

Abstract.—Female cobia, *Rachycentron canadum*, were sampled on their spawning grounds in the northern Gulf of Mexico to study changes in proximate analysis (protein, lipid, carbohydrate, and ash) of the ovaries during gonadal maturation. Four major stages of oocyte development were studied: stage 1, previtellogenesis; stage 2, vitellogenesis; stage 3, final maturation; and stage 4, postovulation. Cobia are multiple spawning fish; therefore, ovaries engaged in a sequential round of oogenesis were distinguished as stages 1' and 2'. Protein was the major constituent of cobia ovaries and its contribution remained fairly constant (49–55% of the dry weight) throughout all stages of development. Lipid was the second most abundant component but the levels, ranging from 21 to 41%, changed depending on the stage of ovarian development. Lipid concentration increased from stage 1 through 3 and decreased slightly in stage 4; it was lower in stage-1 than in stage-1' ovaries but was the same in stages 2 and 2'. Carbohydrate was the least abundant component (3–4%) whereas ash ranked third (6–20%). Most cobia were in prespawning condition (stages 1–3) when they arrived in the northern Gulf of Mexico in April and May; some prespawning fish (stages 1 and 2) were also observed in August and September about a month or two before migration to the overwintering grounds normally occurs. Cobia undergoing sequential spawning episodes (stages 1' and 2') were captured from April through August. Gonosomatic indices (GSI) were calculated both for ovarian developmental stage and for month of capture. Mean GSI increased as ovarian development proceeded and decreased during postovulation; GSI for month of capture was highest during April and May when the prespawning fish first appeared in northern Gulf of Mexico waters.

Biochemical and histological changes during ovarian development of cobia, *Rachycentron canadum*, from the northern Gulf of Mexico

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Cobia, *Rachycentron canadum*, are large migratory fish with a worldwide distribution in tropical and subtropical seas, except for the Pacific coast of North America (Migdalski and Fichter, 1983). In the western Atlantic, cobia are found from Massachusetts and Bermuda to Argentina (Briggs, 1958) but are most common in the Gulf of Mexico (Migdalski and Fichter, 1983), ranging from Key West along the entire coast to Campeche, Mexico (Dawson, 1971). Cobia support a popular sport fishery wherever they are present. Total mortality rates for cobia, including sport and commercial catches plus natural mortality, may be high (Richards, 1967) and it has been questioned whether cobia in the Gulf of Mexico are being exploited at rates beyond which maximum sustainable yields can be maintained.

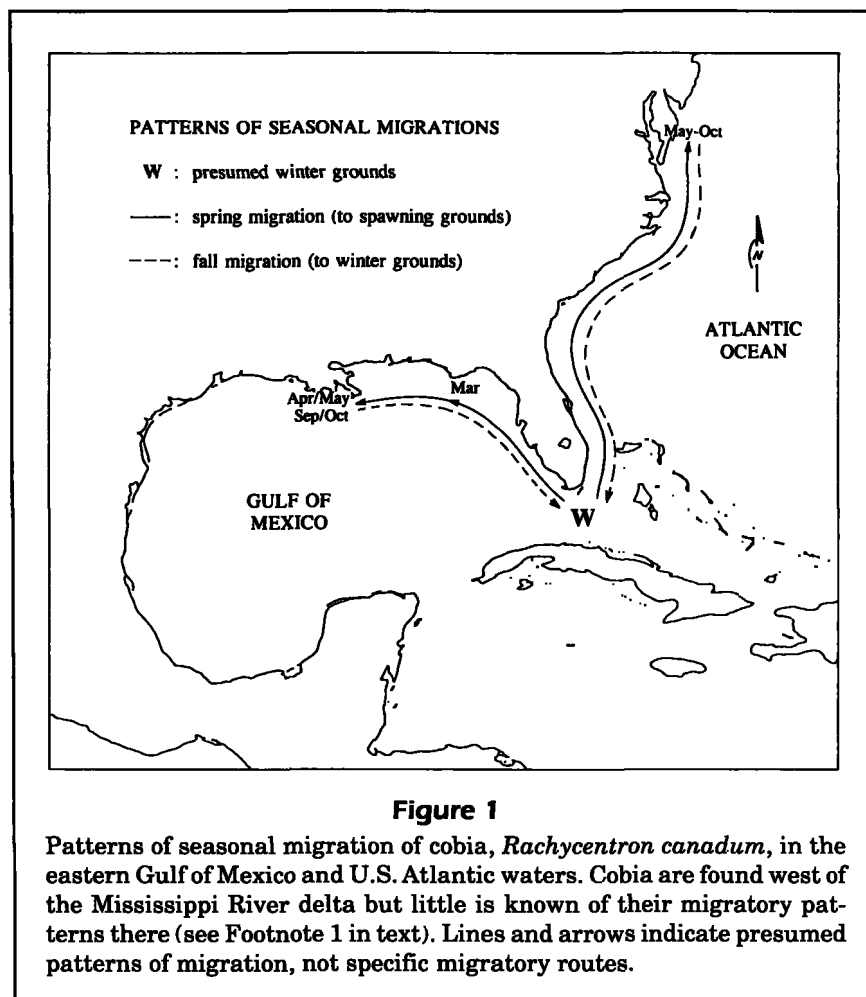
Cobia undergo extensive seasonal migrations (Fig. 1), moving from overwintering grounds to distant spawning/feeding grounds during the spring and summer (Briggs, 1958). They are usually absent from the U.S. fishery in more northerly latitudes during fall and winter months (Dawson, 1971) and are believed to spend their winters near the

Florida Keys.¹ During the spring, cobia move northwest into Gulf waters¹ or north along the eastern seaboard of the United States (Richards, 1977). Cobia usually enter north-central Gulf waters (Alabama and Mississippi) in March or April and begin the return to their wintering grounds in late September.¹

Female cobia with ripe ovaries have been collected in the northern Gulf of Mexico from April to May through October.² Spawning takes place throughout the summer with

¹ Franks, J. S., J. T. McBee, and M. T. Allen. 1992. Studies on the seasonal movements and migratory patterns of the cobia, *Rachycentron canadum*, in Mississippi marine waters and adjacent Gulf waters. Gulf Coast Res. Lab., Ocean Springs, MS 39566-7000. Interim Contract Rep. to Miss. Dept. Wildl., Fish. and Parks/Bur. Mar. Res. and U.S. Fish Wildl. Serv., Atlanta, GA 30303, Project No. F-91, 62 p.

