Inshore spawning of cobia (*Rachycentron canadum*) in South Carolina

Lefebvre and Denson, 2012

SEDAR28-RD10

5 January 2012

INSHORE SPAWNING OF COBIA (*RACHYCENTRON CANADUM*) IN SOUTH CAROLINA

LYNDSEY S. LEFEBVRE

* Grice Marine Laboratory
College of Charleston
205 Fort Johnson Road
Charleston, South Carolina 29412

**Fisheries Ecology Division
Southwest Fisheries Science Center
National Marine Fisheries Service
110 Shaffer Road
Santa Cruz, California 95060
Phone: (831) 345-0827
Fax: (831) 420-3977
Lyndsey.Lefebvre@noaa.gov

MICHAEL R. DENSON

Marine Resources Research Institute
South Carolina Department of Natural Resources
217 Fort Johnson Road
Charleston, South Carolina 29412

Key words: cobia, spawning, embryo development, ovarian development, ichythyoplankton

***** Work conducted while affiliated with

****** Current contact information

ABSTRACT

Inshore spawning of the recreationally important cobia, *Rachycentron canadum*, was documented in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina between April and June of 2007 and 2008. Histological analysis of ovaries confirmed the presence of gravid females inshore, and gonadosomatic index (GSI) values from females collected inshore (mean=7.8) were higher than those caught offshore (mean=5.6), both of which indicate spawning was occurring locally. Additionally, an ichthyoplankton survey found cobia eggs and larvae as far as 10 and 15 km upriver from the mouths of SHS and PRS, respectively. An egg development study of hatchery-reared cobia eggs provided descriptions of embryological development of cobia. The comparison of visual and quantitative characteristics allowed for positive identification of eggs collected in plankton samples. We show that the inshore migration of cobia during April through June, presence of gravid females, significantly higher GSI values, and collection of eggs inside the estuaries, all confirm that PRS and SHS provide spawning habitat for cobia. Due to the potential for heavy exploitation by recreational anglers as cobia move inshore to spawn in South Carolina, current management strategies may need review.

INTRODUCTION

Cobia, *Rachycentron canadum*, is a migratory, stenohaline benthopelagic species distributed worldwide in tropic, subtropic, and warm temperate waters, except the eastern Pacific (Briggs, 1960). In the United States, cobia are found throughout the Gulf of Mexico and along the Atlantic coast from Florida to Massachusetts (Shaffer and Nakamura, 1989). Cobia are moderately long-lived, with a maximum reported age of 15 years (Shaffer and Nakamura, 1989), and have fast growth rates, with both sexes reaching sexual maturity by age two (males 60 cm fork length [FL]; females 80 cm FL; (Smith, 1996; Burns et al., 1998). Currently, no substantial commercial fishery exists for cobia in the United States, with most commercial landings resulting from incidental catch in other fisheries (Shaffer and Nakamura, 1989). Cobia are sought recreationally throughout their range, and the majority of the annual catch in the U.S. comes from the recreational fishery (National Marine Fisheries Services Statistics Division, personal communication). The current fishery management plan, which imposes a bag limit of two fish per person per day and a minimum FL of 84 cm (33 inches), was established by the Gulf of Mexico (GMFMC) and South Atlantic Fishery Management Councils (SAFMC) in 1983[1] with the aim of conserving a population that was considered overexploited at that time. The restrictions were enacted under the assumptions that cobia are widely dispersed, are primarily commercial bycatch, constitute a recreational fishery, and comprise a single population in the U.S. These restrictions were meant to reduce catches and allow females the opportunity to reproduce before entering the fishery. Regional fishing effort and

[1] SAFMC and GMFMC. 1983. Fishery management plan final, environmental impact statement, regulatory impact review, final regulations for coastal migratory pelagic resources (mackerels). SAFMC, 4055 Faber Place, Suite 201, North Charleston, SC 29405. 321 pp.

catch data indicate fishing pressure for cobia has increased over the past decade (Steele[2]). Cobia continues to gain socioeconomic importance as a game fish throughout much of its range, supporting an expanding charterboat industry; however, the current health of the stock is unknown along the southeastern U.S.

In the spring and early summer months, cobia in the western North Atlantic are thought to migrate with warming waters from Florida to as far as Massachusetts (Shaffer and Nakamura, 1989). During this presumed northward migration, cobia enter high salinity bays and estuaries, including Port Royal Sound (PRS) and St. Helena Sound (SHS) in South Carolina, Pamlico Sound in North Carolina (Smith, 1996), and the Chesapeake Bay (Shaffer and Nakamura, 1989), where they are more readily available to recreational anglers. Reasons for the inshore movement are not fully understood, but it is hypothesized they may be following prey species or aggregating to spawn.

On the east coast of the U.S., the cobia spawning season extends from April though September (Lotz et al., 1996; Smith, 1996; Burns et al., 1998; Brown-Peterson et al., 2001) with cobia being batch spawners, capable of spawning multiple times during a season (Biesiot et al., 1994; Lotz et al., 1996). Regional peaks in spawning, as designated by peaks in the gonadosomatic index (GSI), correlate with the migration of cobia from Florida to Massachusetts. Spawning peaks along the southeastern U.S. Atlantic coast in May (Shaffer and Nakamura, 1989; Burns et al., 1998), in North Carolina in June (Smith, 1996), and in the Chesapeake Bay region in June and July (Joseph et al., 1964). In South Carolina the peak in spawning activity in May corresponds to the highest fishing effort in the region, as evidenced by the increased

[2] Steele, G. 2009. Personal communication. South Carolina Department of Natural Resources, Charleston, SC 29412.

landings during this month (Steele[1]). The South Carolina recreational cobia fishery, in which the fish are easily accessible to anglers April through June when located inside PRS and SHS, may be similar to the fishery in other states where cobia enter inland waters. Because the inshore migrations correspond with the spawning season of cobia, it is probable the sounds serve as spawning habitat.

Beyond the general knowledge of spawning season, a paucity of information exists on spawning habitat and time of day of wild cobia spawning because much of the previous research has focused on age and growth (Smith, 1996; Franks et al., 1999), feeding habits (Smith, 1996; Arendt et al., 2001), and general reproductive biology (Biesiot et al., 1994; Lotz et al., 1996; Smith, 1996; Brown-Peterson et al., 2001; van der Velde et al., 2010). Collection of eggs in the Gulf Stream off North Carolina by Hassler and Rainville (1975) suggested spawning took place offshore. Offshore spawning was also proposed by Burns et al. (1998), due to a scarcity of fish with histological signs of final oocyte maturation (FOM) collected in nearshore waters of the Gulf of Mexico and the southeast U.S. Atlantic coast. Inshore spawning of cobia has been proposed in the lower regions of Chesapeake Bay, based on the collection of eggs immediately south of the bay (Joseph et al., 1964) and the ovarian condition of females collected in the bay (Richards, 1967). In North Carolina Smith (1996) suggested cobia spawned adjacent to inlets, by reason of neuston net collection of eggs within inlets and a lack of females caught inshore undergoing FOM.

The main goal of the current study was to determine if cobia spawning occurs within two high salinity South Carolina estuaries, PRS and SHS. Histological analysis of ovarian tissue was used to evaluate the reproductive status of female cobia collected by

the recreational fishery both inshore and offshore of these estuaries. A development study using hatchery-reared cobia eggs provided embryological development characteristics and insight on time of day of spawning of wild cobia. An ichthyoplankton survey targeting cobia eggs and larvae in PRS and SHS was conducted during the spawning season to provide further evidence of spawning locations. Inshore spawning of cobia in South Carolina may suggest that spawning aggregations occur in other inland and nearshore waters that cobia frequent during the spawning season and that cobia may be subject to substantial fishing pressure before having the opportunity to reproduce.

