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

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Abstract—We documented inshore spawning of the recreationally important cobia (*Rachycentron canadum*) in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina, during the period from April to June in both 2007 and 2008. Histological analysis of ovaries confirmed the presence of actively spawning females inshore, and gonadosomatic index (GSI) values from females collected inshore (mean=7.8) were higher than the values from females caught offshore (mean=5.6); both of these mean values indicate that spawning occurred locally. Additionally, we conducted an ichthyoplankton survey in 2008 and found cobia eggs and larvae as far as 10 and 15 km inshore from the mouths of SHS and PRS, respectively. A study of egg development that we conducted in 2007 and 2008 using hatchery-reared cobia eggs provided descriptions of embryological development of cobia. Comparison of visual and quantitative characteristics of the field-collected eggs with those of the hatchery-reared eggs allowed positive identification of eggs collected in plankton samples. The ages of field-collected eggs and presence of females with hydrated oocytes in PRS and SHS observed in our ichthyoplankton survey and histological analysis indicated that wild cobia spawn in the afternoon and early evening. The inshore migration of cobia from April to June, the presence of actively spawning females, significantly higher GSI values, and the collection of eggs inside PRS and SHS all confirm that these estuaries provide spawning habitat for cobia. Because of the potential for heavy exploitation by recreational anglers as cobia move inshore to spawn in South Carolina, current management strategies may require review.

Manuscript submitted 12 November 2011.
Manuscript accepted 30 July 2012.
Fish. Bull. 110:397–412 (2012).

Cobia (*Rachycentron canadum*) is a migratory, euryhaline benthopelagic species distributed worldwide in tropic, subtropic, and warm temperate waters, except in 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 2 (males 60 cm fork length [FL]; females 80 cm FL [Smith, 1996; Burns et al.[1]]). Currently in the United States, most commercial landings of cobia result from incidental catch in other fisheries (Shaffer and Nakamura, 1989). Cobia are sought recreationally throughout their range, and the majority of the annual catch for cobia in the United States comes from the recreational fishery: 957 of the combined 1070 metric tons (t) from commercial and recreational landings in 2010 (National Marine Fisheries Service [NMFS[2]]). The current fishery management plan for cobia, which imposes a bag limit of 2 fish per person per day and a minimum FL of 84 cm (33 in), was established by the Gulf of Mexico Fishery Management Council (GMFMC) and South Atlantic Fishery Management Council (SAFMC) in 1983 to conserve a population considered overexploited at that time (SAFMC and GMFMC[3]). The restrictions were enacted under the assumptions that cobia are widely dispersed, are primarily commercial bycatch, constitute a recreational fishery, and compose a single population in the United States. These restrictions were meant to reduce catches and allow females the opportunity to reproduce before entering the fishery. Data on regional fishing effort and catch indicate fishing pressure

[1] 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. Final Rep., MARFIN Coop. Agreement NA57FF0294 to NMFS (NOAA), 108 p. Mote Marine Laboratory, 1600 Thompson Parkway, Sarasota, FL 34236.

[2] NMFS (National Marine Fisheries Service.) 2012. Personal commun. Fisheries Statistics Division, Silver Spring, MD.

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

for cobia has increased over the past decade (Steele[4]). Cobia continues to gain socioeconomic importance as a game fish throughout much of its range, supporting an expanding charterboat industry; however, the current status of this stock is unknown along the southeastern United States.

In spring and early summer, cobia in the western North Atlantic are thought to migrate along with warming waters from Florida to as far north as the mid-Atlantic Bight (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 Chesapeake Bay (Shaffer and Nakamura, 1989), where they are readily available to inshore recreational anglers. Reasons for the inshore movement are not fully understood, but it is hypothesized that they may be following prey or aggregating to spawn.

On the east coast of the United States, the spawning season for cobia extends from April to September (Lotz et al., 1996; Smith, 1996; Burns et al.[1]; Brown-Peterson et al., 2001); the cobia is an indeterminate batch spawner with group-synchronous oocyte development and is capable of spawning multiple times during a season (Biesiot et al., 1994; Lotz et al., 1996; Brown-Peterson et al., 2001; van der Velde et al., 2010). Regional peaks in spawning, as designated by maxima in the gonadosomatic index (GSI), correlate with the migration of cobia from Florida northward. Spawning peaks along the Atlantic coast of the southeastern United States in May (Shaffer and Nakamura, 1989; Burns et al.[1]), off the coast of North Carolina in June (Smith, 1996), and in Chesapeake Bay in June and July (Joseph et al., 1964). In South Carolina, maximal spawning activity of cobia in May corresponds to peak fishing effort, as evidenced by increased landings during this month (Steele[4]). During the spring recreational fishery for cobia in South Carolina, cobia are easily accessible to anglers in the major coastal sounds, as they are in other states where they enter inland waters. Because the inshore migrations of this fish into PRS and SHS correspond with its spawning season, it is probable that these estuaries serve as spawning habitat.

Beyond a knowledge of spawning season, a paucity of information exists on spawning habitat and daily spawning periodicity of wild cobia, because much of the previous research on this species 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). Hassler and Rainville (1975) collected eggs in the Gulf Stream off North Carolina and suggested that spawning took place offshore. Burns et al.[1] also proposed offshore spawning because of a scarcity of fish with histological signs of final oocyte maturation (FOM) collected in nearshore waters of the Gulf of Mexico and the Atlantic coast of the southeastern United States. It has been proposed that inshore spawning of cobia occurs in the lower Chesapeake Bay on the basis of egg collections immediately south of this bay (Joseph et al., 1964) and the ovarian condition of females collected in this bay (Richards, 1967). In North Carolina, Smith (1996) suggested that cobia spawned adjacent to inlets, on the basis of the presence of eggs in neuston net collections within inlets and a lack of females caught inshore that were undergoing FOM. Additionally, Ditty and Shaw (1992) reported eggs and larvae from another high-salinity bay, Crystal Bay, Florida.

Our objective was to determine whether cobia spawn within 2 high-salinity estuaries in South Carolina, PRS and SHS. Histological analysis of ovarian tissue collected in 2007 and 2008 was used to evaluate the reproductive status of female cobia caught in the recreational fishery both inshore and offshore of these estuaries. A study of egg development conducted in 2007 and 2008 with hatchery-reared cobia eggs provided embryological development characteristics and insight on time of day of spawning for wild cobia. An ichthyoplankton survey targeting cobia eggs and larvae in PRS and SHS was conducted during the 2008 spawning season to provide additional evidence of spawning locations. We discuss the results of this project, particularly with respect to the occurrence of springtime inshore aggregations of cobia, in light of the increasing fishing pressure cobia are experiencing and in regard to current management regulations.

Materials and methods

Fish collections and reproductive biology

Fresh and frozen cobia specimens from PRS and SHS, South Carolina (Fig. 1), were collected by members of the Estuarine Finfish Research Group at the South Carolina Department of Natural Resources (SCDNR) at fishing tournaments and from cooperating anglers, recreational fishing guides, and employees of the SCDNR during the period from April to June in both 2007 and 2008. Specimens were measured for total length (TL, millimeters) and fork length (FL, millimeters) and weighed (fish weight [FW]) to the nearest kilogram. Sex was determined macroscopically, and gonads were excised, stored on ice, and transported to the SCDNR Marine Resources Research Institute (MRRI; Charleston, SC). Date, time, and location of capture were noted when available: fish collected within PRS and SHS were designated as "inshore" specimens, and those fish collected in the ocean beyond the barrier islands were designated as "offshore" specimens. Some cobia carcasses were accumulated in freezers located at marinas and tackle stores: anglers donated the carcasses after filleting, and the aforementioned information was voluntarily recorded.

