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GONADAL MATURATION IN THE COBIA, *RACHYCENTRON CANADUM*, FROM THE NORTHCENTRAL GULF OF MEXICO

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ABSTRACT Gonadal maturation of cobia, *Rachycentron canadum*, was evaluated by examining 508 specimens from its recreational fishery. Specimens were collected off southeast Louisiana to northwest Florida by hook-and-line during February through October 1987-1991. Fork lengths (FL) of these fish ranged from 580-1,530 mm, with corresponding weights of 2.0-43.5 kg. The female:male ratio was 1:0.37. Using a combination of oocyte size-frequency and histological assessment of many of the fish, we determined that females were ripe from May through September, with atretic oocytes occurring in some fish from July through October. Degenerating hydrated oocytes in July and October and the presence of resting ovaries in July suggest two major spawning periods; however, monthly gonosomatic indices peaking in May, followed by a steady decline, do not support that finding. Ovaries were placed into undeveloped, early developing, mid-developing, or late developing categories based upon oocyte size-frequency distributions. Developing ovaries had two or three modes of oocytes larger than 30 µm. Batch fecundity was estimated to be 2.6×10^6 to 1.91×10^8 oocytes, depending on the size of fish/ovaries. The smallest female with oocytes exhibiting vitellogenesis was 834 mm FL. This fish was 2 years old based its otolith evaluation. The smallest male with an abundance of spermatozoa in its testes was 640 mm FL and 1 year old based on otolith evaluation; smaller males were not examined. Females larger than 840 mm FL had vitellogenic oocytes in March and April. A few fish still had vitellogenic oocytes in early October, but none did by late October. When Gilson's fluid was used to assess ovarian tissue, the fresh weight of the tissue was reduced by 20% after being stored for 3 months. The diameter of oocytes shrunk about 25% in Gilson's fluid which was 11% less than those fixed in formalin, embedded in paraffin, and sectioned. Tissue sections from specific individuals, each demonstrating a variety of different developmental stages, were similar regardless of whether they were obtained from the anterior, middle, or posterior portion of either ovary.

INTRODUCTION

The cobia, *Rachycentron canadum* (Goode 1884), is a pelagic fish that is found throughout most of the warm ocean waters of the world, except for the Pacific coast of North America (Migdalski and Fichter 1983). In the western Atlantic Ocean, *R. canadum* occurs from Massachusetts to Argentina and is common in the Gulf of Mexico (Shaffer and Nakamura 1989). In the Gulf of Mexico (Gulf), cobia migrate from their wintering grounds off the southern Florida coast into the waters of the northern Gulf in late March and April and return to their wintering grounds in late autumn and early winter (Biesiot et al. 1994; Franks 1991b). However, a relatively large number of fish appears to remain in the northcentral Gulf during winter months at depths of 100-125 m (Howse et al. 1992). The cobia is a highly prized recreational species in the Gulf and U.S. South Atlantic Ocean. Most of the U.S. cobia landings come from Gulf waters (Shaffer and Nakamura 1989). Although most cobia are caught by recreational anglers, some are caught incidentally in U.S. commercial fisheries (Shaffer and Nakamura 1989).

Relatively little is known about the reproductive biology of cobia. Joseph et al. (1964) described eggs and juveniles collected from Chesapeake Bay and the nearby Atlantic Ocean and suggested that spawning occurred during summer. Richards (1967), also working in Chesapeake Bay, documented sexual dimorphism in size at maturity, presented evidence for spawning from late June through mid-August and postulated that multiple spawnings might occur. Dawson (1971) suggested that spawning occurred during spring in the coastal waters of the northern Gulf of Mexico. Finucane et al. (1978) reported evidence that cobia spawned off the coast of Texas in July and September, and Thompson et al. (1992) observed peak spawning in cobia from May through July in Louisiana coastal waters. Biesiot et al. (1994) described the biochemical changes in developing ovaries in cobia from the northern Gulf of Mexico and reported that spawning occurred during spring and summer.

Our study was undertaken to answer the following questions for cobia in the northcentral Gulf of Mexico: 1) what is the minimum size (length) of fish at maturity; 2) what is the temporal period of reproductive activity; 3) does the cobia spawn more than once per spawning season, and if so, what is the estimated batch fecundity of a female? We attempted to answer these questions through an analysis of oocyte size-frequency distributions, gonadal histology and the gonosomatic index (GSI).