MATERIALS AND METHODS

Reproductive Biology

Fresh and frozen cobia specimens were collected via hook and line from PRS and SHS, South Carolina (Fig. 1) in April-June 2007 and 2008, from fishing tournaments, cooperating anglers, recreational fishing guides, and SCDNR employees. For all whole fish collected, total length (TL, mm), FL (mm), and fish weight (FW, kg) were measured. Sex was determined macroscopically and gonads were excised, stored on ice, and transported to the SCDNR Marine Resources Research Institute (MRRI). Date, time, and location of capture were noted when available, with fish collected within PRS and SHS designated as “inshore” and fish collected outside the two estuaries (defined as outside the barrier islands) designated as “offshore”. For carcasses collected through SCDNR’s freezer program, in which recreational anglers donate the fish carcass after filleting, the aforementioned information was recorded when provided by anglers.

At the MRRI, gonads were weighed to the nearest gram and the GSI was calculated as

$$GSI = \left({}^{GW}/_{SW} \right) \times 100$$

where GW = gonad weight (g) and SW = somatic weight (g). To determine if GSI values for male and female cobia were significantly different, a Wilcoxon rank test was performed. Cobia are batch spawners with indeterminate fecundity and group-synchronous oocyte development (Lotz et al., 1996; Brown-Peterson et al., 2001; van der Velde et al., 2010). Ovarian growth in other species with the same reproductive strategy is isometric with body growth (Taylor et al., 1998; Somarakis et al., 2004). Additionally, previous work from cobia ovaries with FOM has shown that there is no relationship between relative fecundity and either body weight or fork length (Brown-Peterson et al., 2001; van der Velde et al., 2010). Therefore, female GSI was used as a proxy for ovarian maturation for statistical purposes. To evaluate if ovarian maturation differed between cobia collected inshore and offshore, a Wilcoxon rank test was performed using GSI as a proxy for maturation. Non-parametric Wilcoxon tests were performed due to non-normality in the data resulting from the capture of two gravid females. A t-test was used to determine if differences in GSI between inshore and offshore females persisted with the exclusion of the GSI values from the two gravid females.

Histological analyses were limited to samples collected from female cobia, as males are capable of spawning year-round (Brown-Peterson et al., 2001). Homogenous ovarian development has been documented previously for cobia (Lotz et al., 1996); therefore, a single portion of tissue (approximately 50-100 mg) from the middle of one ovarian lobe was fixed in 10% neutral buffered formalin before being rinsed in freshwater and stored in 50% isopropyl alcohol at least 24 hours prior to processing. Tissue samples were dehydrated; infiltrated and blocked in paraffin; sectioned to 6 µm

using a rotary microtome; mounted on glass slides; and stained using hematoxylin and eosin-y according to standard histological techniques (Humason, 1972). Slides were examined under a compound microscope at 100x magnification and staged according to ovarian development. Ovarian stages (Table 1) are based on descriptions of teleost oocyte development in Wallace and Selman (1981) and with modifications from Roumillat and Brouwer (2004). When present, postovulatory follicles (POFs) were categorized as either less than or equal to, or greater than 12 hours old, based on rates of POFs atresia found in *Cynoscion nebulosus* (spotted sea trout; Roumillat and Brouwer, 2004). All samples were staged by a second, independent reader. Discrepancies were resolved by simultaneous viewing of the slides by both readers, with all discrepancies resolved. Percent composition (PC) of females in each of the ovarian maturation stages was calculated separately for females collected inshore and offshore as

$$PC = \left(n_s / T\right) * 100,$$

where n_s = the number of female samples in stage *s*, and T = the total number of female samples.

Egg Development

To obtain a time-series reference collection of cobia eggs to assist in positively identifying and aging candidate eggs from plankton collections, development studies were conducted in 2007 and 2008 using fertilized eggs obtained from wild-caught adult cobia spawned in the laboratory. Cobia yolk-sac larvae from the 2007 study served as reference for the identification of young larvae in plankton collections. The experimental temperature treatments covered the measured range of surface temperatures encountered in PRS and SHS during May and June of both years.

In 2007 at the Hollings Marine Laboratory (HML), four fiberglass hatching cones (170 L) were equipped with aerators and heaters, and filled with seawater (34.5 parts per thousand [ppt]) from Charleston Harbor that had been filtered (5 µm) and UV sanitized after settling for three days. The water was heated to 22.5, 25.0, 26.5, and 29.0 ºC, respectively, and maintained for 48 hours prior to the start of the study. Four 50 mL aliquots of eggs (~2 x 10^4 eggs) were used in the trial. Prior to the addition of eggs to the water baths at approximately 11 hours post-spawn, digital micrographs of eggs were recorded using a Nikon SMZ1500 stereo microscope (Nikon Instruments Inc., Melville, NY) mounted with a Micropublisher 3.3 camera (Q Imaging, Surrey, BC, Canada). Thereafter, approximately 10 eggs and/or larvae were collected from each tank and micrographs were recorded every four hours until 61 hours post-spawn. After images were recorded, eggs and/or larvae were preserved in 10% neutral buffered formalin.

In order to capture earlier stages of egg development and to expand experimental temperatures, a second development study was conducted in 2008. Three water baths were heated to and maintained at 24.0, 26.0, and 28.0 ºC in a temperature-controlled laboratory 30 hours prior to the beginning of the study. At two hours post-spawn, a 25 mL aliquot of cobia eggs (~1 x 10^4 eggs) was divided between petri dishes in each temperature bath. Every hour, until 13 hours post-spawn, and every other hour thereafter, to 25 hours post-spawn, a sample of approximately 10 eggs was removed from each water bath, digital micrographs were recorded, and eggs were preserved in 10% neutral buffered formalin.

Micrographs of live eggs, egg diameters and oil droplet diameters were measured to the nearest µm using ImageJ image analysis software (ImageJ, vers. 1.38, Bethesda,

MD). To determine if damage and diameter changes occurred with preservation, changes in appearance were noted and measurements of egg and oil droplet diameters were measured to the nearest µm using ImageJ from micrographs of preserved eggs taken approximately one year from the date of collection in 2007 and two months from the date of collection in 2008. Diameters were only measured in undamaged, preserved eggs. Percent shrinkage was calculated as change in egg and oil droplet diameter between the live and corresponding preserved eggs multiplied by 100. To determine if there was a significant decrease in diameter due to preservation, two-sample t-tests were performed independently for 2007 egg and oil droplet diameters and 2008 egg diameters. Due to non-normality of the data, a Wilcoxon rank test was performed using 2008 oil droplet diameters to determine if there was significant shrinkage after preservation.

Based on the micrographs of live eggs from the development studies, coarse stages of embryological development were described. Because only the 2008 study captured the earliest stages of development and larvae only hatched in the 2007 study, the two temperature treatments that were closest in temperature (26.5 ºC and 26 ºC in 2007 and 2008, respectively) were used for the description of development to capture the earliest stages of development through hatching.