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

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

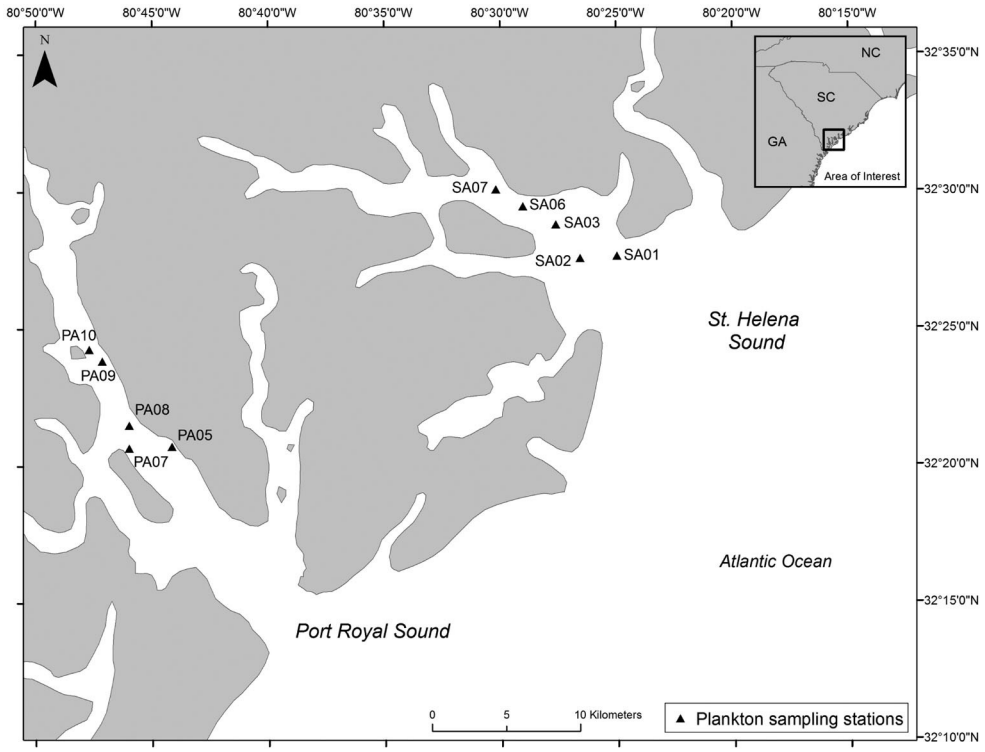

Figure 1

Coastal South Carolina and estuaries that cobia (*Rachycentron canadum*) enter during their annual northward spring migration. For this study of inshore spawning, cobia were collected from 2 inshore estuaries, Port Royal Sound and St. Helena Sound, and offshore in 2007 and 2008. Triangles indicate stations sampled in each estuary during the ichthyoplankton survey conducted in 2008. Map by Jessica Boyton (South Carolina Department of Natural Resources).

$$GSI = \left(GW/SW\right) \times 100,$$

where GW = gonad weight (grams); and
SW = somatic weight (grams).

To determine whether GSI values for male and female cobia were significantly different, a Wilcoxon rank test was performed. To evaluate if ovarian maturation differed between inshore and offshore collections of female cobia, a Wilcoxon rank test was performed with the GSI as a proxy for maturation for statistical purposes. Nonparametric Wilcoxon tests were performed because the data displayed non-normality, which resulted from the capture of 2 actively spawning females. A *t*-test was used to determine if differences in GSI between inshore and offshore females persisted after exclusion of the GSI values from the 2 actively spawning females.

Histological analyses were limited to samples collected from female cobia because 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; van der Velde et al., 2010); therefore, a single portion of tissue (~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 hr before processing. Tissue samples were dehydrated, infiltrated and blocked in paraffin, sectioned to 6 µm with a rotary microtome, mounted on glass slides, and stained with hematoxylin and eosin-y according to standard histological techniques (Humason, 1972). Slides were examined under a compound microscope at 100× magnification and staged according to ovarian development. Ovarian phases (Table 1) were determined on the basis of descriptions of teleost oocyte development in Wallace and Selman (1981), with modifications from Roumillat and Brouwer (2004) and Brown-Peterson et al. (2011). When present, postovulatory follicles (POFs) were categorized as either less than or equal to or greater than 12 hr old, on the basis of rates of POF atresia found in spotted seatrout (*Cynoscion nebulosus* [Roumillat and Brouwer, 2004]). Vitellogenic oocytes of specimens that had been frozen or had begun to decay before collection superficially resembled oocytes at the beginning stages of FOM, with nucleus migration and early lipid coalescence. To avoid confusion between FOM and damaged tissue, FOM stages earlier than the hydration stage were not addressed. All samples were staged by a second, independent reader. All discrepancies were resolved by both readers simultaneous view-

Table 1

Stage of ovarian development was determined for female cobia (*Rachycentron canadum*) collected during the period from April to June in both 2007 and 2008 for this study of inshore spawning of cobia in South Carolina. Phases were based on descriptions of teleost oocyte development by Wallace and Selman (1981) and modified from Roumillat and Brouwer (2004) and Brown-Peterson et al. (2011). FOM=final oocyte maturation; POF=postovulatory follicle.

Phase	Subphase	Description
Immature		Only oogonia and primary oocytes present. Fish has not yet reached sexual maturity and is incapable of spawning.
Developing		Primary growth oocytes dominate. A few early vitellogenic oocytes may be present and are <500 µm in diameter. Cortical alveoli visible. On the basis of diameter, there is a dominant batch of small vitellogenic oocytes and a few larger vitellogenic oocytes. Fish has not yet spawned this season.
Spawning capable	Late developing	Primary growth, cortical alveolar, early vitellogenic and advanced vitellogenic oocytes present, with the diameter of oocytes in the largest batch at 500–850 µm. On the basis of 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.
	Actively spawning	One batch of oocytes undergoing FOM (through hydration), as evidenced by lipid coalescence and a diameter >850 µm. More advanced stages of FOM also will show migration of nucleus to animal pole. Oocytes in the next-largest batch are 300–500 µm in diameter. Spawning imminent.
	Past spawner 1—recent spawning	Recent POFs are abundant and distinguished by size (>250 µm across longest axis) and morphological features. Recent POFs are amorphous and clearly show multiple infoldings of thecal and granulose cells Oocytes in the largest batch are 300–550 µm in diameter. Fish has spawned within 0–24 hr.
	Past spawner 2—previous spawning	Degradation of POFs indicates spawn was >24 hr prior. Older POFs are triangular in shape, condensed, and less numerous compared with recent counterparts. Oocytes in the largest batch are 550–700 µm in diameter.
Regressing		Majority of largest batch of vitellogenic oocytes undergoing atresia. Oogonia and primary growth oocytes may be present. This stage is indicative of cessation of spawning for the season.
Regenerating		Oogonia and primary growth oocytes dominate. Other oocytes are in late stages of atresia.

ing the questionable slides. Percent composition (PC) of females in each of the ovarian maturation phases was calculated separately for females collected inshore and for females collected offshore:

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

where n_s = the number of female samples in phase s; and
T = the total number of samples.