² Lotz, J. M., R. M. Overstreet, and J. S. Franks. 1991. Reproduction of cobia, *Rachycentron canadum*, from the north-eastern Gulf of Mexico. In J. S. Franks, T. D. McIlwain, R. M. Overstreet, J. T. McBee, J. M. Lotz, and G. Meyer, Investigations of the cobia (*Rachycentron canadum*) in Mississippi marine waters and adjacent Gulf waters. Gulf Coast Res. Lab., Ocean Springs, MS 39564-7000. Final Rep. to Miss. Dept. Wildl., Fish. and Parks/Bur. Mar. Res. and U.S. Fish Wildl. Serv., Atlanta, GA 30303, Project No. F-91, p. 2-1 to 2-42.



mature females releasing eggs at least once but possibly twice or more during the breeding season; the population experiences a spawning peak during late spring or early summer. Fertilized cobia eggs are pelagic and egg diameter is between 1.16 and 1.42 mm (Joseph et al., 1964).

Although histological changes during development of cobia ovaries have been described previously,² nothing is known about biochemical changes in the ovary that occur during gonadal maturation. Data on the patterns of change in protein, lipid, and carbohydrate during oocyte development, and on the subsequent utilization of these reserves by the embryos and larvae are important to understanding the early life history of cobia. There is, in fact, very little information about interactions among these three nutrient reserves relative to reproduction despite intensive work on the nutritional requirements of a wide range of fish species.

Few studies have considered the relationship of the major biochemical components (protein, lipid, carbohydrate, and ash) throughout the course of fish

ovarian development. Dawson and Grimm (1980) showed that protein was higher and more constant than lipid during gonadal development of plaice, *Pleuronectes platessa*; ash was low and carbohydrate was not measured. Other authors have studied only the ripe (prespawning) stage of fish ovaries. Ripe mullet, *Mugil cephalus* (Lu et al., 1979), and Atlantic cod, *Gadus morhua* (Kjesbu et al. 1991), ovaries also had higher protein than lipid levels and had low ash and carbohydrate. On the other hand, lipid was the major component of ripe anabantid *Trichogaster pectoralis* ovaries (Hails, 1983).

The present study addresses changes in biochemical composition of the cobia ovary throughout the course of gonadal development. Total protein, lipid, carbohydrate, and ash were measured and compared among fish sampled on their spawning grounds in the northern Gulf of Mexico; the different stages of gonadal development were confirmed histologically. In addition, gonosomatic indices (GSI) were calculated on the basis of ovarian developmental stage and month of capture.

Table 1
Stages of ovarian development in cobia, *Rachycentron canadum*.

Stage	Characteristics
1 Previtellogenesis	Germinal vesicle develops; evaginations appear in nuclear envelope; cortical alveoli form in ooplasm.
2 Vitellogenesis	Lipid vacuoles form; uneven dispersal of protein and lipid yolk.
3 Final maturation	Clearing of lipid around periphery of oocytes; enlarged size; chromosomes condense.
4 Postovulation	Oocytes become distorted and compacted; presence of postovulatory follicles; frothy residual lipid vacuoles.
1' Sequential previtellogenesis	Sequential development of previtellogenic oocytes after a spawning episode; presence of postovulatory follicles and resorbing oocytes in addition to characteristics of stage 1.
2' Sequential vitellogenesis	Sequential development of vitellogenic oocytes after a spawning episode; presence of postovulatory follicles and resorbing oocytes in addition to characteristics of stage 2.

Materials and methods

Sample collection

Cobia examined in this study were collected from coastal waters of Florida, Alabama, Mississippi, Louisiana, and Texas, mostly through fishing tournaments held along the northern Gulf Coast during April through September of 1991 and 1992, although a few fish were caught by project personnel during that same time period. Fish were stored on ice from the time of capture. Immediately after each fish was weighed and measured (total and fork lengths), the ovaries were removed, placed in plastic resealable bags, and stored on ice for 4 to 20 hours until gonad total weights could be recorded and aliquots of the tissue taken. Separate aliquots of each ovary sample were placed in 10% phosphate-buffered formalin and stored at room temperature until the tissues were processed for microscopic examination (see below). Additional aliquots of each ovary were stored at -80°C until the biochemical analyses (see below) were performed.

Histology

Ovaries were processed according to techniques modified from Humason (1979). Tissues were dehydrated in ethyl alcohol and embedded in paraffin by means of a Histomatic automatic tissue processor. The embedded tissues were sectioned at 4 or 5 μm . Sections were stained with Delafield's hematoxylin and eosin (95% ethyl alcohol) (Humason, 1979).