Ichthyoplankton Survey

Field Collection

Five stations in each estuary (Fig. 1), encompassing known cobia fishing grounds, were sampled weekly from May 6, 2008 through June 15, 2008 in PRS and May 8 through June 8, 2008 in SHS. Collections were made from 0745 to 1945 hours to accomplish plankton captures during similar stages of the incoming tide. Stations in PRS

were located between 12.1 and 20.6 km from the mouth of the estuary, as measured from a navigation buoy (GC “25) located in the channel between St. Helena Island and Hilton Head Island. Stations in SHS were located between 5.1 and 14.3 km from the mouth of the estuary, as referenced from navigation buoy GC “9. Stations were positioned upriver of particular bathymetric features where anglers typically target cobia. These are areas where sand bars and banks result in water depth rising from 10 to 12 m mean low water (mlw) to 2 to 3 m (mlw). Depths of stations ranged from 5.5 to 9.6 m at the time of net deployment. Anchored plankton nets (Fig. 2) were deployed at slack water prior to the daylight flood tide. Floats attached to the frame maintained the position of the net at approximately 1 m below the surface. Flow meters were mounted in the center of the nets at the most seaward and most upriver stations. At the time of net deployment, ancillary water data were collected (temperature, salinity, dissolved oxygen) at the surface and bottom of the water column using a handheld YSI model 556 (YSI Inc., Yellow Springs, OH). Plankton nets were deployed for the length of time required to set all nets and return to retrieve the first (70-150 minutes). The average current speed during the time of collection was calculated using the flow meter reading. Volume of water filtered was calculated directly from flow meter readings in nets. For nets without flow meters, current speeds and volumes filtered were estimated by taking the average of the values from the most seaward and most upriver stations.

Egg and Larval Identification

Plankton samples were rinsed and sorted under a dissecting microscope. When settled plankton volumes were greater than 1 L, samples were split using a Burrell plankton splitter (Burrell et al., 1974) until a settled plankton volume of 0.5 L or less was

attained. All non-clupeiform larvae were removed, identified to lowest possible taxonomic level, and preserved in 70% isopropyl alcohol. Cobia larvae were identified from descriptions of Ditty and Shaw (1992). All eggs measuring between 1.0 and 1.4 mm on an ocular micrometer and with one or more of the morphological characteristics corresponding to cobia eggs (single, large [300 to 600 µm], pigmented oil droplet; heavily pigmented embryo; narrow perivitelline space; (Ditty, 2006) were removed and preserved in 10% neutral buffered formalin.

Digital micrographs of preliminarily identified cobia eggs were recorded. Egg and oil droplet diameters of preserved eggs were measured to the nearest µm using ImageJ. To aid in identification, eggs from the two development studies were viewed as references. Eggs were positively identified as cobia if their diameter fell within the range noted in the literature and if the morphological characteristics, with the exception of number and diameter of oil droplets, matched the hatchery-reared eggs and published description of cobia eggs (Ditty, 2006). The number and diameter of oil droplets were excluded as positive identifying characters because of damage occurring due to preservation. For further analyses eggs were labeled as early- (from fertilization to blastopore closure; embryo not visible in preserved eggs; Ahlstrom and Moser, 1980) or late- (from blastopore closure to hatching; embryo evident in preserved eggs; mid and late stages in Ahlstrom and Moser 1980) stage.

Maternal condition in other species has been shown to have a significant effect on oil droplet size of eggs (Gagliano and McCormick, 2007) and larvae (Berkeley et al., 2004; Sogard et al., 2008), but no significant effect on egg size. As secondary evidence for correct identification of eggs, an analysis of covariance (ANCOVA) was conducted to

test for differences in the relationship of egg and oil droplet diameters between known cobia eggs (early- and late-stage eggs from the 2007 and 2008 development studies) and eggs identified from plankton samples. If the relationship was not significantly different between hatchery-reared and wild eggs, the wild eggs were confirmed as cobia. Only measurements from eggs with single, intact oil droplets were used in the ANCOVA.

Ages of cobia eggs collected from the plankton samples were estimated via side-by-side comparison to eggs reared at the closest matching temperature from either the 2007 or 2008 development study. For a given sample, time of spawning was approximated using the estimated age of eggs by back-calculating from the time of sample collection. Numbers of cobia eggs and larvae found in samples that had been split were estimated based on the fraction of the sample sorted. Concentrations of cobia eggs and larvae collected in the ichthyoplankton survey were obtained using calculated and estimated sample volume filtered for the corresponding sample.

All statistical analyses were conducted using R statistical software (R Development Core Team 2009). The significance level of $\alpha = 0.05$ was used for all tests.

RESULTS

Cobia (278 females, 283 males) were collected between April 2007 and August 2008. Two-hundred sixty-one fish came from fishing tournaments, 37 from SCDNR efforts, and 263 from donations to the SCDNR freezer program. Specimens ranged in size from 850 to 1425 mm FL (mean=1042 mm) for females and 386 to 1215 mm FL (mean=930 mm) for males. Weights ranged from 6.7 to 38.3 kg (mean=15.0 kg) for females and 0.5 to 23.0 kg (mean=9.9 kg) for males. Eighty percent of the fish 1000 mm FL or larger and 79% of the fish with a total weight equal to or greater than 10 kgs were female. Capture locations were available for 183 fish (44 offshore, 101 PRS, 39 SHS).

Reproductive Biology

GSIs were calculated for 278 cobia (164 females; 114 males) and were combined for April, May and June of both years. GSI for all cobia ranged between 0.7 and 22.5 (mean=6.1), and females had a significantly higher mean (7.3) than males (4.4; $p<0.05$). Females collected inshore (mean=7.8; *n*=64) had a statistically significant higher average GSI than females collected offshore (mean=5.6; *n*=34; $p=0.002$), suggesting the ovaries were in a more developed state in inshore fish. The comparatively higher mean GSI from females collected inshore was not biased by the collection of gravid females inshore or spent females offshore as the mean inshore GSI (7.4) remained statistically higher than the mean offshore GSI (5.6; $p=0.005$) with removal of the gravid and spent females.

Histological analysis of 213 ovaries showed that female cobia were capable of spawning during May and June, when they are also most heavily targeted by anglers in South Carolina (Steele[1]). All ovarian stages were represented in the female cobia samples examined except for the immature and recovering stages (Fig. 3). Immature specimens were absent because current fishing regulations impose a minimum FL (84 cm) larger than the length at female first maturity (80 cm). The lack of females in the recovering state is a result of sample collection occurring only within the known spawning season of cobia. Further histological analysis of female ovarian samples was limited to the 98 specimens for which location of capture was known (64 inshore and 34 offshore). The majority (72%) of female cobia collected from both inshore and offshore waters had ovaries in the late stage of development (Table 2). Two females with ovaries

in the early developing stage were collected offshore in early April 2007 and inshore in early May 2007. Both females (89 cm FL and 93 cm FL, respectively) were larger than the size at maturity, and had no evidence of prior spawns (POFs), and were, therefore, likely maturing for the first time of the spawning season. A single female in the spent stage was collected in offshore waters in early June 2008. Females with histological signs of a previous spawn (visible POFs) were found in cobia collected inshore as well as offshore. All POFs were estimated to be greater than 12 hours old (Hunter and Macewicz, 1985; Roumillat and Brouwer, 2004), with the exception of one specimen collected in the morning hours in May 2007, in which the POFs were estimated to be 12 hours old.