Egg-development study

Identification and aging of candidate eggs from the plankton collections were accomplished by using a time-series reference collection of cobia eggs obtained from wild-caught adult cobia spawned in the laboratory in 2007 and 2008 for the egg-development study described below. Cobia yolksac larvae from the egg-development study in 2007 served as reference for the identification of young larvae in the plankton collections. The temperature treatments during the egg-development study of hatchery-reared eggs fell within the measured range of surface temperatures (20.1–30.0°C) encountered in PRS and SHS during May and June of both years (2007–2008).

In 2007 at the Hollings Marine Laboratory in Charleston, South Carolina, 4 fiberglass hatching cones (170 L) were equipped with aerators and heaters and filled with seawater (34.5 psu) from Charleston Harbor that had been filtered (5 µm) and UV-sanitized after settling for 3 days. The water in these cones was heated to 22.5°C, 25.0°C, 26.5°C, and 29.0°C, respectively, and

maintained for 48 hr before the start of this study. Four 50-mL aliquots of eggs (~2×10^4 eggs) were used in the trial. Before the addition of eggs to the water baths at ~11 hr postspawn, digital micrographs of eggs were recorded with a Nikon[5] SMZ1500 stereo microscope (Nikon Instruments, Inc., Melville, NY) mounted with a Micropublisher 3.3 camera (QImaging, Surrey, BC, Canada). Thereafter, ~10 eggs or larvae were collected from each tank, and micrographs were recorded every 4 hr until 61 hr after spawning. After images were recorded, eggs and larvae were preserved in 10% neutral buffered formalin.

To capture earlier stages of egg development and to expand the range of experimental temperatures, we expanded the egg-development study in 2008. Three water baths were heated to and maintained at 24.0°C, 26.0°C, and 28.0°C in a temperature-controlled laboratory 30 hr before the beginning of this second study. Two hours after time of spawning, a 25-mL aliquot of cobia eggs (~1×10^4 eggs) was divided between petri dishes in each temperature bath. Every hour, until 13 hr after spawning, and every other hour thereafter up to 25 hr after spawning, a sample of ~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 were taken, and both egg diameters and oil-droplet diameters were measured to the nearest micrometer by using ImageJ image-analysis software (vers. 1.38, ImageJ, 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 micrometer from micrographs of preserved eggs taken ~1 year from the date of collection in 2007 and 2 months from the date of collection in 2008. Diameters were measured only for undamaged, preserved eggs. Percent shrinkage was calculated as the change in egg and oil-droplet diameters between the live and corresponding preserved eggs multiplied by 100. To determine if a significant decrease in diameter because of preservation had occurred, 2-sample *t*-tests were performed independently for 2007 egg and oil-droplet diameters and 2008 egg diameters. Because of non-normality of the 2008 oil-droplet-diameter data, a Wilcoxon rank test was performed with these data to determine if significant shrinkage had occurred after preservation.

With the use of the micrographs of live eggs from the egg-development studies, coarse stages of embryological development were described. Because the earliest stages of egg development were captured only in the 2008 study and larvae hatched only in the 2007 study, the 2 temperature treatments that were closest in temperature (26.5°C in 2007 and 26°C in 2008) were used for the characterization of egg-development stages from the earliest ones to the hatching stage.

[5] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Ichthyoplankton survey

Field collections Five stations in each of the 2 estuaries (Fig. 1), located in known cobia fishing grounds, were sampled weekly from 6 May to 15 June 2008 in PRS and 8 May to 8 June 2008 in SHS. Collections occurred between 0745 and 1945 hr. Survey stations in PRS were located 12.1 to 20.6 km inshore from the mouth of that estuary. Stations in SHS were located 5.1 to14.3 km inshore from the mouth of that estuary. Stations were positioned upriver of particular bathymetric features where anglers typically target cobia. These stations were adjacent submerged sand bars and banks where water depths rose from 10 to 12 m at mean low water to 2 to 3 m at mean low water. Depths of stations ranged from 5.5 to 9.6 m at the time of net deployment. Anchored plankton nets, made of Nitex nylon mesh (Sefar Holding AG, New York, New York), were deployed at slack water before the daylight flood tide. These plankton nets had a mouth 0.5-m in diameter and were 2.5 m long, with a mesh size of 505 µm along the length and 303 µm at the codend. Floats attached to circular frames maintained the position of these nets at ~1 m below the surface. Flow meters were mounted in the center of these nets at the most seaward and farthest inshore 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 with a handheld instrument, YSI 556 MPS (YSI, Inc., Yellow Springs, Ohio). Plankton nets were deployed for the length of time required to set all nets and return to retrieve the first one (70–150 min). The average current speed during the time of collection was calculated by using the flow meter readings. 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 averaging the values from the most seaward and farthest inshore stations.

Egg and larval identification Plankton samples were rinsed and then sorted under a dissecting microscope. When settled plankton volumes were greater than 1 L, samples were split with a Burrell plankton splitter (Burrell et al., 1974) until a settled plankton volume of 0.5 L or less was attained. All nonclupeiform larvae were removed, identified to lowest possible taxonomic level (Moser et al., 1984; Richards, 2005), 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 with an ocular micrometer and having one or more of the morphological characteristics corresponding to cobia eggs—single, large (300 to 600 µm), pigmented oil droplet, heavily pigmented embryo, or narrow perivitelline space (Ditty and Shaw, 1992; Ditty, 2006)—were removed and preserved in 10% neutral buffered formalin.

Digital micrographs of preliminarily identified cobia eggs were recorded. Diameters of all eggs and of single, intact oil droplets from preserved eggs were measured to the nearest micrometer with ImageJ. To aid in iden-

tification, eggs from the 2 development studies were viewed as references. Eggs were positively identified as cobia if their diameter fell within the range noted in the literature (1.0–1.4 mm) 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 and Shaw, 1992; Ditty, 2006). The number and diameter of oil droplets were excluded as positive identifying characters because of damage incurred during preservation. For further analyses, eggs were labeled as early stage or late stage. The early stage comprised eggs from fertilization to blastopore closure, with the embryo not visible in preserved eggs (Ahlstrom and Moser, 1980). The late stage comprised eggs from blastopore closure to hatching, with the embryo evident in preserved eggs, including eggs at mid and late stages as described in Ahlstrom and Moser (1980).

For 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 egg-development studies) and eggs identified from plankton samples. If the relationship was not significantly different between hatchery-reared and field-collected eggs, the field-collected eggs were confirmed as cobia. Only measurements from eggs with single, intact oil droplets were used in the ANCOVA.