Aspects of fish ovarian development as described by Blaxter (1969), Wallace and Selman (1981), Overstreet (1983, a and b), Guraya (1986), and Mommsen and Walsh (1988) were used to determine the stages of development in cobia ovaries. Four categories of development were observed in this study: stage 1, previtellogenesis; stage 2, vitellogenesis;

stage 3, final maturation, and stage 4, postovulation (Table 1). Some ovaries appeared to have entered another, sequential round of oocyte maturation. Because we were interested in biochemical differences that might exist between successive clutches of oocytes, the following additional categories were studied: stage 1', a sequential previtellogenesis, and stage 2', second (or sequential) vitellogenesis.

Biochemistry

The frozen tissues were thawed on ice and homogenized with either a Virtis tissue homogenizer or a hand-held ground glass mortar and pestle. Protein was measured according to Hartree (1972) with bovine serum albumin as the standard. Carbohydrate was measured according to Dubois et al. (1956) with glucose as the standard. Dry weight was determined after drying the samples overnight at 80°C to constant weight. The same samples were then combusted overnight at 500°C to determine ash content. Lipid extraction was performed according to Sasaki and Capuzzo (1984) which is a modification of Folch et al. (1957) and Bligh and Dyer (1959); total lipid was measured gravimetrically with a Cahn C-31 microbalance.

Calculations and statistics

A gonosomatic index (GSI) was calculated as

$$\text{GSI} = \frac{\text{ovary weight}}{\text{total fish weight} - \text{ovary weight}} \times 100$$

(DeVlaming et al., 1982).

Nonparametric Kruskal-Wallis analysis of variance by ranks (Zar, 1984) was performed with the SPSS-X2.1 statistical software package in order to test the null hypothesis that there were no significant differences among the means being compared. In cases

where the null hypothesis was rejected ($\alpha < 0.05$), nonparametric Tukey-type multiple comparisons were performed according to Zar (1984) in order to determine between which of the mean values significant differences occurred.

Results

Histology

Histological analyses were performed on the gonads of 115 female cobia collected from the northern Gulf of Mexico over the course of two breeding seasons ($n=42$ in 1991 and $n=73$ in 1992). Of these fish, 14 were caught in Florida waters, 6 in Alabama, 60 in Mississippi, 26 in Louisiana, and 7 in Texas; location data could not be obtained for two fish, but they were probably caught in either Mississippi or Louisiana waters. We observed, as had Lotz et al.,² that cobia oocyte production appeared to be group synchronous as defined by Wallace and Selman (1981), such that each ovary examined contained oocytes at different stages of maturation. However, ovaries could be assigned to specific categories based on the dominant oocyte maturity stage.

In stage-1 previtellogenesis, the oocytes were small, compact, and irregularly shaped (Fig. 2A). The previtellogenic stage comprised three substages: a) early previtellogenesis, characterized by small oocytes in which the nucleus had swollen to form a large germinal vesicle; b) middle previtellogenesis, characterized by nucleoli developing within the nucleus and causing evaginations to form in the nuclear envelope; and c) late previtellogenesis, characterized by the presence of cortical alveoli. The latter substage marked the beginning of the transition to stage 2.

In stage-2 vitellogenesis, the oocytes increased in size as the yolk material increased (Fig. 2B) and formed unevenly dispersed lipid vacuoles. Vitellogenic oocytes were somewhat more rounded and were not as compacted as previtellogenic oocytes.

During stage-3 final maturation, the oocytes were larger and the lipid vacuoles and proteinaceous yolk material had become more evenly dispersed (Fig. 2C). The lipid droplets fused and congregated around the periphery of the oocytes, resulting in a clearing of that region of the cell. Note that although most of the oocytes in Fig. 2C were stage-3 oocytes; some stage-1 oocytes and late stage-2 oocytes were also present. Chromosomes condensed during stage 3 for the initiation of meiosis (Fig. 2D).

During stage-4 postovulation, unspent oocytes and postovulatory follicles (POF) were resorbed (Fig. 3A). The oocytes became distorted and compacted, as did

the POF. Residual lipid vacuoles were observed in the resorbing oocytes. (A few early previtellogenic oocytes can also be seen in Figure 3A, concurrent with the resorption process.)

A sequential round of ovarian development was observed in some cobia ovaries categorized as stage 1' (Fig. 3B). The presence of resorbing oocytes and POF in ovaries suggested that a prior spawning episode had recently occurred. Early previtellogenic oocytes, resorbing oocytes, and POF were not seen simultaneously in ovaries categorized as stage 1. A sequential vitellogenic stage, stage 2', characterized by oocytes with numerous small lipid vacuoles, was also observed (Fig. 3C). Previtellogenic and resorbing oocytes as well as resorbing POF were also present in the ovary during this stage.

Timing of ovarian development

The stages of cobia ovarian development were tabulated according to month of capture for 1991 and 1992 data combined (Table 2). In both April and May, 14–15% of the ovaries were developing (stages 1 and 2), ~60% of the ovaries were ripe and about to be spawned (stage 3), ~20% were postspawning (stage 4), and 3–5% had already spawned but were preparing for a sequential spawning episode (stages 1' and 2'). The similarity in ovarian developmental stages for these two months is not surprising because all the April fish were collected in the last week of the month whereas all the May fish were collected in the first week of that month. During July (first and second weeks of the month), again ~15% of the ovaries were developing (stages 1 and 2) but only ~30% were ripe (stage 3); 15% were postspawning (stage 4) and over 40% had already spawned at least once and were in the process of developing for a subsequent spawning (stages 1' and 2'). Fewer numbers of fish were collected in August (last week of the month) and September (first week of the month) but the predominant stages of ovarian development were dramatically different from fish collected earlier in the season. The majority of ovaries (over 80%) were previtellogenic or vitellogenic (stages 1, 2, and 2') whereas fewer than 20% were ripe (stage 3) or postspawn (stage 4); no stage-1' ovaries were seen.