Two gravid females were collected from inshore waters in 2007. The first (121.6 cm FL and 27 kg TW) was collected in mid-morning on May 12, 2007 in the Broad River (PRS). The second (93.4 cm FL and 11 kg TW) was caught by SCDNR employees in SHS on June 8 at 1030 hours. Both of these samples showed ovaries in the late stage of final oocyte maturation (FOM), in which yolk coalescence and hydration occurs immediately prior to ovulation. The time of collection of both the females, the state of hydration of the oocytes, and the rapid nature of FOM found in other multiple spawning species with distributional overlaps with cobia (Brown-Peterson et al., 1988; Fitzhugh et al., 1993; Roumillat and Brouwer, 2004) indicates they would have spawned the afternoon of capture. Damage occurred to some tissue samples that had either been hard frozen or that had decayed prior to collection, which resulted in all size groups of vitellogenic oocytes superficially resembling oocytes undergoing the beginning stages of FOM (early lipid coalescence and migration of the nucleus). In order to avoid confusion

between FOM and damage caused to the tissue due to decay or freezing, as well as to maintain consistency across all samples, FOM stages earlier than hydration were not addressed.

Egg Development

Micrographs of cobia eggs from the 2007 and 2008 development studies were taken at 13 and 19 time periods, respectively. In 2007, the eggs hatched between 39 and 43 hours post-spawn at 22.5 ºC; 29 and 33 hours at 25.0 ºC; and 25 and 29 hours at 26.5. Eggs incubated at 29 ºC were observed hatching at 26 hours post-spawn as micrographs were being recorded. At the end of the 2007 study, all larvae still had yolk-sacs and were 3.8 to 4.6 mm TL. The 2008 development study ended before any eggs hatched. Mean egg diameters of live cobia eggs were 1241 and 1337 µm in 2007 and 2008, respectively. Mean oil droplet diameters were 359 µm in 2007 and 403 µm in 2008.

Micrographs of preserved eggs from development studies from each year revealed damage to the oil droplet in many specimens due to preservation and/or handling (Fig. 4). Deformation of the oil droplet (irregularly shaped oil droplet) occurred most often in early-stage eggs. Damage in late-stage eggs included split oil droplets or droplets in which the pigmented portion had detached from the lipid. Measurements of preserved eggs were limited to specimens that had a single, intact oil droplet. The mean egg diameter of preserved eggs from both years was 1280 µm, and the mean oil droplet diameter was 380 µm. The averages of egg and oil droplet diameters for both preserved and live cobia eggs fell within the published range (Table 3; Ditty, 2006). There was significant shrinkage in cobia egg diameters with preservation, with a rate of 1.7% in

2007 ($p<<0.05$) and 1.0% in 2008 ($p<<0.05$). No shrinkage occurred in the oil droplet diameters during either year (2007 $p=0.13$; 2008 $p=0.92$).

Five stages of embryological development were described for cobia (Fig. 5) and are detailed below. Duration of each stage is approximated in hours. There is overlap in the durations of stages III and IV due to minor differences between eggs in 2007 and 2008 development studies.

Stage I (0-7 h): Newly fertilized eggs have a distinct, translucent oil droplet. Early cell divisions are evident at the animal pole (opposite of the oil droplet) and eventually form the blastodisc. Continuing divisions progress until individual blastomeres are no longer distinguishable. Stage ends when germ ring is visible and encloses approximately 1/3 of the yolk mass. *Stage II* (7 -13 h): The germ ring becomes distinct and epiboly proceeds until the germ ring is in center of the egg. Stage ends when the blastopore is closed and the optic cups distinguish head and tail regions of the embryo. *Stage III* (13-19 h): The embryo is greater than ¼ internal circumference of the egg. Somites are distinct. Stellate melanophores are scattered around the outside of the yolk. Through the stage, pigmentation on the embryo (from head to anterior of caudal region) increases. Stage ends with first appearance of melanophores on oil globule and when embryo is approximately ½ internal circumference of egg. *Stage IV* (14-21 h): The embryo continues to become more heavily pigmented. "Free" melanophores continue to congregate on oil globule until there are few to no free melanophores around outside of yolk. The embryo begins to move, as evidenced by flexion of the body. Stage ends when tail is visibly detached from yolk, and embryo is approximately ¾ internal circumference of egg. *Stage V* (21-29 h): The embryo continues to become more heavily pigmented.

More movement of the embryo is evident. The embryo is greater than 100% internal circumference of the egg before hatching, with the tail extending beyond the head.

Ichthyoplankton Survey

A total of 52 anchored plankton samples (26 PRS; 26 SHS) were collected between May 6 and June 18, 2008. All samples from PRS were sorted completely. Seventeen of the 26 samples from SHS were split to 1/2 (*n*=1), 1/4 (*n*=14), or 1/8 (*n*=2) the original settled plankton volume. Measured current speeds ranged from 0.08 to 0.93 m/s (0.29 to 3.35 km/hr), with most speeds falling between 0.14 and 0.71 m/s (0.50 to 2.56 km/hr). Volumes filtered through the plankton nets ranged from 120 to 1156 m^3. Surface water temperatures in both estuaries ranged from 20.1 to 30.0 ºC. Salinities ranged from 31.6 to 34.3 ppt in PRS and 28.4 to 32.7 ppt in SHS, which are within tolerable ranges for larval, juvenile, and adult cobia (Hassler and Rainville, 1975; Shaffer and Nakamura, 1989; Denson et al., 2003). The water columns in both PRS and SHS appeared to be well mixed, with surface and bottom temperatures and salinities varying by no more than 0.8 ºC and 0.8 ppt, respectively.

A total of 923 eggs were identified as cobia based on size and morphological characteristics (562 early-stage and 364 late-stage; Tables 3 and 4; Fig. 6). Late-stage eggs were found in samples collected at all stations in PRS (*n*=59). The 27 early-stage eggs from PRS were collected at 1930 hours on June 5, 2008 at a single station (PA08, Fig. 1; Table 4) approximately 15 km upriver in the Broad river. The majority of the eggs were collected in SHS, with 535 early-stage eggs and 305 late-stage eggs collected among all stations. Four hundred ninety-six early-stage eggs found in SHS came from a single sample collected at 1900 hours on June 3, 2008 at a station (SA03) approximately

9.7 km inshore (Fig. 1; Table 4). Late-stage eggs were collected between 1230 and 1945 hours in both estuaries on 8 sampling days. Egg concentrations ranged from 0.14 to 62.51 per 100 m^3 (Table 4).

Oil droplets in many of the preserved eggs collected in the plankton survey resembled damaged oil droplets of preserved cobia eggs from the development study. All egg diameters were measured from micrographs, but oil droplet diameter was only measured when a single oil droplet was present (*n*=73). Egg diameters ranged from 1116 to 1393 µm, and oil droplet diameters ranged from 275 to 420 µm. The mean egg and oil droplet diameters were similar to those for hatchery-reared cobia eggs and to those reported elsewhere (Table 3; Ditty and Shaw, 1992; Ditty, 2006).

Early- (*n*=48) and late-stage (*n*=25) egg measurements were combined for the ANCOVA. Due to the significant shrinking of egg diameter with preservation, only measurements from preserved hatchery-reared eggs were used for the ANCOVA. Mean oil droplet diameters for field-collected eggs (357 µm) was lower than that for hatchery-reared cobia eggs (380 µm); however, the ANCOVA showed the slopes of the regression lines were not statistically different (p=0.35; overall R^2=0.61; Fig. 7). The lack of a significant difference in the relationship of egg to oil droplet diameter between hatchery-reared cobia eggs and eggs collected in the plankton samples supports the visual identification of those from plankton collections.