MATERIALS AND METHODS

Cobia examined in this study were caught by hook-and-line in the recreational fishery off southeast Louisiana, Mississippi, Alabama, and northwest Florida during February through October 1987-1991. Additional gonad samples from six cobia caught from the Gulf side of the Florida Keys in January 1991 were used for histological evaluation only. In addition to those fish we caught, some were provided by recreational fishermen as well as state and federal fisheries agencies.

Fish were stored on ice from the time of capture until examined dockside or when received at coastal fishing tournaments. Fork length (FL) and total length (TL) were measured in mm, and total body weight (TW) was recorded to the nearest 0.1 kg. The pair of gonads was removed, placed in a resealable plastic bag, and stored in an ice slurry for up to 20 h. Total gonad weight was recorded to the nearest 0.1 g. A small subsample of each gonad was weighed to the nearest 0.1 g and fixed in 10% buffered formalin for histological examination. A second subsample of each ovary was weighed to the nearest 0.1 g and fixed in Gilson's fluid to facilitate estimation of oocyte numbers and size-frequency distributions.

A gonosomatic index (GSI) was calculated for both males and females: GSI = gonad weight/total fish weight) x 100.

Shrinkage of oocytes due to fixation was estimated by measuring the largest oocytes from fresh gravid ovaries, formalin-fixed-paraffin-embedded gravid ovaries, and Gilson's-fixed gravid ovaries. An estimate of the weight loss due to Gilson's fixative was determined by weighing a sample of fresh ovary at the time of collection, and then reweighing the same sample after 3 months in Gilson's fixative.

Ovarian tissue remained in Gilson's fluid for at least 3 months prior to estimating oocyte density and oocyte size-frequency distribution. A Bioquant® image analysis system was used to expedite oocyte counts and measurements.

The number of oocytes in an aliquot was determined using a counting chamber. Oocyte density was determined from the number of oocytes in corrected-weight aliquots of Gilson's fixed tissue and expressed as the number of oocytes per gram of fresh ovarian tissue. The total number of oocytes per female was obtained by multiplying the oocyte density by the total ovarian weight.

The frequency distribution of oocyte sizes was obtained by measuring the maximum distance across 100-200 randomly selected oocytes greater than 30 µm in diameter from an aliquot of Gilson's-fixed tissue. Presumptive batch fecundity, the presumed number of eggs released during each spawning episode, was determined on the basis of the percentage of oocytes appearing as the most advanced standing stock of oocytes in late developing ovaries.

Samples for histological analysis were embedded in paraffin from Hemo-De® xylene substitute, chilled, sectioned at 4 µm, stained with Gills hematoxylin, and counterstained with eosin-phloxine. Oocytes from the coverslipped slides were then staged according to sexual maturity. To determine whether the distribution of oocyte stages was homogeneous between ovaries and among anterior, middle, and posterior portions of each ovary; we examined histologically wedge-shaped samples from the wall to the lumen at those sites.

Size (length) at maturity was determined as the smallest fish which exhibited vitellogenesis or spermatogenesis. The ages of several fish examined during this study were determined as part of a concurrent study estimating age by otolith analysis (Franks et al. 1991a).

Statistical analyses were performed using Systat® software (Wilkinson 1990). Overall significance among group means was determined by the Kruskal-Wallis test ($P<0.05$); significance between pairs of means was determined by the Mann Whitney U-test using Bonferroni's correction ($P<0.05$). Batch fecundity data were transformed to logarithms ($\log_{10}$) to normalize the data before correlation analyses were performed.

RESULTS

A total of 508 cobia (374 females and 134 males) was sampled for reproductive analyses. The sex ratio of fish examined in this study, 1:0.36 (female:male), was representative of the sex ratio of cobia entered in fishing tournaments within our study area. Total weight and FL ranges among all specimens were 2.0-43.5 kg and 580-1,530 mm, respectively.

Seasonal pattern of maturation

All adult males ≥640 mm FL (N=134) and females ≥834 mm FL (N=361) were used for GSI calculations (Figure 1). Ovarian weights from the adult females ranged from 0.3% to 12.5 % of total body weight. Results of graphing GSI against month of collection revealed that the ovaries comprised an increasing proportion of body weight in spring, with a marked peak in GSI mean value of 5.0 in May, followed by a steady decline throughout summer and into autumn (Figure 1). Figure 1 also shows that the GSI for males is essentially the same as that for females.

Figure 2 shows the seasonal dynamics of vitellogenesis. Three of four females caught off Mississippi during March 1991 were in early vitellogenesis. In April, when cobia first appeared in near shore waters of the northern Gulf, all females ≥ than 834 mm FL were vitellogenic. The peak period of ovarian development occurred from April through June during which all females ≥834 mm FL were vitellogenic. Ovarian development in our samples decreased in late summer and autumn. Although a few fish were vitellogenic in early October, none were vitellogenic in late October.