Gonosomatic index

Cobia with ovaries in stages 1, 2, and 3 had increasing mean GSI's of 1.1 ± 0.6 , 5.0 ± 2.2 , and 5.4 ± 2.2 , respectively (Fig. 4A). GSI declined to 3.5 ± 1.6 in cobia with stage-4 ovaries (postovulation); the lower GSI reflects the loss of oocytes to spawning. Almost all of the pairwise comparisons were significantly different (Tukey-type multiple comparison test,

$\alpha < 0.05$). The exceptions were that stage-1 ovaries were not significantly different from stage-1' or stage-2' ovaries nor were stage-1' ovaries significantly different from stage-2' ovaries.

Mean GSI of female cobia was low in both January and March (1.1 ± 0.2 for each month) (Fig. 4B). The January fish were caught on the winter grounds in south Florida waters whereas the March fish were early arrivals in Mississippi waters. GSI increased to 5.5 ± 2.9 in April but declined slightly in May to 4.7 ± 2.0 ; the largest number of cobia enter Missis-

issippi waters during these two months.¹ Mean GSI continued to decline during July (2.9 ± 1.9) and August (1.7 ± 1.6). Slightly more than half of the pairwise comparisons showed significant differences (Tukey-type multiple comparison test, $\alpha < 0.05$). The GSI of January and March fish was significantly different from April, May, and July fish; the GSI of April and May fish was significantly different from July, August, and September fish; and the July and August fish were significantly different from each other.

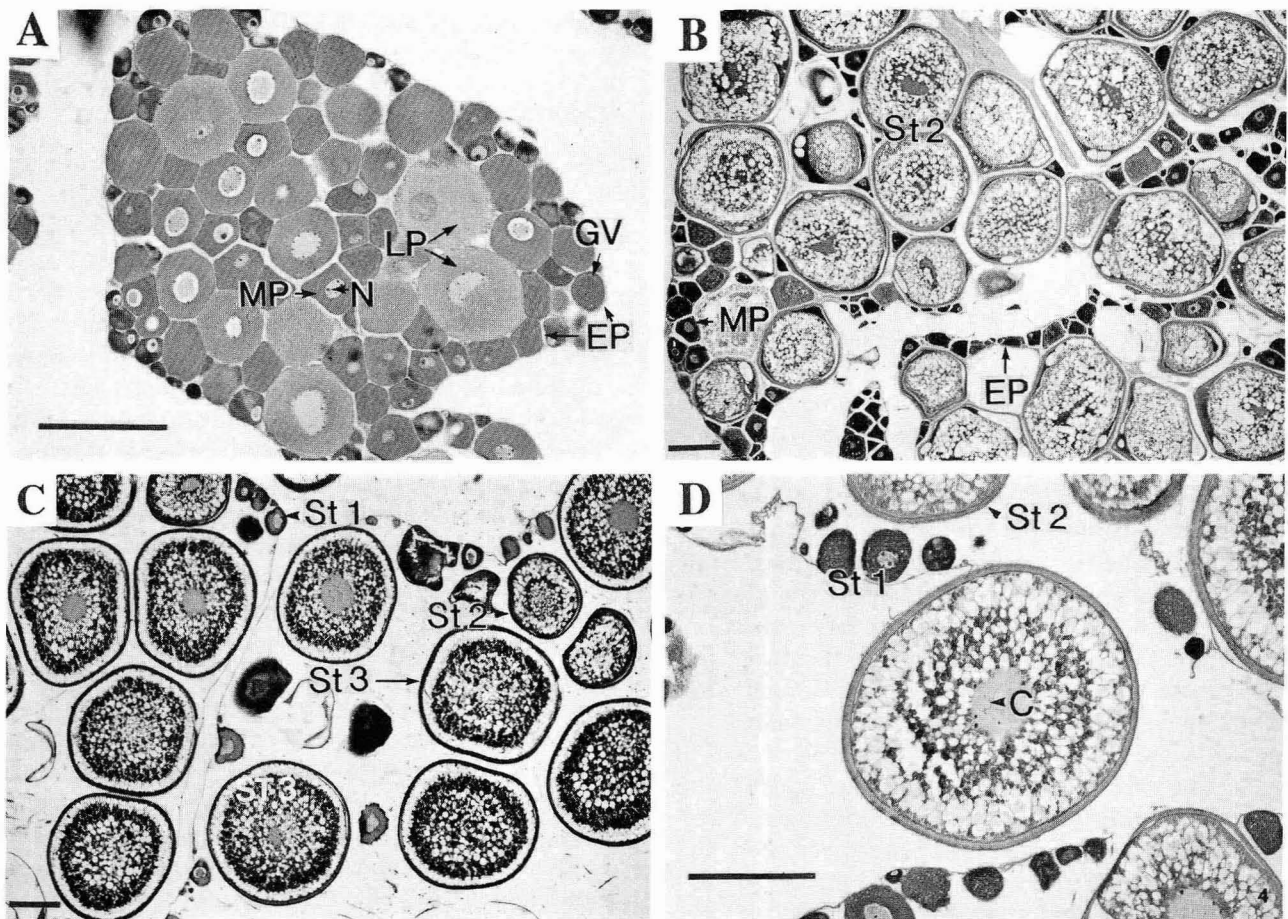


Figure 2

(A) Stage-1 cobia, *Rachycentron canadum*, ovary, previtellogenesis. Early previtellogenesis (EP) with large germinal vesicle (GV) developing; middle previtellogenesis (MP) characterized by developing nucleoli (N) which cause evaginations to form in the nuclear envelope; late previtellogenesis (LP) characterized by appearance of lipid vacuoles. (B) Stage-2 ovary, vitellogenesis. Vitellogenic oocytes (st 2) have increased in size and in number of lipid vacuoles. Note nonsynchronous formation of oocytes; early previtellogenetic (EP) and middle previtellogenetic (MP) stage-1 oocytes also occur. (C) Stage-3 ovary, final maturation. Oocytes (st 3) enter pre-ovulation stage and become more rounded. Lipid vacuoles concentrate around periphery and cause a clearing. Stage-1 (St 1) and stage-2 (St 2) oocytes are also present. (D) Detail of stage-3 ovary. Lipid vacuoles are aggregated around oocyte periphery. Chromosomes (C) condense for initiation of meiosis. Stage-1 (St 1) and late stage-2 (St 2) oocytes are also present. Scale bars=250 μ m.