For plankton samples that contained more than one undamaged egg, ages of cobia eggs were estimated through side-by-side comparison to eggs reared at the closest matching temperature from either the 2007 or 2008 development study. In early-stage eggs that turned opaque with preservation, it was difficult to discern their stage of cell division or cleavage; therefore, age was estimated only for eggs that were not opaque. For a given sample, time of spawning was approximated on the basis of the age of an egg, which was estimated by back-calculating from the time of sample collection. When field-collected eggs resembled an intermediate stage between 2 sampling time periods within the egg development study, ages were estimated to be halfway between the 2 periods. Numbers of cobia eggs and larvae from split samples were estimated from the fraction of the sample sorted. Concentrations of cobia eggs and larvae collected in the ichthyoplankton survey were obtained by 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 α=0.05 was used for all tests.

Results

Between April 2007 and June 2008, 554 cobia (275 females, 279 males) were collected. Of these fish, 262 came from carcass donations, 261 from fishing tournaments, and 31 from SCDNR. 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. Most of the largest fish were female: 80% of the fish 1000 mm FL or longer and 79% of the fish 10 kg or greater in total weight. 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 because of small sample sizes collected in April and June. GSI for all cobia ranged between 0.7 and 22.5 (mean=6.1), and females had a significantly higher mean GSI (7.3) than males (4.4; P<0.05). Ovarian growth in other group-synchronous, batch-spawning species is isometric with body growth (Taylor et al., 1998; Somarakis et al., 2004), and previous work on 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 deemed appropriate as a proxy for ovarian maturation for statistical purposes. The average GSI from females collected inshore (mean=7.8; n=64) was significantly higher than the average GSI from females collected offshore (mean=5.6; n=34; p=0.002), indicating that ovaries were in a more developed state in inshore fish than in offshore fish. The mean inshore GSI (7.4) remained statistically higher than the mean offshore GSI (5.6; P=0.003) with removal of the actively spawning females. Therefore, the higher mean GSI from females collected inshore was not biased by the collection of actively spawning females inshore or spent females offshore.

Histological analysis of 213 ovaries showed that female cobia were in the middle of their reproductive season and capable of spawning during May and June. All ovarian phases, except the immature and regenerating phases, were represented in the female samples examined (Fig. 2). Immature specimens were unavailable because current fishing regulations impose a minimum FL (84 cm), which is larger than the approximate length at first maturity for female cobia (80 cm). Females in the regenerating state were likely absent because sampling was done during the spawning season.

Further histological analysis of female ovarian samples was limited to 98 specimens for which capture location 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 development subphase of the spawning capable phase (Table 2). Two females with ovaries in the developing phase were collected offshore in early April 2007 and inshore in early May 2007. Both of these females (89 cm FL and 93 cm FL, respectively) were larger than the typical size at maturity, had no evidence of prior spawning (POFs), and were, therefore, likely maturing

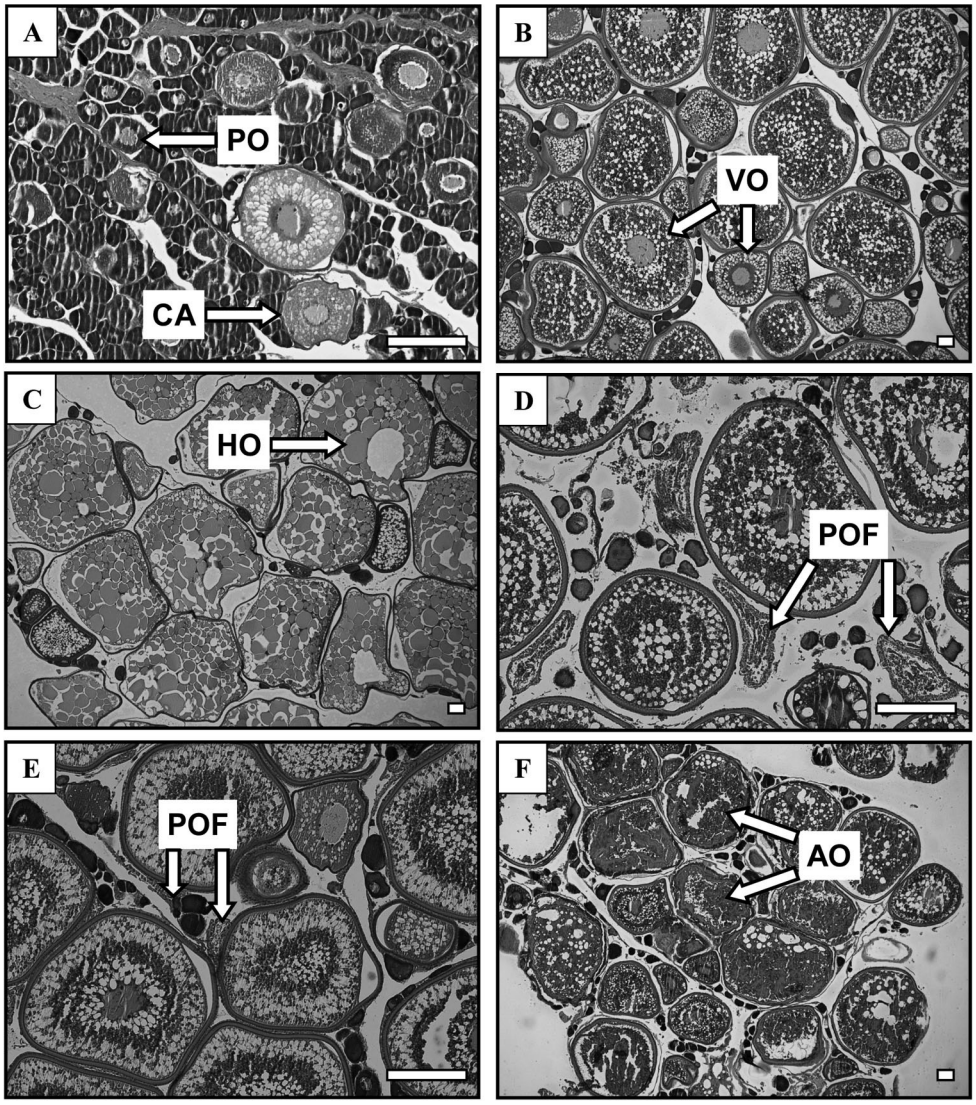

Figure 2

Histological micrographs of the ovarian phases of cobia (*Rachycentron canadum*) collected in South Carolina waters in 2007 and 2008. Scale bars=200 µm. (**A**) Developing: primary oocytes (PO) dominate with few cortical alveolar (CA) present. Spawning capable: (**B**) late developing (subphase): vitellogenesis continues; multiple batches of vitellogenic oocytes (VO) are present; (**C**) actively spawning (subphase): hydrated oocytes (HO) show lipid coalescence; (**D**) past spawner 1—recent spawning (subphase): most recent postovulatory follicles (POF) are 250 µm across longest axis; multiple batches of VO present; and (**E**) past spawner 2 (subphase)—previous spawning: POF are less numerous and are smaller, more condensed, and triangular in shape; multiple batches of VO present. (**F**) Regressing: largest batch of VO are undergoing atresia (AO).

for the first time in this spawning season. A single female in the regressing phase was collected in offshore waters in early June 2008. Females with histological signs of previous spawning (visible POFs) were found in cobia collected inshore and offshore. All POFs were estimated to be greater than 12 hr old (Hunter and Macewicz, 1985; Roumillat and Brouwer, 2004), with the exception of one specimen collected during morning hours in May 2007, for which the POFs were estimated to be ~12 hr old.

