned females with mean ovum diameters ≥ 0.46 mm, but used few fish and provided no statistical treatment of oocyte data. The present study yielded an oocyte maturation size of 0.45 mm. The estimated oocyte maturation size was corroborated by the mean ovum diameter of 0.45 mm found in spotted seatrout which were successfully strip-spawned following HCG injection. The median number of eggs/g stripped from each female was not statistically different from relative batch fecundity estimates indicating strip-spawning allows efficient utilization of available ova following induced ovulation.

Spotted seatrout are unique among fishes currently under consideration for hatchery production. Unlike annual spawning fishes, traditional fecundity measurements do not consistently predict egg production. Further, spotted seatrout do not release millions of eggs at each spawning event like other fractional spawners which have been hatchery reared (Lasswell et al. 1977). As a result, hatchery scale spawning will require a relatively intense period of broodfish collection and spawning to accomplish production goals. However, labor requirements can be reduced by concentrating broodfish collection efforts during April and May and only using females displaying mean ovum diameter ≥ 0.45 mm for hormone-induced strip spawning.

Literature Cited

Arnold, C. R., J. L. Lasswell, W. H. Bailey, T. D. Williams, and W. A. Fable, Jr. 1976. Methods and techniques for spawning and rearing spotted seatrout. Proc. Annu. Conf. Southeast. Assoc. Fish and Wild. Agencies 30:167–178.

Bagenal, J. B. and E. Braum. 1971. Eggs and early life history. Pages 166–198 *in* W. E. Ricker, ed. Methods for assessment of fish production in fresh waters. IBP Handbook No. 3, Blackwell Sci. Publ., Oxford and Edinburgh, Great Britain.

Brown, N. J., P. Thomas, and C. R. Arnold. 1984. Reproductive biology of female spotted seatrout in a South Texas embayment. Annu. Proc. Texas Chap. Am. Fish. Soc. 7:4 (abstract).

Cochran, W. G. 1977. Sampling techniques. John Wiley and Sons. New York. 428pp.

Colura, R. L. 1974. Induced spawning of the spotted seatrout *Cynoscion nebulosus* (Cuvier). Proc. World Maricult. Soc. 5:319–330.

———, C. W. Porter, A. F. Maciorowski. 1984. Preliminary evaluation of the scale method for describing age and growth of spotted seatrout (*Cynoscion nebulosus*) in the Matagorda Bay system, Texas. Texas Parks and Wildl. Dep. Manage. Data Ser. No. 57, Austin 17pp.

Conover, W. J. and R. L. Inman. 1981. Rank transformations as a bridge between parametric and nonparametric statistics. The Am. Stat. 35:124–129.

Hegen, H. E. and G. C. Matlock. 1980. Monitoring of coastal finfish resources for sport fish management October 1978-September 1979. Texas Parks and Wildl. Data Series No. 17, Austin. 245pp.

Hein, S. H. and J. A. Shepard. 1979. Spawning of spotted seatrout in Louisiana estuarine ecosystem. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 33:451–465.

Higham, J. R. and V. R. Nicholson. 1964. Sexual maturation and spawning of Atlantic menhaden. Fish. Bul. 63:255–271.

Hoff, F., C. Powell, and T. Pulver. 1972. Artificially induced spawning of the Florida pompano, *Trachinotus carolinus,* under controlled conditions. Proc. World Maricult. Soc. 3:53–69.

Kuo, C. M., C. E. Nash, and Z. H. Shehadeh. 1973. A procedural guide to induce spawning. Pages 1–16 *in* The grey mullet (*Mugil cephalus* L.): Induced breeding and larval rearing research 1972–73 Volume II. Report No. OI-73-128, Oceanic Inst. Waimanalo, Hi.

Lasswell, J. L., G. Garza, and W. H. Bailey. 1977. Status of marine fish introductions into the fresh waters of Texas Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 31:399–403.

McEachron, L. W. and A. W. Green. 1985. Trends in relative abundance of selected finfishes along the Texas coast: November-June 1984. Texas Parks and Wildl. Dep. Manage. Data Series No. 79, Austin. 189pp.

Morawska, B. 1984. The effect of water temperature elevation on incipient and cumulative fecundity of batch-spawning Tench, *Tinca tinca* (L.). Aquaculture 42:273–288.

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. Bul. Bur. Fish. 44:129–214.

Porter, C. W. and A. F. Maciorowski. 1984. Spotted seatrout fingerling production in saltwater ponds. J. World Maricult. Soc. 15:222–232.

SAS. 1985. SAS/STAT Guide for personal computers, Version 6 ed. SAS Inst. Inc. Cary, N.C. 378pp.

Simpson, A. C. 1951. The fecundity of plaice. Fish. Invest. London, Ser. 2. 17:1–27.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. W. H. Freeman Company. San Francisco. 859pp.

Stewart, K. W. 1961. Contributions to the biology of the spotted seatrout (*Cynoscion nebulosus*) in the Everglade National Park, Florida. M.S. Thesis, Univ. Miami, Coral Gables, Fla. 103pp.

Sundararaj, B. I. and R. D. Suttkus. 1962. Fecundity of the spotted seatrout *Cynoscion*

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

Tabb, D. C. 1961. A contribution to the biology of the spotted seatrout *Cynoscion nebulosus* (Cuvier) of east-central Florida. Fla. Board of Conser. Tech. Ser. No. 35:1–24.

Tucker, J. W. and B. E. Faulkner. 1987. Voluntary spawning patterns of captive spotted seatrout. North. Gulf. Sci. 9:59–63.