Ages of field-collected eggs were estimated for samples that contained more than one undamaged egg (Table 5). Early-stage eggs tended to turn opaque in preservation, and, as a result, it was difficult to discern their stage of cell division or cleavage: therefore, ages were estimated for early eggs from a single sample collected June 5 in

PRS. When eggs resembled an intermediate between two time periods, ages were estimated to be midway. Late-stage eggs were estimated to be between 18 and 26 hours old. Time of spawning was estimated to range from the late afternoon (1530 hours) to late evening (2145 hours) hours (Table 5), with most of the spawning between 1530 and 1800 hours. The three exceptions were from a single sample day in SHS, when spawning was estimated to have occurred near midnight. While the estimates of the ages were determined subjectively by comparing wild-caught plankton to hatchery reared eggs, eggs collected from multiple stations on the same day were aged independently and estimated all to be nearly the same age.

A total of 42 cobia larvae (18 PRS; 24 SHS; Fig. 8) were collected on eight sampling days. Larval concentrations ranged between 0.169 and 1.989 larva per 100 m^3 (Table 5). Five yolk-sac cobia larvae were collected, two on May 14 (stations PA05 and PA08), one on May 29 (station PA07), and two on May 21 (station SA01).

DISCUSSION

The results of this study indicate cobia spawn within PRS and SHS, South Carolina, as evidenced by the high mean GSI value of females caught, the collection of gravid females, the presence of recently fertilized eggs, and the presence of larvae in these two estuaries. Using estimated ages of field-collected eggs, in combination with time and location of capture data from adult cobia collected at fishing tournaments, this study provides evidence of spawning of wild cobia in inshore waters in the late afternoon and early evening hours.

The mean GSI of female cobia collected in this study peaked at a higher value (7.3) than previously reported for cobia in the Gulf of Mexico by Biesiot et al. (1994; 5.5)

and Lotz et al. (1996; 5), and North Carolina by Smith (1996; 5.7). The higher mean GSI value reported in this study is not biased by the collection of two gravid females, as Smith (1996) and Biesiot et al. (1994) also collected females with ovaries undergoing FOM. Even when these females were removed from analyses, the GSI values still yielded an average value well above that reported elsewhere. It is unlikely the high mean GSI was due to regional differences between collected specimens. Brown-Peterson et al. (2001) found that female cobia collected from the southeast Atlantic U.S. had higher mean GSI values (~5.5) than those from either the eastern (~5) or north-central Gulf of Mexico (~4.5). However, their reported peak mean GSI value for fish from the southeast Atlantic coast was comparable to the peak GSI values reported in fish from the Gulf of Mexico by Biesiot et al. (1994; 5.5) and Lotz et al. (1996; 5). In addition to a higher GSI value, the size range of oocytes (500-850 µm) in late developing ovaries, which dominated collections in this study, was higher than the group with the most developed oocytes reported elsewhere (500-650 µm; Lotz et al., 1996). Fully developed and fertilized cobia eggs in this study and Ditty and Shaw (1992) ranged in size from 1.15 mm to 1.42, so the larger oocyte sizes found in developing ovaries suggest the oocytes were more highly developed in the current study. Brown-Peterson et al. (2001) and van der Velde et al. (2010) reported that monthly GSI values corresponded with histological evidence of when spawning was occurring, supporting GSI as a good proxy of the developmental state of ovaries. In the current study, the larger size range of oocytes together with the higher GSI values indicate female cobia collected in South Carolina were closer to the time of spawning than previous studies.

Differences in the stage of cobia ovarian development between this and other studies may stem from female capture location. In previous work, females were collected from coastal waters in the Gulf of Mexico (Lotz et al., 1996; Brown-Peterson et al., 2001), as well as off the southeast Atlantic coast of the U.S. (Smith, 1996; Brown-Peterson et al., 2001). Of the samples we examined, the mean GSI of females collected offshore (5.6) was closer to the means reported elsewhere (Biesiot et al., 1994; Lotz et al., 1996; Smith, 1996; Brown-Peterson et al., 2001). The dissimilarity of GSI values between female cobia collected inshore and offshore is likely because those caught inshore were part of a spawning aggregation whereas those caught offshore were caught before or after spawning or intercepted while migrating to spawning grounds. This hypothesis is supported by recent genetic evidence showing the presence of distinct population segments in South Carolina (Darden[3]). In a study of the genetic structure of cobia caught offshore from Florida to Virginia and from inshore locations in South Carolina and Virginia, Darden et al. found that fish collected offshore comprised a single population. Furthermore, the fish collected in inshore waters were genetically distinct from the offshore group and from other estuaries. If there is a distinct inshore South Carolina population segment, it could be maintained through spawning of cobia in their natal estuaries.

Histological analysis of the ovaries demonstrated that the fish were in the middle of their reproductive season, with the majority in the late developing stage. The distribution of females between the reproductive states may not represent the actual distribution in the population because the primary means of specimen collection was

[3] Darden, T. 2009. Personal communication. South Carolina Department of Natural Resources, Charleston, SC 29412.

fishery-dependent. In particular, the low percentage of females with early developing and spent ovaries is likely result of fishery practices. Cobia are generally thought to enter inshore waters when water temperatures reach 20 ºC (Richards, 1967; Smith, 1996), and water temperatures in PRS and SHS reached 20 ºC before the end of April in 2008. Recreational anglers, in contrast, targeted cobia most heavily in May. Similarly, the fishing season for cobia may end before the fish leave inshore waters: the final plankton collections in PRS and SHS in mid-June contained cobia eggs and larvae, respectively, indicating spawning was still occurring in the area despite a decrease in availability of adult carcasses from recreational anglers. Female cobia with histological signs of recent spawns (POFs) were collected inshore, but may be underrepresented, again, due to the primary means of collection being heavily dependent on recreational fishermen. Evidence from both the histological analysis and plankton collections of eggs in this study suggest that spawning of wild cobia in South Carolina inshore waters occurred from mid afternoon to late evening, with most spawning activity occurring between 1530 and 1800 hours. Unfortunately, fish collected through tournament donations, where weigh-ins occurred between 1500 and 1800 hours, were the primary means of attaining samples. Few fish were collected during this critical period of time, when females would have shown indications of a recent spawn, and, as expected, most POFs were found to be > 12 hours old.

The best evidence produced from histological analysis that cobia were spawning inshore in South Carolina was the collection of gravid females. The duration of FOM is unknown for cobia, though this hormonally controlled “point-of-no-return” on the path to spawning has been found to commence as late as 13 or as early as six hours prior to

spawning in spotted sea trout (Brown-Peterson et al., 1988; Roumillat and Brouwer, 2004), which spawn in estuaries during the same time of year and within the same range as cobia. Because of potential damage to tissues caused by freezing or decay prior to sample collection, the earliest stages of FOM were not examined in this study and we, therefore, do not have an estimate as to the time of onset of FOM in cobia. However, both gravid females we collected had oocytes that were in the late stages of FOM (near the mid-point of hydration), suggesting they both would have spawned in the mid afternoon, only a few hours after their time of capture. The nearness to spawning of these two females provides strong evidence of spawning activity occurring in the proximity of their capture.

Few studies have collected females undergoing FOM, although there are exceptions (Biesiot et al., 1994; Smith, 1996; van der Velde et al., 2010). Gravid females may, again, comprise a larger percentage of the South Carolina inshore cobia population than reported here, but capture of them may be precluded due to pre-spawning behavior. As oocytes hydrate during FOM, their volume can nearly quadruple (Wallace and Selman, 1981). In the PRS gravid female, the ovaries comprised 1/6 of the total body weight, nearly filling the entire body cavity of the fish. It is probable that feeding activity reduces or ceases altogether by the time ovaries are this far developed, which would result few of these females being available to the hook-and-line fishery.