Biochemistry

Biochemical analyses were performed on about one third of the fish sampled for the histological study ($n=43$). Protein was the major biochemical component (Fig. 5A), representing from 49 to 55% of the ovary total dry weight (507.5–550.5 $\mu\text{g}/\text{mg}$ dry weight). There were no statistically significant dif-

ferences in protein concentration among ovarian developmental stages (Kruskal-Wallis, $\alpha>0.05$).

Lipid concentration ranged from 209.3 to 412.5 $\mu\text{g}/\text{mg}$ dry weight (21–41% dry weight) during ovarian development, increasing from stage 1 through stage 3 (Fig. 5B). The increase was likely due to the formation of lipid yolk during oocyte maturation. Lipid concentrations then decreased after ovulation but not to the low level of stage 1, probably reflecting the residual lipid that had not been resorbed during stage 4. The only statistically significant difference in lipid concentration during the course of ovarian development was between stages 1 and 3 (Tukey-type multiple comparison test, $\alpha<0.05$).

Carbohydrate concentration was very low during all stages of oogenesis in cobia, ranging from 27.2 to 45.2 $\mu\text{g}/\text{mg}$ dry weight (3–4% dry weight) (Fig. 5C). It decreased from stage 1 to 2, increased from stages 3 through 1', and declined slightly in stage 2'. Almost all of the pairwise comparisons of carbohydrate concentration were significant (Tukey-type multiple comparison test, $\alpha<0.05$) except that stage 1 was not significantly different from stages 4 and 2' nor was stage 2 significantly different from stage 3.

Ash concentration decreased from a high of 196.3 $\mu\text{g}/\text{mg}$ dry weight to a low of 55.3 $\mu\text{g}/\text{mg}$ dry weight (6–20% dry weight) (Fig. 5D); it increased in stage 4 and stage 1' but declined again in stage 2'. Stage-1 ash concentration was significantly different from stages 3, 4, and 2'; whereas stage 2 was significantly different from stage 3 (Tukey-type multiple comparison test, $\alpha<0.05$). All of the other pairwise comparisons were not significant.

Discussion

Protein was the major constituent of cobia ovaries and its contribution remained fairly constant (49–55%) throughout all stages of gonadal devel-