Two actively spawning females were collected from inshore waters in 2007. The first (121.6 cm FL and 27 kg FW) was collected in mid-morning on 12 May in the Broad River (PRS). The second (93.4 cm FL and 11

Table 2

State of ovarian development of female cobia (*Rachycentron canadum*) collected during the period from April to June in both 2007 and 2008 for this study of inshore spawning of cobia in South Carolina. No.=number of fish; PC=percent composition.

		Inshore		Offshore		Unknown	
Phase	Subphase	No.	PC	No.	PC	No.	PC
Immature		0	0	0	0	0	0
Developing		1	2	1	3	1	1
Spawning capable	Late developing	51	80	20	59	97	84
	Actively spawning	2	3	0	0	3	3
	Past spawner 1—recent spawning	3	5	1	3	4	4
	Past spawner 2—previous spawning	7	11	11	32	9	8
Regressing		0	0	1	3	1	1
Regenerating		0	0	0	0	0	0

kg FW) was caught by SCDNR employees in SHS on 8 June at 1030 hr. Both had ovaries in the late stage of FOM, during which yolk coalescence and hydration occurs immediately before ovulation. The time of collection, state of oocyte hydration, and rapid nature of FOM found in other multiple-spawning species with similar geographic distributions (Brown-Peterson et al., 1988; Fitzhugh et al., 1993; Roumillat and Brouwer, 2004) indicate that these 2 specimens would have spawned the afternoon of capture.

Egg-development study

Micrographs of cobia eggs from the 2007 and 2008 development studies of hatchery-reared eggs were taken at 13 and 19 sampling times, respectively. In 2007, eggs hatched between 39 and 43 hr postspawning at 22.5°C; between 29 and 33 hr at 25.0°C; and between 25 and 29 hr at 26.5°C. Eggs incubated at 29°C were observed hatching 26 hr after having been spawned as micrographs were being recorded. At the end of the study in 2007, 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 each year revealed damage to the oil droplet in many specimens because of preservation or handling (Fig. 3). Irregularly shaped oil droplets 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 (Fig. 3B). 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 diameter of oil droplets was 380 µm. The average diameters of eggs and oil droplets for both preserved and live cobia eggs fell within the published range (Table 3 [Ditty and Shaw, 1992; Ditty, 2006]). Upon preservation, cobia eggs shrank significantly: 1.7% in 2007 ($P<0.05$) and 1.0% in 2008 ($P<0.05$). No shrinkage occurred in the diameters of oil droplets during either year (2007, $P=0.13$; 2008, $P=0.92$).

Five stages of embryological development were described for cobia (Fig. 4) and are detailed below. Duration of each stage was approximated in hours. Overlap was observed in the durations of stages III and IV because of minor differences between eggs in the 2007 and 2008 development studies.

Stage I (0–7 hr; Fig. 4, A and B): newly fertilized eggs had a distinct, translucent oil droplet. Early cell divisions were evident at the animal pole (opposite of the oil droplet) and eventually formed the blastodisc. Divisions progressed until individual blastomeres were no longer distinguishable. Stage I ended when the germ ring was visible and enclosed approximately one-third of the yolk mass.

Stage II (7–13 hr; Fig. 4C): the germ ring became distinct and epiboly proceeded until the germ ring was in center of the egg. Stage II ended when the blastopore was closed and the optic cups distinguished head and tail regions of the embryo.

Stage III (13–19 hr; Fig. 4D): the embryo was greater than one-quarter of the internal circumference of the egg. Somites were distinct. Stellate melanophores were scattered around the outside of the yolk. Pigmentation on the embryo increased (from head towards anterior of caudal region). Stage III ended with first appearance of melanophores on the oil globule and when the embryo was approximately one-half the internal circumference of the egg.

Stage IV (14–21 hr; Fig. 4E): the embryo continued to become more heavily pigmented. "Free" melanophores continued to congregate on the oil globule until there were few to no free melanophores around the outside

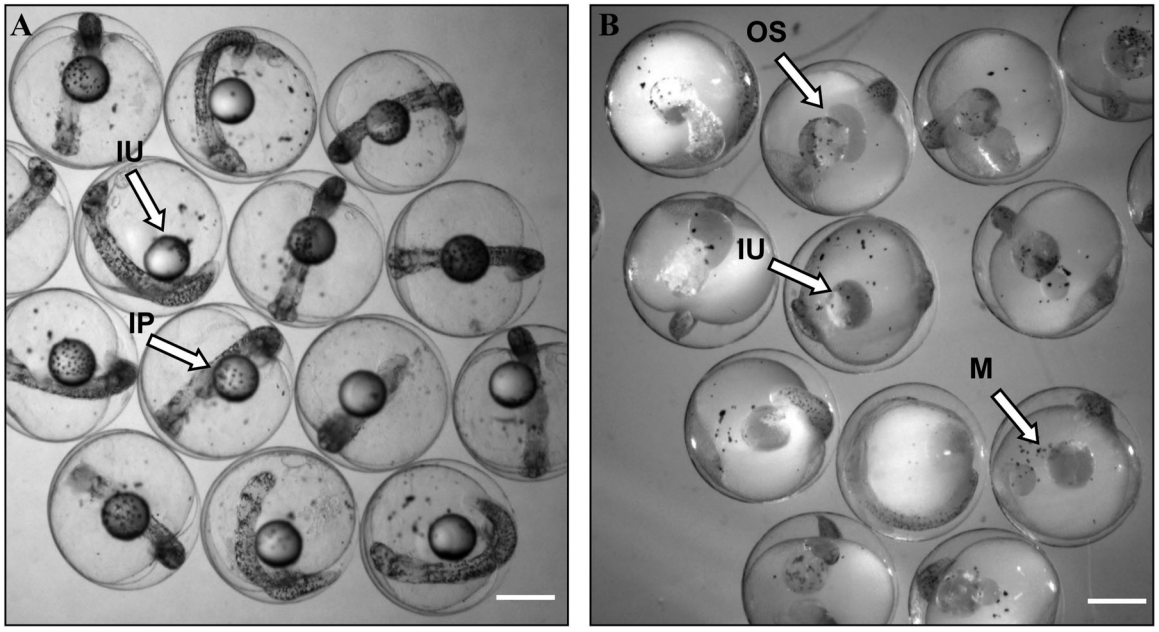

Figure 3

Micrographs of late-stage hatchery-reared cobia (*Rachycentron canadum*) eggs from the egg-development study conducted in 2008. Scale bars=500 µm. (**A**) 21-hr-old eggs before preservation. IU=intact oil droplet (OD), unpigmented; IP=intact OD, pigmented. (**B**) The same eggs after 2 months in 10% neutral buffered formalin. OS=oil separating from pigmented portion; M=multiple OD.

Table 3

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

	Hatchery		Plankton samples	
Parameter	Live	Preserved*	Preserved*	Literature
Diameter	1.21–1.38 mm (mean: 1.30 mm)	1.18–1.37 mm (mean: 1.29 mm)	1.15–1.39 mm (mean: 1.28 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

of the yolk. The embryo began to move, as evidenced by flexion of the body. Stage IV ended when the tail was visibly detached from the yolk and the embryo was approximately three-quarters the internal circumference of the egg.