Supporting evidence of inshore spawning was also found by the collection of eggs identified as cobia in both PRS and SHS. Identification of fish eggs by morphological characteristics can be problematic for several reasons. First, the egg stage is the most difficult life stage to identify due to transitory features and a small number of permanent

attributes as compared to larvae (Fuiman, 2002). Secondly, preservation can alter morphological features, such as the oil droplet, which may detach or rupture (Gates et al., 1987; Klinger and Van Den Avyle, 1993). These two challenges were manageable due in large part to the availability of preserved hatchery-reared cobia eggs, which provided references for the identification of eggs collected in plankton samples. While preservation damage did occur in hatchery eggs and appeared to have occurred in the plankton samples, there were a number of eggs in both groups that remained intact. The identification of late-stage eggs from the plankton samples, due to the distinct features and size of cobia eggs, was done confidently, and side-by-side examination of undamaged cobia and plankton eggs demonstrated the two were visually indistinguishable.

The positive morphological identification of cobia eggs in plankton collections is supported with the quantitative comparison of egg characteristics with known cobia eggs. The eggs identified from the plankton samples had egg and oil droplet diameters within the range reported for cobia (Ditty and Shaw, 1992); however, the oil droplet diameters were smaller compared to the hatchery-reared eggs. The hatchery-reared eggs came from the spawns of only two females that had been hormonally induced to spawn, and maternal condition is known to affect egg quality and size in fishes (Berkeley et al., 2004; Gagliano and McCormick, 2007; Sogard et al., 2008). Gagliano and McCormick (2007) compared egg and oil droplet sizes from wild *Pomacentrus ambionensis* that were kept on experimental reefs that were either supplemented with additional food or not, and found that while egg size was not affected, females at supplemented reefs had eggs with significantly larger oil droplets. Additionally, oil droplet size of larvae is significantly

correlated with maternal age and length in several *Sebastes* species (Berkeley et al., 2004; Sogard et al., 2008). It is likely that eggs collected in the plankton samples came from several females which encountered a variety of environmental conditions and whose physiological conditions were variable, as compared to the eggs from females reared in a controlled environment with a regular, strictly controlled diet.

The collection of eggs and larvae within PRS and SHS alone, while highly suggestive of spawning, does not alone positively confer spawning habitat. Origin of large larvae (those without a yolk-sac) cannot be determined due to the ability of these larvae to migrate (Clark and Levy, 1988) through selective tidal stream transport (Boehlert and Mundy, 1988). However, the presence of cobia larvae in PRS and SHS suggests that both inshore environments provide favorable habitat for larval survival and development. The best evidence of inshore spawning of cobia comes from eggs collected inshore. Cobia eggs in PRS occurred 12 to 20.5 km landward of the mouth of the estuary. Based on the measured current speeds, a floating object would travel 7-15 km over the course of a single flood tide, making it improbable that all eggs were spawned outside of the system. The most conclusive evidence for spawning within PRS came from eggs that were estimated to be only two to three hours old collected in the Broad River, 15.0 km from the mouth. With an average current speed of 0.7 m/s measured during the time of collection, transport could not have carried these eggs to this location from outside of the estuary. In SHS, the early evening (1900 hours) collection of 496 early-stage eggs in a single sample 9.7 km (station SA03) from the mouth of the sound lends compelling evidence that spawning had occurred recently and in the immediate vicinity in that estuary as well.

CONCLUSION

Based on the evidence provided here, cobia spawn in PRS and SHS during the months of May and June. The collection of cobia eggs in early stages of development, the high average inshore GSI value and the presence of gravid females in PRS and SHS makes this the first study to positively document spawning of cobia in inshore waters. The examination of embryological development and subsequent aging of wild-caught eggs concurs with estimates based on histology of spawning in the afternoon and evening hours (primarily between 1530 and 1800 hours). While other studies suggest daytime and evening spawning (Shaffer and Nakamura, 1989; Ditty and Shaw, 1992; Weirich et al., 2006), this is the first study with a specific aim of documenting spawning and to positively document it through multiple methods.

Recreational fishing is generally considered to be less harmful to fish populations than commercial fishing; however, intense recreational fishing can produce changes in fish populations and communities in ways similar to commercial fishing (Coleman et al., 2004; Cooke and Cowx, 2006). Additionally, hyperstability, whereby catches remain constant as population declines are occurring, may mask population declines at spawning aggregations (Sadovy and Domeier, 2005), and fishing of spawning aggregations has been shown to be unsustainable in other aggregating species such as orange roughy (Koslow et al., 2000; Clark et al., 2000) and Nassau grouper (Sala et al., 2001). Cobia found on the Atlantic coast of the U.S. was considered to be overfished by the SAFMC and GMFMC in 1983, although no recent fishery assessments have been conducted in the region. Since that time, cobia has gained popularity among recreational anglers, as

evidenced by the continual increase in effort by South Carolina charter boats since1997 (G. Steele, SCDNR, personal communication).

Further research needs to be conducted to determine the contribution of inshore spawning cobia to the overall U.S. Atlantic population, as other studies have hypothesized spawning may occur offshore as well (Hassler and Rainville, 1975; Smith, 1996; Burns et al., 1998). However, the documented spawning in PRS and SHS, suggests inshore waters elsewhere in their region may also provide critical habitat for cobia. In combination with the documented spawning, the discovery of a unique population segment within South Carolina inshore waters (Darden 2009[4]) provides a compelling reason for management agencies to reconsider current management strategies. Treating all cobia in U.S. waters as a single population may no longer be appropriate, and the possible existence of regional, self-sustaining population segments should be taken into account.

ACKNOWLEDGEMENTS

A sincere thanks to Bill Roumillat (SCDNR) for his guidance with the development of histology criteria and staging of ovarian samples. A special thanks to the late John Olney (Virginia Institute of Marine Science [VIMS], Department of Fisheries Science) for helping with the design of the ichthyoplankton study, providing sampling equipment, and assisting with the sorting and identification of eggs and larvae; and to Pat Crewe (VIMS) for substantial assistance in the sorting of plankton samples. We would like to thank the members of the Estuarine Finfish Research group at SCDNR who helped with data collection, especially Justin Yost, Matt Perkinson, and Brock Renkas; Allison Williams

[4] Darden, T. Personal communication. South Carolina Department of Natural Resources

(SCDNR) and Lauri DiJoy (SCDNR) for conducting the second reads on all histology slides; Colden Battey (NOAA) and Karl Brenkert (SCDNR) for assistance with setup for the development study at HML; Nora Sturgeon for plankton sorting assistance; and David Knott and the Southeastern Regional Taxonomic Center at SCDNR for providing sorting space. Tanya Darden, John Leffler, and Tracey Smart (SCDNR) provided helpful suggestions on the manuscript. This project was funded by the SCDNR. This is Contribution No. XXX of the Grice Marine Laboratory, College of Charleston, Charleston, South Carolina and Contribution XXX of the South Carolina Department of Natural Resources.

LITERATURE CITED

Ahlstrom, E. H. and G. H. Moser. 1980. Characters useful in identification of pelagic marine fish eggs. Cal. Coop. Ocean Fish.. 21:121-131.

Arendt, M. D., J. E. Olney, and J. A. Lucy. 2001. Stomach content analysis of cobia, *Rachycentron canadum*, from lower Chesapeake Bay. Fish. Bull. 99:665-670.

Berkeley, S. A., C. Chapman, and S. M. Sogard. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology. 85:1258-1264.

Biesiot, P. M., R. E. Caylor, and J. S. Franks. 1994. Biochemical and histological changes during ovarian development of cobia, Rachycentron canadum, from the northern Gulf of Mexico. Fish. Bull. 92:686-696.