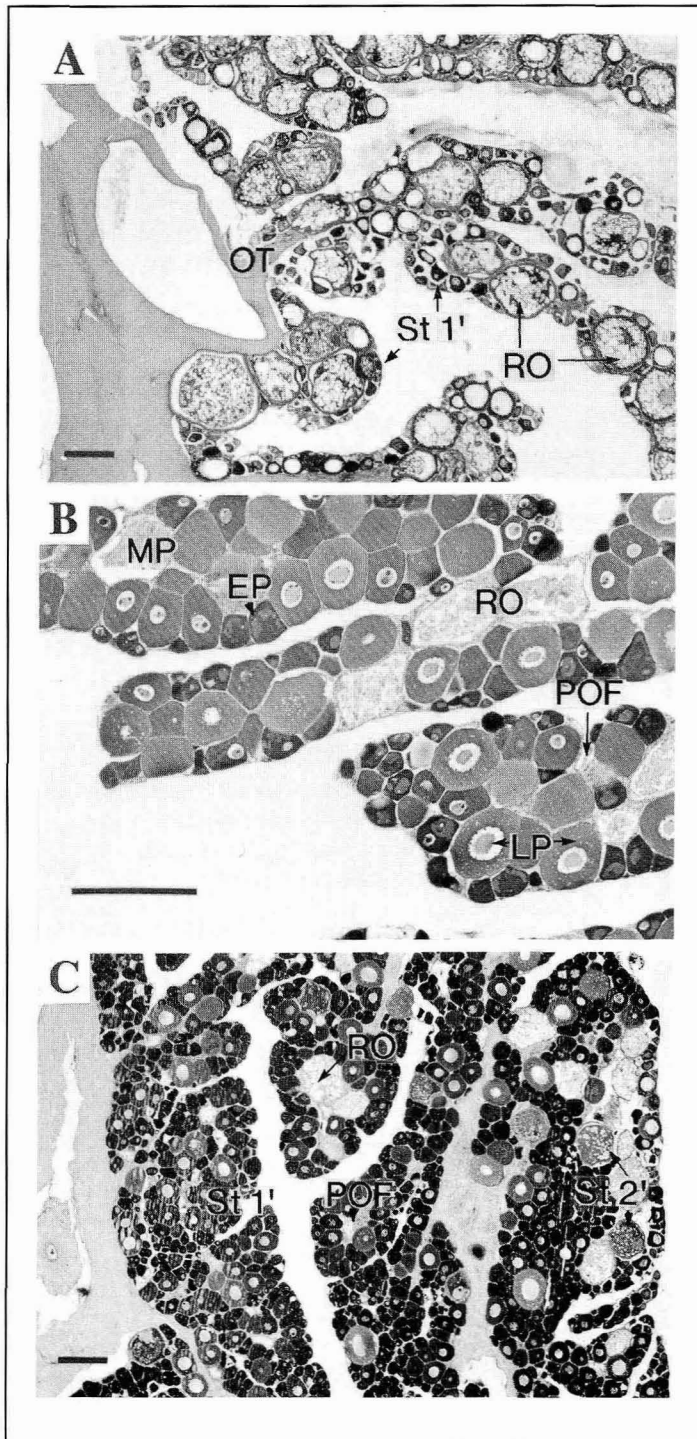


Figure 3

(A) Stage-4 ovary, postovulation. Resorption of unspent stage-3 oocytes (RO) into ovarian tissue (OT); oocytes are distorted and compacted. There is residual lipid in the resorbing oocytes. Stage-1' oocytes (St 1') occur. (B) Stage-1 ovary, second previtellogenesis. Early previtellogenic (EP), middle previtellogenic (MP), and late previtellogenic (LP) stage-1' oocytes develop. Resorbing oocyte (RO) and resorbing postovulatory follicle (POF) are remnants from stage 4. (C) Stage-2' ovary, second vitellogenesis (early). Formation of second round of vitellogenic oocytes (St 2'). Note resorbing oocytes (RO), postovulatory follicles (POF), and stage-1' oocytes (St 1'). Scale bars=250 μm .

Table 2

Percentage of cobia, *Rachycentron canadum*, ovaries at each stage of development by month of capture. Stages are as described in Table 1. Data are combined from 1991 and 1992.

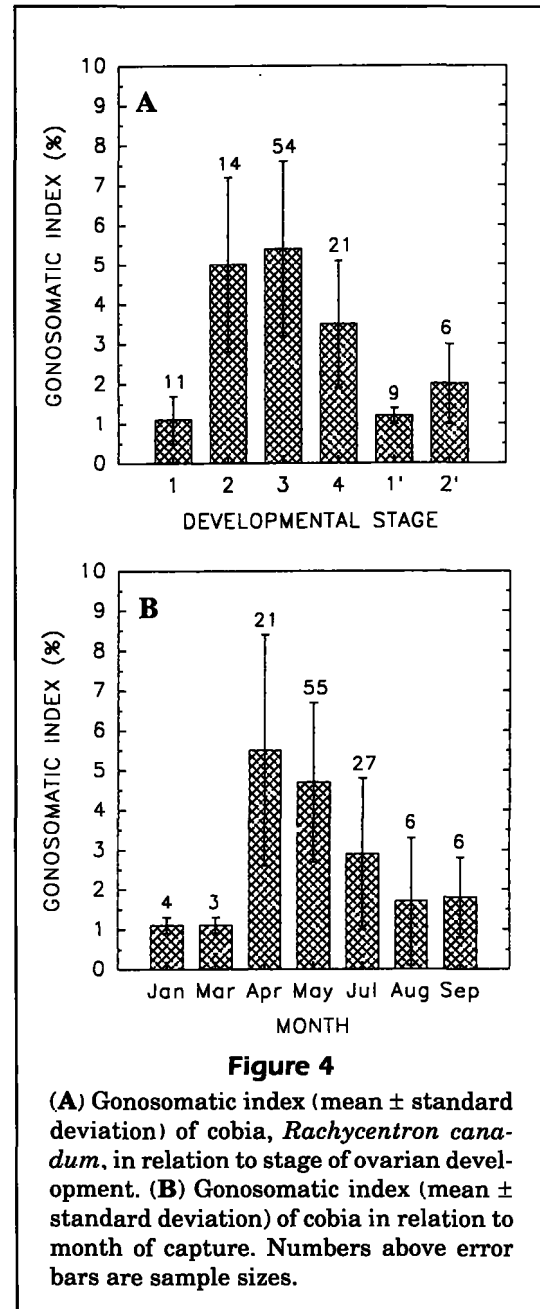
Stage	Month of capture					Total (n=118)
	Apr (n=21)	May (n=58)	Jul (n=27)	Aug (n=6)	Sep (n=6)	
1	—	5.2	3.7	50.0	66.7	9.3
2	14.3	10.3	11.1	16.7	16.7	11.9
3	61.9	60.3	29.6	—	16.7	48.3
4	19.0	20.7	14.8	16.7	—	17.8
1'	4.8	—	29.6	—	—	7.6
2'	—	3.4	11.1	16.7	—	5.1

opment. We believe that any putative increase in the proteinaceous yolk as oocytes ripened was not detectable by the methods used in this study because the follicles were also increasing in size as oocytes matured. That is, protein concentration was relatively stable because structural protein (follicles, etc.) contributed far more to the total protein concentration than did yolk proteins.

Lipid was the second most abundant component but the levels changed from stage to stage, ranging from 21 to 41%. The fluctuations in lipid concentration during ovarian maturation can be explained by the increasing amount of lipid yolk reserves that are deposited as oocytes mature from stages 1 to 3 followed by the subsequent loss of ripe oocytes from the ovary after ovulation and spawning.