Stage V (21–29 hr; Fig. 4F): the embryo continued to become more heavily pigmented. More movement of the embryo was evident. The embryo was greater than 100% the internal circumference of the egg before hatching, with the tail extending beyond the head.

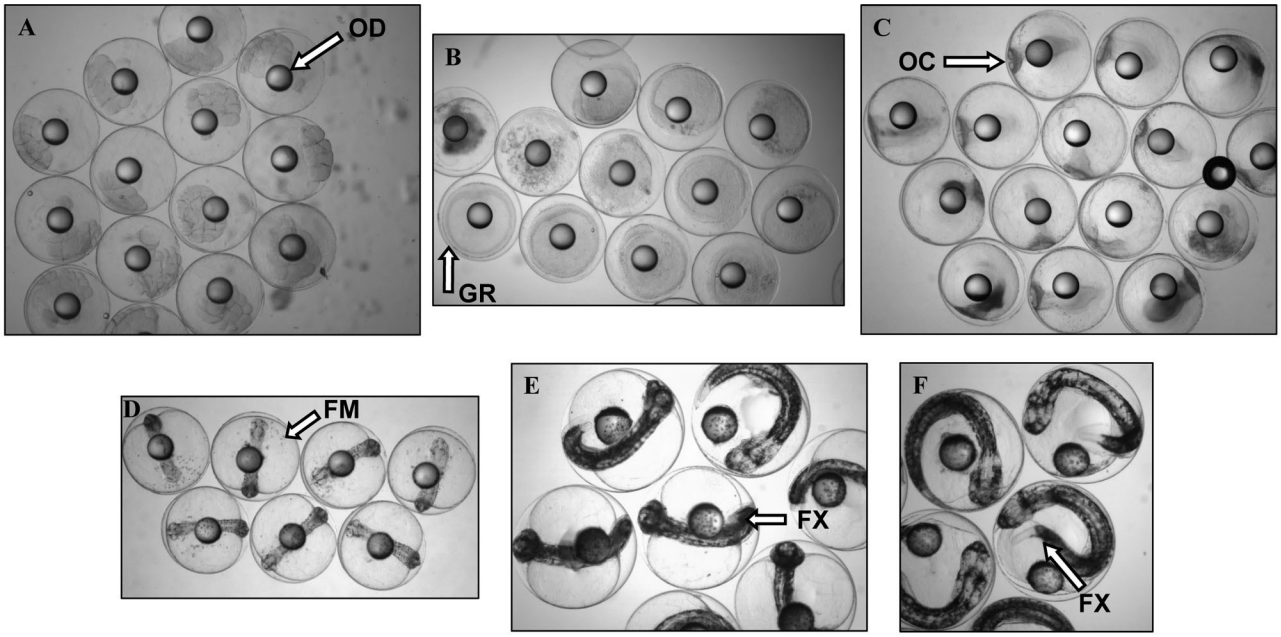

Figure 4

Micrographs of embryonic development of cobia (*Rachycentron canadum*) eggs (A, B, C, and D) from the 2007 egg-development study of hatchery-reared eggs (26.5°C) at 20× magnification and of eggs (E and F) from the 2008 development study (26°C) at 40× magnification. (**A**) Stage I (1 hr postfertilization [pf]): newly fertilized egg with single oil droplet (OD) and first cellular divisions occurring. (**B**) End of stage I, start of stage II (7 hr pf): germ ring (GR) has formed. (**C**) Stage II (13 hr pf): optic cups (OC) distinguish head and tail regions of embryo. (**D**) Stage III (17 hr pf): embryo is acquiring pigmentation, and somites are visible; free melanophores (FM) in yolk begin to migrate toward OD. (**E**) Stage IV (21 hr pf): embryo is heavily pigmented and is >50% the internal circumference of the egg; flexion (FX) of embryo is evident. (**F**) Stage V (25 hr pf): embryo fills more of the egg and greater movement is evident.

Ichthyoplankton survey

Between 6 May and 18 June 2008, 52 anchored plankton-net samples (26 PRS, 26 SHS) were collected. All samples from PRS were sorted completely. Of the 26 samples from SHS, 17 were split to one-half (1 sample), one-quarter (14 samples), or one-eighth (2 samples) the original volume. Measured current speeds ranged from 0.08 to 0.93 m/s (0.29 to 3.35 km/hr), and most speeds ranged 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°C to 30.0°C. Salinities ranged from 31.6 to 34.3 psu in PRS and from 28.4 to 32.7 psu in SHS; these values 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 were well mixed, with little variation of surface and bottom temperatures and salinities (±0.8°C and 0.8 psu, respectively).

On the basis of size and morphological characteristics, 926 eggs were identified as cobia (562 early stage and 364 late stage [Tables 3 and 4; Fig. 5]). Late-stage eggs occurred in samples collected at all stations in PRS (59 eggs). At a single station (PA08) ~15 km inshore, 27 early-stage eggs from PRS were collected at 1930 hr on 5 June 2008 (Table 4). The majority of the eggs identified as cobia were from SHS, with 535 early-stage eggs and 305 late-stage eggs collected among all stations. Of the early-stage eggs found in SHS, 496 came from a single sample collected at 1900 hr on 3 June 2008 at a station (SA03) ~9.7 km inshore (Table 4). Late-stage eggs were collected between 1230 and 1945 hr in both estuaries on 8 sampling days. Egg concentrations ranged from 0.14 to 62.51 eggs/100 m^3 (Table 4).

Egg diameters ranged from 1116 to 1393 µm (mean=1292 µm). The diameters of 73 intact oil droplets ranged from 275 to 420 µm (mean=357 µm). The mean diameters of eggs and oil droplets were similar to the diameters observed for hatchery-reared cobia eggs and to diameters reported elsewhere (Table 3) (Ditty and Shaw, 1992; Ditty, 2006). Measurements of early- (48 eggs) and late-stage (25 eggs) eggs were combined for the ANCOVA. Because of egg shrinkage, only measurements from preserved hatchery-reared eggs were used for the ANCOVA. The mean diameter of oil droplets for field-collected eggs (357 µm) was lower than the mean for hatchery-reared cobia eggs (380 µm); however, the

ANCOVA showed that the slopes of the regression lines were not statistically different (P=0.35; overall coefficient of determination [r^2]=0.61; Fig. 6). This result supports our visual identifications of cobia eggs in the plankton collections.

Most early-stage eggs turned opaque: ages were estimated from a single sample collected 5 June 2008 in PRS, and eggs were ~2–3 hr old. The ages of late-stage eggs were estimated at between 18 and 26 hr old. Time of spawning was estimated to range from the late afternoon (1530 hr) to late evening (2145 hr), and most spawning occurred between 1530 and 1800 hr (Table 5). The 3 exceptions were from one day in SHS, when spawning probably occurred near midnight. Although age estimates of the field-collected eggs were admittedly subjective, eggs collected from multiple stations on the same day were aged independently, and all of them were estimated to be nearly the same age.

Over 8 sampling days, 42 cobia larvae (18 PRS; 24 SHS) were collected. Larval concentrations ranged between 0.17 and 1.99 larva/100 m^3 (Table 5). Five yolksac cobia larvae were collected: 2 on 14 May 2008 (stations PA05 and PA08), 1 on 29 May 2008 (station PA07), and 2 on 21 May 2008 (station SA01).