Boehlert, G. W. and B. C. Mundy. 1988. Roles of behavioral and physical factors in larval and juvenile fish recruitment to estuarine nursery areasp. Am. Fish. Soc. Symp. 3:51-67.

Briggs, J. C. 1960. Fishes of worldwide (circumtropical) distribution. Copeia. 1960:171-180.

Brown-Peterson, N., P. Thomas, and C. R. Arnold. 1988. Reproductive biology of the spotted seatrout, *Cynoscion nebulosus*, in South Texas. Fish. Bull. 86:373-388.

Brown-Peterson, N. J., R. M. Overstreet, J. M. Lotz, J. S. Franks, and K. M. Burns. 2001. Reproductive biology of cobia, Rachycentron canadum, from coastal waters of the southern United States. Fish Bull. 99:15-28.

Burns, K. M., C. Neidig, J. Lotz, and R. Overstreet. 1998. Cobia (*Rachycentron canadum*) stock assessment study in the Gulf of Mexico and in the South Atlantic. Mote Marine Lab. Tech. Rep.. No. 571:pp. 108.

Burrell, V. G., W. A. Van Engel, and S. G. Hummel. 1974. A new device for subsampling plankton samples. J. Conseil Int. Exp. Mer. 35:364-366.

Clark, C. W. and D. A. Levy. 1988. Diel vertical migrations by juvenile sockeye salmon and the antipredation window. Am Nat. 121:271-290.

Clark, M. R., O. F. Anderson, R. I. C. Chris Francis, and D. M. Tracey. 2000. The effects of commercial exploitation on orange roughy (Hoplostethus atlanticus) from the continental slope of the Chatham Rise, New Zealand, from 1979 to 1997. Fish. Res. 45:217-238.

Coleman, F. C., W. F. Figueira, J. S. Ueland, and L. B. Crowder. 2004. The impact of United States recreational fisheries on marine fish populations. Science. 305:1958-1960.

Cooke, S. J. and I. G. Cowx. 2006. Contrasting recreational and commercial fishing: Searching for common issues to promote unified conservation of fisheries resources and aquatic environments. Biol. Conserv. 128:93-108.

Denson, M. R., K. R. Stuart, T. I. J. Smith, C. R. Weirich, and A. Segars. 2003. Effects of salinity on growth, survival, and selected hematological parameters of juvenile cobia Rachycentron canadum. J. World Aquacult. Soc. 34:496-504.

Ditty, J. G. 2006. Rachycentridae: Cobias, p. 1429-1431. *In:* Early Stages of Atlantic Fishes: An Identification Guide for the Western Central North Atlantic. Vol. 2. W. J. Richards (ed.). Taylor and Francis, Boca Raton.

Ditty, J. G. and R. F. Shaw. 1992. Larval development, distribution, and ecology of cobia Rachycentron canadum (Family: Rachycentridae) in the northern Gulf of Mexico. Fish. Bull. 90:668-677.

Fitzhugh, G. R., B. A. Thompson, and T. G. Snider Iii. 1993. Ovarian development, fecundity, and spawning frequency of black drum *Pogonias cromis* in Louisiana. Fish Bull. 91:244-253.

Franks, J. S., J. R. Warren, and B. V. Buchanan. 1999. Age and growth of cobia, Rachycentron canadum, from the northeaster Gulf of Mexico. Fish. Bull. 97:459-471.

Fuiman, L. A. 2002. Special Considerations of Fish Eggs and Larvae. *In:* Fishery Science: The Unique Contributions of Early Life Stages. L. A. Fuiman and R. G. Wenner (eds.), pp. 1-32. Blackwell Science, Ltd., Oxford.

Gagliano, M. and M. I. Mccormick. 2007. Maternal condition influences phenotypic selection on offspring. J. Anim. Ecol. 76:174-182.

Gates, D. W., J. S. Bulak, and J. S. Crane. 1987. Preservation of striped bass eggs collected from a low-hardness, freshwater system in South Carolina. Prog. Fish-Cult. 49:230-232.

Hassler, W. W. and R. P. Rainville. 1975. Techniques for hatching and rearing cobia, *Rachycentron canadum*, through larval and juvenile stages. University of North Carolina Sea Grant Program, p. 26.

Humason, G. L. 1972. Animal tissue techniques. W. H. Freeman and Co., San Francisco London.

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. L. Lasker (ed.), pp. 79-94. NOAA Technical Report.

Joseph, E. B., J. J. Norcross, and W. H. Massmann. 1964. Spawning of the cobia, *Rachycentron canadum*, in the Chesapeake Bay Area, with observations of juvenile specimens. Chesapeake Sci. 5:67-71.

Klinger, R. C. and M. J. Van Den Avyle. 1993. Preservation of striped bass eggs: effects of formalin concentration, buffering, stain, and initial stages of development. Copeia. 1993:1114-1119.

Koslow, J.A., G.W. Boehlert, J.D.M. Gordon, R.L. Haedrich, P. Lorance, and N. Parin. 2000. Continental slope and deep-sea fisheries: implications for a fragile ecosystem. ICES J. Mar. Sci. 57:548-557.

Lotz, J. M., R. M. Overstreet, and J. S. Franks. 1996. Gonadal maturation in the cobia, *Rachycentron canadum*, from the Northcentral Gulf of Mexico. Gulf Res. Rep. 9:147-159.

Richards, C. E. 1967. Age, growth and fecundity of the cobia, *Rachycentron canadum*, from Chesapeake Bay and adjacent mid-Atlantic waters. Trans. Am. Fish. Soc. 96:343-350.

Roumillat, W. A. and M. C. Brouwer. 2004. Reproductive dynamics of female spotted seatrout (*Cynoscion nebulosus*) in South Carolina. Fish. Bull. 102:473-487.

Sadovy, Y. and M. Domeier. 2005. Are aggreation-fisheries sustainable? Reef fish fisheries as a case study. Coral Reefs. 24:254-262.

Sala, E., E. Ballesteros, and R. M. Starr. 2001. Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. Fisheries. 26:23-30.

Shaffer, R. V. and E. L. Nakamura. 1989. Synopsis of biological data on the cobia, *Rachycentron candum* (Pisces: Rachycentridae). *In:* NOAA Tech. Rep. NMFS82 FAO Fish. Synop. 153.

Smith, J. W. 1996. Life history of cobia, *Rachycentron canadum* (Osteichthyes: Rachycentridae), in North Carolina waters. Brimleyana. 23:1-23.

Sogard, S. M., S. A. Berkeley, and R. Fisher. 2008. Maternal effects in rockfishes *Sebastes* spp.: a comparison among species. Mar. Ecol. Progr. Ser. 360: 227–236.

Somarakis, S., K. Ganias, G. Tserpes, and C. Koutsikopoulos. 2004. Ovarian allometry and the use of the gonosomatic index: a case study in the Mediterranean sardine, *Sardina pilchardus*. Mar. Biol. 16:181-189.

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

Van Der Velde, T. D., S. P. Griffiths, and G. C. Fry. 2010. Reproductive biology of the commercially and recreationally important cobia *Rachycentron canadum* in northeastern Australia. Fisheries Sci. 76:33-43.

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

Weirich, C. R., A. D. Stokes, T. I. J. Smith, W. E. Jenkins, and M. R. Denson. 2006. Outdoor tank and pond spawning of cobia, *Rachycentron canadum*, in coastal South Carolina. J. Appl. Aquaculture. 18:1-16.

Table 1. Stages of ovarian development, based on descriptions of teleost oocyte development by Wallace and Selman (1981) and modified from Roumillat and Brouwer (2004). FOM = final oocyte maturation; POFs = postovulatory follicle.