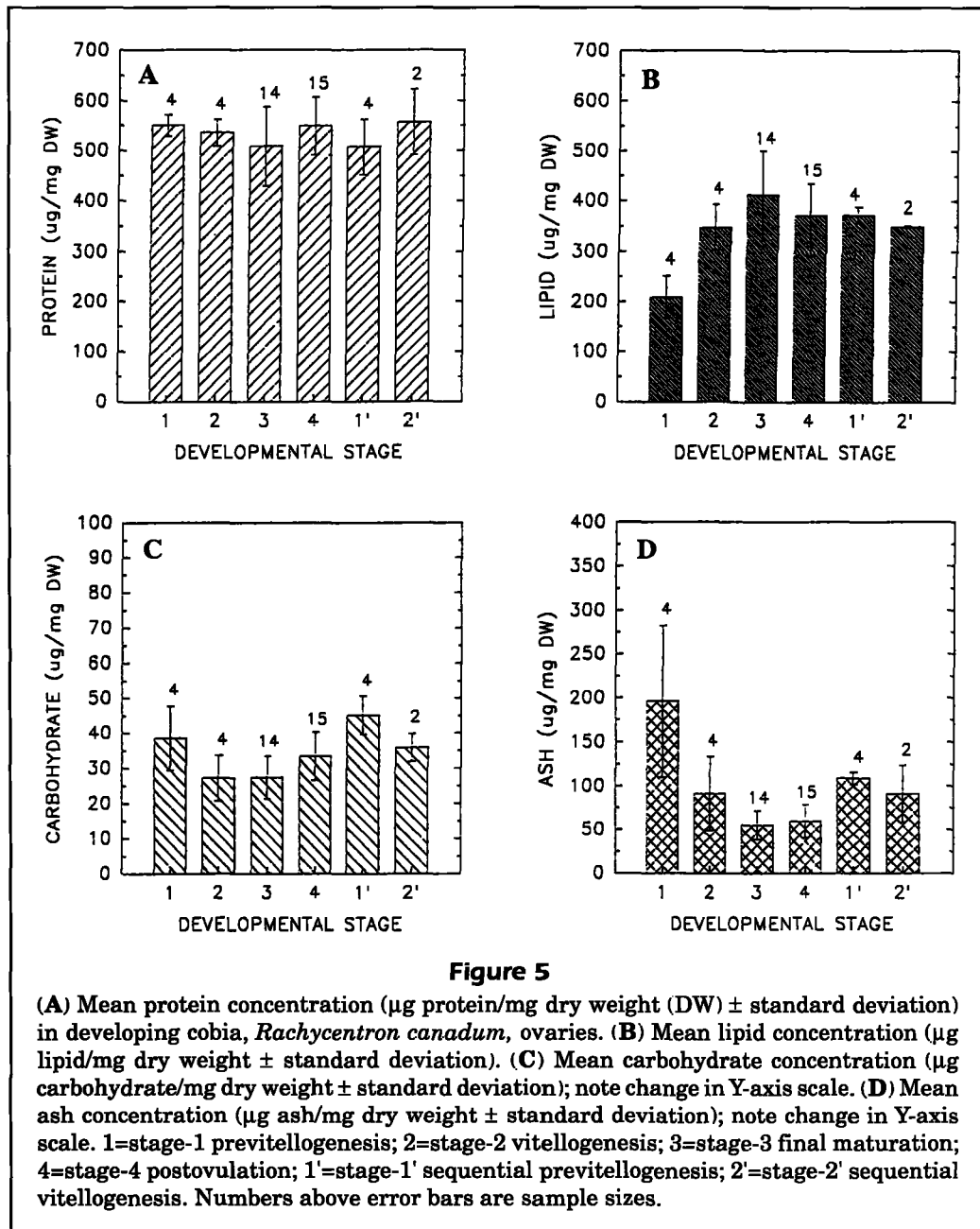
Carbohydrate was the least abundant component (3–4%) of cobia ovaries and ash ranked third (6–20%). Boulekbache (1981) noted that the enzymes of carbohydrate metabolism increased in activity during oogenesis. Carbohydrate concentration, therefore, may be low due to constant catabolism. In the present study, it is not known whether carbohydrate was constantly being catabolized and replaced, or whether concentrations were low. In most fish, however, carbohydrate is not readily available for use until after fertilization occurs (Boulekbache, 1981). The trend in ash concentrations was the inverse of lipid concentrations; that is, ash concentration declined when the lipid concentration increased and vice versa.

Results of biochemical analysis of ripe ovaries from similar studies using other species of fish are given in Table 3. Protein was the major component of ripe ovaries followed by lipid, ash, and carbohydrate for cobia, *Rachycentron canadum* (this study), striped mullet, *Mugil cephalus* (Lu et al., 1979), plaice, *Pleuronectes platessa* (Dawson and Grimm, 1980), and Atlantic cod, *Gadus morhua* (Kjesbu et al., 1991). The primary dif-



ferences among the four species of fish were the relative proportions of protein and lipid. Ripe cod ovaries had less than half the amount of lipid than either mullet or cobia; cobia ovaries had ~1.1 times more lipid than mullet ovaries. Only for the anabantid *Trichogaster pectoralis* (Hails, 1983) was lipid the major component of ripe ovaries.

Since lipid is the most efficiently stored energy reserve, supplying 9.5 cal/mg, whereas protein liberates 5.7 cal/mg and carbohydrate 4.1 cal/mg (Crisp, 1984), one might expect fish eggs to have large amounts of lipid to supply the energy needed for growth and metabolism during embryogenesis and



subsequent early larval development before first-feeding. The lipid:protein (L:P) ratio of ripe cobia oocytes (not ovaries as reported in the present study) was 1:0.7 (Caylor, 1992), which is similar to both striped bass, *Morone saxatilis*, eggs (1:0.6) (Eldridge et al., 1982) and red drum, *Sciaenops ocellatus*, eggs (1:0.8) (Vetter et al., 1983). Winter flounder, *Pseudopleuronectes americanus*, eggs, however, had a much higher L:P ratio of 1:5.2 (Cetta and Capuzzo, 1982).