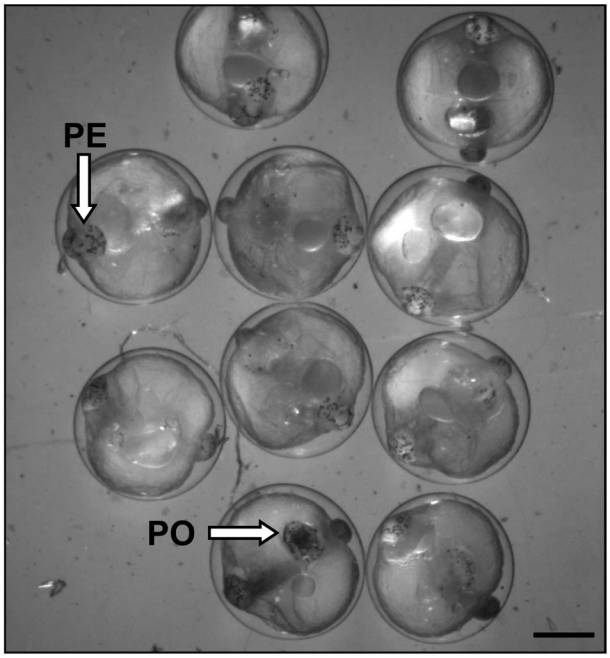

Figure 5

Micrograph of late-stage cobia (*Rachycentron canadum*) eggs collected from ichthyoplankton surveys conducted in Port Royal Sound, South Carolina, on 29 May 2008. PO=pigmented oil droplet; PE=pigmented embryo. Scale bar=500 µm.

Discussion

The results of this study indicate that cobia spawn within PRS and SHS, South Carolina, as evidenced by the high mean GSIs of females, the collection of actively spawning females, the presence of recently fertilized eggs, and the presence of larvae in these 2 estuaries. Through the use of the estimated ages of field-collected eggs, in combination with time and location of capture from adult cobia collected at fishing tournaments, this study provides evidence of spawning of wild cobia in estuarine 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 was previously reported for cobia in the Gulf of Mexico by Biesiot et al. (1994; 5.5), Lotz et al. (1996; 5) and Brown-Peterson et al. (2001; 4.5); in North Carolina by Smith (1996; 5.7); and along the Atlantic coast of the southeastern United States by Brown-Peterson et al. (2001; 5.5). The higher mean GSI value reported in this study is not biased by the collection of 2 actively spawning females: when these females were removed from analyses, the mean GSI values were still well above the values reported elsewhere. In addition to a higher GSI value, the size range of oocytes (500–850 µm) in ovaries in the late developing subphase (the phase that dominated collections in this study) was greater than the size range reported elsewhere for the group with the most developed oocytes (500–650 µm [Lotz et al., 1996]). Fully developed and fertilized cobia eggs in this study and in Ditty and Shaw (1992) ranged in size from 1.15 to 1.42 mm; therefore the larger oocyte sizes found in developing ovaries suggest that oocytes were more highly developed in our current study. Brown-Peterson et al. (2001) and van der Velde et al. (2010) reported that monthly GSI values corresponded with histological evidence of spawning times, supporting GSI as a proxy for the developmental state of ovaries. In our current study, the larger size range of oocytes, together with the higher GSI values, indicate that female cobia collected in South Carolina were closer to actually spawning than were specimens from previous studies.

Differences in the stage of cobia ovarian development between this and other studies may stem from locations where females were captured. In previous work, females were collected from coastal waters in the Gulf of Mexico (Lotz et al., 1996; Brown-Peterson et al., 2001) and off the Atlantic coast of the southeastern United States (Smith, 1996; Brown-Peterson et al., 2001). For 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 those caught offshore was observed likely because those fish caught inshore were part of a spawning aggregation and those fish caught offshore were caught before or after spawning or were intercepted as they were migrating to spawning grounds. This hypothesis is supported

Table 4

The number of cobia (*Rachycentron canadum*) eggs (early-stage and late-stage) and larvae identified from plankton collections made in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina, in May and June 2008. Station codes: PA=PRS anchored net; SA=SHS anchored net; numbers correspond to a specific station. Station numbers with an asterisk were subsampled: numbers of eggs and larvae are estimated from the fraction sorted. No.=number of eggs or larvae; C=concentration (number/100 m^3).

		Early-stage eggs		Late-stage eggs		Larvae	
Date	Station	No.	C	No.	C	No.	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

by recent genetic evidence that indicates the presence of distinct population segments along the southeastern coast of the United States (Darden[6]). 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 found that fish collected offshore were a single population segment. Furthermore, the fish collected in inshore waters were genetically distinct from the offshore group and from cobia in other estuaries. If there is a distinct inshore South Carolina population segment, it could be maintained through spawning of cobia in their natal estuaries.

The distribution of female cobia among the reproductive phases may not represent the actual distribution in the population because the primary means of specimen collection was fishery dependent. In particular, the low percentages of females with developing and regressing ovaries and those females with histological signs of a recent spawn (POFs) are likely a result of fishery practices, particularly the seasonality of the recreational fishery. Cobia generally are thought to enter inshore waters when water temperatures reach 20°C (Richards, 1967; Smith, 1996), and water temperatures in PRS and SHS reached 20°C before the end of April in 2008. Recreational anglers, in contrast, targeted cobia most heavily in May (Steele[4]). 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 these areas despite a decrease in availability of specimens from recreational anglers. Female cobia with ovaries containing POFs were underrep-

[6] Darden, T. L. 2009. Personal commun. South Carolina Dept. Natural Resources, Charleston, SC 29412.

resented likely because of the time of day anglers were targeting fish. Evidence from both the histological analysis and plankton collections of eggs in this study suggests that wild cobia in South Carolina inshore waters spawn from mid-afternoon to late evening, with most activity occurring between 1530 and 1800 hr. Unfortunately, fish collected at tournaments were probably caught from morning to mid-afternoon, before the peak in daily spawning activity.

The best evidence produced from histological analysis that cobia were spawning inshore in South Carolina was the collection of actively spawning females. The duration of FOM is unknown for cobia, although this hormonally controlled "point-of-no-return" on the path to spawning commences 6 to 13 hr before spawning in spotted seatrout (Brown-Peterson et al., 1988; Roumillat and Brouwer, 2004), a co-estuarine inhabitant with cobia in the southeastern United States that spawns the same time of year. Because the earliest stages of FOM were unavailable to us, we were unable to estimate the time of FOM onset in cobia. However, both of the actively spawning females that we collected had oocytes that were in the late stages of FOM (near the mid-point of hydration), indicating that they would have spawned in the mid-afternoon and likely in the proximity of their capture.