Stage	Description
Immature	Only oogonia and primary oocytes present. Fish has not yet reached sexual maturity and is incapable of spawning.
Early Developing	Primary growth oocytes dominate. A few early vitellogenic oocytes may be present and are <500 µm in diameter. Cortical alveoli visible. Based on diameter, there is a dominant batch of small vitellogenic oocytes and a few larger vitellogenic oocytes. Fish has not yet spawned this season.
Late Developing	Primary growth and advanced vitellogenic oocytes present, with the diameter of the largest batch between 500-850 µm. Cortical alveoli visible. Based on diameter, there are at least 2 distinct batches of vitellogenic oocytes. Some atresia may be present. Fish is capable of spawning and may have previously spawned.
Gravid	One batch of oocytes undergoing FOM (through hydration), as evidenced by lipid coalescence and diameter (>850 µm). More advanced stages of FOM will also show migration of nucleus to animal pole. Next largest batch of oocytes is 300-500 µm in diameter. Spawn imminent.
Postovulatory 1-Recent Spawn	Recent POFs are abundant, and distinguished by size (>250 µm across longest axis) and morphology. Recent POFs are amorphous that clearly show multiple infoldings of thecal and granulose cells. Largest batch of oocytes is 300-550 µm in diameter. Fish has spawned within hours 0-24 hours.
Postovulatory 2-Prior Spawn	Degradation of POFs indicate spawn was >24 hours prior. Older POFs are triangular in shape, condensed, smaller, and less numerous compared to recent counterparts. Largest batch of oocytes is 550-700 µm in diameter.
Spent	Majority of largest batch of vitellogenic oocytes undergoing atresia. Oogonia and primary growth oocytes may be present. Indicative of cessation of spawning for the season.
Recovering	Oogonia and primary growth oocytes dominate. Other oocytes are in late stages of atresia.

Table 2. State of ovary development of female cobia caught in South Carolina in 2007 and 2008. *n* = number of fish; PC = percent composition.

STAGE	INSHORE		OFFSHORE		UNKNOWN	
	n	PC	*n*	PC	*n*	PC
Immature	0	0	0	0	0	0
Early developing	1	2	1	3	1	1
Late Developing	51	80	20	59	97	84
Gravid	2	3	0	0	3	3
Postovulatory 1-Recent spawn	3	5	1	3	4	4
Postovulatory 2-Prior spawn	7	11	11	32	9	8
Spent	0	0	1	3	1	1
Recovering	0	0	0	0	0	0

Table 3. Descriptions of cobia eggs from hatchery-reared eggs, eggs collected in plankton samples in 2008 and from Ditty (2006). Asterisks indicate only preserved eggs with a single oil droplet were measured.

PARAMETER	HATCHERY		PLANKTON SAMPLES	LITERATURE
	Live	Preserved*	Preserved*	
Diameter	1.21 - 1.38 mm (Mean: 1.30 mm)	1.18 - 1.37 mm (Mean: 1.28 mm)	1.15 - 1.39 mm (Mean: 1.29 mm)	1.15 - 1.42 mm (Mean: 1.24 mm)
Number of Oil Droplets	1	1 to several, often irregular in shape	1 to several, often irregular in shape	1
Oil Droplet Diameter	0.34 - 0.42 mm (Mean: 0.38 mm)	0.32 - 0.42 mm (Mean: 0.38 mm)	0.28 - 0.42 mm (Mean: 0.36 mm)	0.34 - 0.65 mm (Mean: 0.45 mm)
Oil Droplet Pigment	present	present; in some eggs, pigment separated from oil	present; in some eggs, pigment separated from oil	present
Perivitelline Space	narrow	narrow	narrow	narrow
Embryonic Pigment	heavy, except on caudal peduncle	heavy, except on caudal peduncle	heavy, except on caudal peduncle	heavy, except on caudal peduncle

Table 4. Cobia eggs and larvae identified from plankton collections in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina in May and June 2008. Station codes: PA = PRS anchored net; PT = PRS towed net; SA = SHS anchored net; ST = SHS towed net; numbers correspond to specific station. Early eggs are from fertilization to blastopore closure; late eggs are from blastopore closure to hatching. Concentrations (number per 100 m^3) were calculated for all anchored stations. Station numbers with an asterisk were subsampled: numbers of eggs and larvae are estimated based on the fraction sorted. *n* = number of eggs or larvae; C = concentration; na = not applicable due to no volume filtered estimates.

Date	Station	Early eggs		Late eggs		Larvae	
		n	**C**	***n***	**C**	***n***	**C**
6-May	PA-5			3	0.61		
8-May	SA-3	1	0.14				
14-May	PA-8					3	1.66
	PA-9					1	0.55
21-May	SA-1*	6	0.93	88	13.58	4	0.62
	SA-2			12	4.6		
	SA-3	2	0.28	9	1.27		
	SA-6*	4	0.56	12	1.69		
28-May	SA-1	2	0.38	16	3.08	1	0.19
	SA-3			13	2.64	5	1.01
29-May	PA-7			10	2.95	1	0.3
	PA-8			15	3.22		
	PA-9			3	0.64		
	PA-10			1	0.17	1	0.17
3-Jun	SA-1*	24	2.08	12	1.04	4	0.35
	SA-2*			56	7.06		
	SA-3*	496	62.51	64	8.07	4	0.5
	SA-6*			16	2.02		
	SA-7*			4	0.93	4	0.93
5-Jun	PA-7			4	0.57		
	PA-8	27	3.18	3	0.35	2	0.24
12-Jun	PA-7			15	4.26		
	PA-8			5	1.42	2	0.57
	PA-9					7	1.99
	PA10					1	0.19
18-Jun	SA-3			3	0.54	2	0.36

Table 5. Estimated age and spawning time of cobia eggs identified from plankton collections in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina, in 2008. Ages were estimated by comparing stage of development to those of cobia eggs reared at temperatures in the range of field water temperatures. Estimated spawn time is rounded down to the nearest quarter hour. Station codes: PA = PRS anchored net; SA = SHS anchored net; numbers correspond to specific station.

STATION	SAMPLE PICK-UP		AGE (HOURS)	ESTIMATED TIME OF SPAWN	
	Date	Time		Date	Time
SA01	5/21/2008	1816	19.5	5/20/2008	2045
SA02	5/21/2008	1835	21.5	5/20/2008	2100
SA03	5/21/2008	1850	21.5	5/20/2008	2115
SA06	5/21/2008	1920	21.5	5/20/2008	2145
SA01	5/28/2008	1237	19.5	5/27/2008	1700
SA03	5/28/2009	1304	19.5	5/27/2008	1730
PA07	5/29/2008	1350	21.5	5/28/2008	1615
PA08	5/29/2008	1405	21.5	5/28/2008	1630
PA09	5/29/2008	1425	21.5	5/28/2008	1700
SA01	6/3/2008	1827	25.5	6/2/2008	1700
SA02	6/3/2008	1850	25.5	6/2/2008	1720
SA03	6/3/2008	1900	25.5	6/2/2008	1730
SA06	6/3/2008	1923	25.5	6/2/2008	1800
PA07	6/5/2008	1912	26.0	6/4/2008	1715
PA08	6/5/2008	1927	2 - 3	6/5/2008	1630 - 1730
		1927	26.0	6/4/2008	1730
PA07	6/12/2008	1255	20.0	6/11/2008	1700
PA08	6/12/2008	1303	21.5	6/11/2008	1530
SA03	6/18/2008	1757	24.0	6/17/2008	1800