One factor affecting the storage of biochemical components is egg size. Many marine fish eggs are relatively small and do not have large stores of energetic

reserves; these small eggs usually hatch quickly. Cobia eggs range from 1.16 to 1.42 mm in diameter (Joseph et al., 1964). Red drum eggs are 0.86–0.98 mm (Vetter et al., 1983) and striped bass oocytes are 3.3–3.4 mm after hydration (Eldridge et al., 1981). Winter flounder eggs are the smallest, 0.74–0.85 mm diameter (Smigielski and Arnold, 1972), yet they are composed of about five times as much protein as lipid. Thus, generalizations about energy reserve storage cannot be made based solely on egg size.

Winter flounder eggs are demersal whereas eggs of cobia, red drum, and striped bass are pelagic. De-

Table 3
 Biochemical composition (% dry weight) of some fish ovaries including cobia, *Rachycentron canadum*.

Species	Protein	Lipid	Carbohydrate	Ash	Reference
<i>Rachycentron canadum</i> (cobia) (ripe ovary)	50.7	41.1	2.7	5.5	present study
<i>Mugil cephalus</i> (mullet) (ripe ovary)	59.3	36.0	—	4.7	Lu et al., 1979 ¹
<i>Pleuronectes platessa</i> (plaice) (ripe ovary)	87.4	8.4	—	3.1	Dawson and Grimm, 1980 ²
(spent ovary)	88.6	3.6	—	7.1	Dawson and Grimm, 1980 ²
<i>Trichogaster pectoralis</i> (anabantid) (ripe ovary)	27.7	72.3	0.16	—	Hails, 1983 ²
<i>Gadus morhua</i> (Atlantic cod) (ripe ovary)	77.7	16.5	0.7	5.1	Kjesbu et al., 1991 ¹

¹ Original data reported as percent wet weight; we converted to percent dry weight.

² Original data reported as dry weight; we converted to percent dry weight.

mersal eggs tend to have more protein than lipid (Flachter and Pandian, 1968), which results in negative buoyancy. This could account for the high proportion of protein in winter flounder eggs in contrast to the high proportion of lipid in cobia, striped bass, and red drum eggs. Another possible explanation for the two very different patterns of biochemical composition is that cobia, striped bass, and red drum are warm-temperate species whereas winter flounder is a cold-water species. Cobia (Ditty and Shaw, 1992), striped bass (Harrell et al., 1990), and red drum (Vetter et al., 1983) have short incubation times: 24 hours at 29°C, 48 hours at 18°C, and 22 hours at 23°C, respectively. Winter flounder has a much longer incubation time, 11–20 days at 4–6°C (Cetta and Capuzzo, 1982). The difference in incubation times for different species is due in part to the effect of temperature on metabolic rate of the developing embryos. Catabolism of specific endogenous energy stores in fish eggs is known to be related to the temperature of incubation. Lipid tends to be consumed in higher quantities at higher temperatures but protein consumption dominates at lower temperatures (Heming and Buddington, 1988). Therefore, it is not surprising to see different patterns of biochemical composition in light of the temperature history during early development of these different species.

In addition to reporting the changes in biochemical composition during cobia ovarian development, we also examined the cyclical variation in ovary size. This was done by means of the gonosomatic index (GSI), a commonly used ratio that normalizes gonad size among animals of different size classes in order to assess their reproductive state. The GSI was determined for each female cobia sampled in this study and compared both to stage of ovarian development

and to month of capture. The majority of cobia landed in April and May had ovaries in stage-3 condition (~60%). This was reflected in the high mean GSI for those months. By July and August fewer cobia, ~30% and 0%, respectively, had stage-3 ovaries; this was reflected by the declining mean GSI. In September the increase in cobia with ovaries in prespawning condition was indicated by the slight increase in GSI.

It is not clear why there was a greater proportion of stage-1 and stage-2 ovaries in August and September. Possible explanations include 1) difficulty in distinguishing stage-2 ovaries from stage-2' ovaries; 2) presence of resident young, small fish that were immature at the beginning of the summer but which grew to maturity late in the season; or 3) an influx of older, late-arriving cobia from unknown areas. We believe that a combination of the first two explanations is most likely. Some of the late summer/early fall fish with ovaries classified as stage-2 fish may well have spawned a batch of eggs earlier in the season and therefore were actually stage 2'. But after the POF and any unspent stage-3 oocytes are resorbed, it is not possible to distinguish between a stage-2 and stage-2' ovary. On the other hand, the late summer and early fall stage-1 fish were small; fork length was 94.8 ± 5.3 cm and 102.8 ± 7.9 cm in August and September, respectively. Based on cobia growth equations,² it is highly unlikely that these fish could have spawned the previous year, and they were probably too immature to have spawned earlier in the same year. It is not known whether these fish would have spawned in the fall or whether they would have overwintered without further ovarian development.

Lotz et al.² suggested that cobia spawn over some unspecified period of time during the May to Sep-

tember season. Their conclusion was based on the observed nonsynchronous formation of oocytes in the ovaries, considered to be strong evidence of multiple spawning (Hunter et al., 1992). Nonsynchronous development of oocytes was also observed in the present study. Data from this study further suggest that cobia resorb unspent stage-3 oocytes after ovulation. This hypothesis is supported both by the biochemical data and the histological evidence of residual lipid in stage-4 and stage-1 ovaries.

In summary, we have determined that lipid concentration, but not protein concentration, changes during cobia ovarian development, presumably as lipid yolk reserves are deposited in the oocytes. Carbohydrate and ash concentrations also varied during development, but they were only minor components of the system. Further research is needed on newly fertilized cobia eggs and developing embryos and larvae in order to answer questions about the patterns and rates of energy reserve utilization during embryogenesis and during larval development before first feeding in this species. Because cobia eggs and larvae are only rarely found in plankton collections in the Gulf of Mexico, we have initiated studies on the spawning of ripe, field-caught cobia (Caylor et al., in press).

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