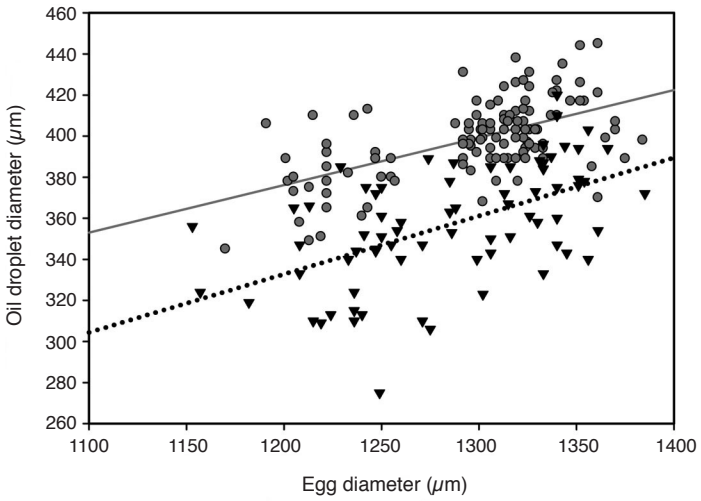

Figure 6

Regression lines for egg (ED) and oil-droplet (OD) diameters from preserved hatchery-reared cobia (*Rachycentron canadum*) eggs from the egg-development studies in 2007 and 2008 (circles, solid line; $y=0.23x+98.46$) and eggs identified as cobia from plankton collections conducted in Port Royal Sound and St. Helena Sound, South Carolina, in 2008 (triangles, dotted line; $y=0.28x-7.63$). Results of the analysis of covariance show no significant difference between the relationship of ED and OD of known cobia and suspected cobia eggs ($P=0.35$; overall coefficient of determination [r^2] $=0.61$).

Female cobia undergoing hydration have been collected in few studies, although there have been exceptions (Smith, 1996; van der Velde et al., 2010). Actively spawning females may compose a larger percentage of the inshore cobia population in South Carolina than the percentage reported here, but prespawners may not take a baited hook. As oocytes hydrate during FOM, their volume can nearly quadruple (Wallace and Selman, 1981). The ovaries of an actively spawning female caught in PRS composed one-sixth of the total body weight of the fish, nearly filling its entire body cavity. It is possible that cobia cease feeding during egg hydration.

Collection of cobia eggs in PRS and SHS also supports the idea that these areas are inshore spawning sites. Morphological features of wild cobia eggs matched closely those of hatchery-reared cobia eggs and were supported with the quantitative comparison of egg characteristics. Cobia eggs found in plankton collections had oil-droplet diameters within the reported range but smaller than the diameters reported for the hatchery-reared eggs. The hatchery eggs came from the spawns of only 2 females that had been hormonally induced to spawn, and maternal condition is known to affect egg and larval quality in fishes, including the size of oil droplets (Berkeley et al., 2004; Gagliano and McCormick, 2007; Sogard et al., 2008). Eggs collected in the plankton samples likely came from several females that encountered a variety of environmental conditions and had variable physiological conditions compared with 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, although highly indicative of spawning, does not alone positively confer evidence of spawning habitat. Origin of late-stage larvae often cannot be determined: active dispersal or transport may be possible because of their increased swimming ability (Clark et al., 2005) or ability to control their vertical position in the water column, which allows migration by selective tidal stream transport (Boehlert and Mundy, 1988; Hogan and Mora, 2005). Cobia eggs were found in PRS from 12 to 20.5 km inshore. On the basis of the measured current speeds, a floating object could have traveled 7–15 km during a single flood tide, making it improbable that all eggs were spawned beyond the estuary. The most conclusive evidence for spawning within PRS comes from eggs that were estimated to be only 2–3 hr old and collected in the Broad River, 15.0 km inshore. With an average current speed of 0.7 m/s measured during the time of their collection, these eggs must have come from this estuary. In SHS, one particular collection of 496 early-stage eggs 9.7 km inshore lends compelling evidence that spawning occurred near the time and in the immediate vicinity of collection.

Table 5

Estimated age and time that cobia (*Rachycentron canadum*) eggs from plankton collections were spawned in Port Royal Sound (PRS) and St. Helena Sound (SHS), South Carolina, in 2008. Estimated spawning time is rounded down to the nearest quarter hour. Station codes: PA=PRS anchored net; SA=SHS anchored net; numbers correspond to a specific station.

		Sample pick-up		Estimated time of spawning	
Station	Date	Time	Age of eggs (hr)	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

Conclusions

On the basis of the evidence provided here, cobia spawn in PRS and SHS during May and June. The collection of cobia eggs in early stages of development, the high average inshore GSI values, and the presence of actively spawning females in PRS and SHS makes this study the first one to positively document spawning of cobia in inshore waters. Spawning occurs in the afternoon and evening (primarily between 1530 and 1800 hr), as indicated by the embryological development of eggs and aging of wild-caught eggs. Some studies have suggested daytime and evening spawning of cobia based on the reported observation of what was believed to be spawning activity of adult cobia (see Shaffer and Nakamura, 1989), developmental stage of wild caught cobia eggs (Ditty and Shaw, 1992), and volitional spawning of a single female cobia held in captivity (Weirich et al., 2006); however, we documented daytime and evening spawning through multiple methods.

Intense recreational fishing can produce changes in fish populations and communities in ways similar to the effects of commercial fishing (Coleman et al., 2004; Cooke and Cowx, 2006). Moreover, hyperstability may mask overfishing conditions (Sadovy and Domeier, 2005), whereby catches remain constant at spawning aggregations as stocks decline, as observed in species such as the orange roughy (*Hoplostethus atlanticus)* (Clark et al., 2000; Koslow et al., 2000) and Nassau grouper (*Epinephelus striatus*) (Sala et al., 2001). Cobia found on the Atlantic coast of the United States were considered to be overfished by the SAFMC and GMFMC[3] in 1983, although the only assessment of the cobia fishery was conducted in the Gulf of Mexico (Williams, 2001). Since 1983, cobia has gained popularity among recreational anglers, as evidenced by the continual increase in effort by South Carolina charter boats since 1997 (Steele[4]).

Additional research needs to be conducted to determine the contribution of fish that spawn inshore to the overall U.S. Atlantic population of cobia because spawning may occur offshore as well, as hypothesized in other studies (Hassler and Rainville, 1975; Smith, 1996; Burns et al.[1]). Documentation of spawning in PRS and SHS indicates that inshore waters elsewhere in their region may also provide critical habitat for cobia. Together, the documented spawning and the discovery of a unique population segment within South Carolina inshore waters (Darden[4]) provide compelling reasons for management agencies to reconsider current management strategies. To treat 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.

Acknowledgments

We thank B. Roumillat of the South Carolina Department of Natural Resources (SCDNR), for his guidance developing the histology criteria and staging of ovarian samples, and J. Olney of the Virginia Institute of Marine Sciences (VIMS), for his help with design of, equipment for, and sorting of samples collected during the ichthyoplankton survey. We also thank members of the Estuarine Finfish Research Group at SCDNR who helped with data collection (J. Yost, M. Perkinson, and B. Renkas); P. Crewe (VIMS) and N. Sturgeon (College of Charleston) for sorting assistance; A. Williams and L. DiJoy (SCDNR) for providing second readings on histology slides; D. Knott (SCDNR) for providing sorting space; and C. Battey (NOAA) and K. Brenkert (SCDNR) for larval husbandry support. T. Darden, J. Leffler, and T. Smart (SCDNR), as well as 3 anonymous reviewers, provided helpful suggestions on the manuscript. This project was funded by the SCDNR. This article is contribution no. 386 of the Grice Marine Laboratory, College of Charleston, Charleston, South Carolina, and contribution no. 693 of the South Carolina Department of Natural Resources.

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