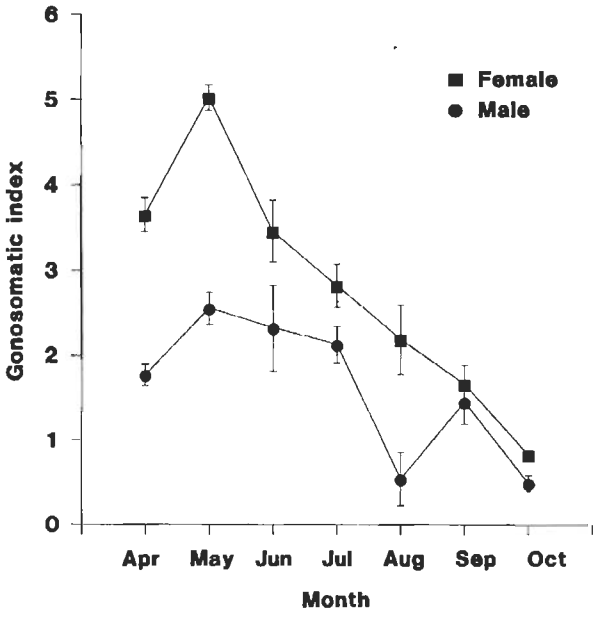


Figure 1. Gonosomatic indices for adult male (N=134) and female (N=361) cobia, *Rachycentron canadum* (means ± 1 standard error of the mean). Figure represents a composite of years 1987-1990.

Weight loss due to Gilson's fluid

The mean weight loss of ovarian tissue fixed in Gilson's fluid was 20% (S.E.=1.1%) of the fresh weight. This number was used as a correction factor for calculating the number of oocytes in fresh ovaries.

Shrinkage of oocytes due to fixation

The largest oocytes observed in fresh, well-developed ovaries were 950-1000 m in diameter. The largest oocytes in groups of large oocytes observed in Gilson's-fixed tissue from sites adjacent to those where fresh material was obtained from the same ovaries were 700-750 µm. Therefore, we estimate that diameter shrinkage due to Gilson's treatment was about 25%.

Examination of ovarian tissue from individual females, both by histological techniques and by Gilson's fixation, allowed for a more precise estimate of the relative shrinkage from the two treatments. Examination of 37 fish revealed that the diameter of oocytes fixed in Gilson's fluid was 11% (S.E.=3%) less than the diameter of oocytes treated by formalin fixation, followed by paraffin embedding.

Oocyte size-frequency distributions

Oocyte size-frequency distributions were estimated for 131 cobia. Inspection of the oocyte size-frequency distributions coupled with examination of histological sections of ovaries allowed fish to be placed into one of four groups representing various stages of ovarian development (Figure 3).

Group I, Undeveloped. Twenty-nine of the 131 fish for which oocyte frequencies were determined were placed into the first group. Figure 3 illustrates the oocyte-size distribution of a representative undeveloped fish. Undeveloped fish exhibited ovaries which contained the greatest proportion of small diameter oocytes. Fish placed into this group possessed ovaries with 90-100% of their Gilson-fixed oocytes with diameters less than 100 µm. The oocyte size-frequency distribution had a single mode of oocyte diameters between 50 and 100 µm, and all eggs were less than 250 µm. Nineteen of the 29 fish were examined histologically and confirmed to be inactive, i.e. not vitellogenic. The mean GSI of fish with undeveloped ovaries was 0.84 (S.E.=0.05). The mean number of oocytes per gram of ovarian tissue in these fish was 1.39×10^7 (S.E.=1.12×10^6) with the mean number of oocytes per female fish of 1.18×10^8 (S.E =1.78×10^7).

Group II, Early Developing. Forty-one of the 131 fish were placed in the early developing group. Figure 3 displays the distribution of oocyte sizes in the ovary of a fish in the early phases of vitellogenesis. Generally, fish in early development had ovaries with 50-90% of the oocytes smaller than 100 µm in diameter. The major mode was between 50 and 100 µm, but most of these fish had a small proportion of oocytes greater than 250 µm. Many fish showed signs of an additional minor mode of oocyte sizes, particularly in the 250-400 µm range. The mean GSI of fish in this group was 2.11 (S.E.=0.18). The mean number of oocytes per gram of ovarian tissue was 4.26×10^6 (S.E.=1.8×10^5), with a mean number of oocytes per female of 1.10×10^8 (S.E.=1.3×10^7).

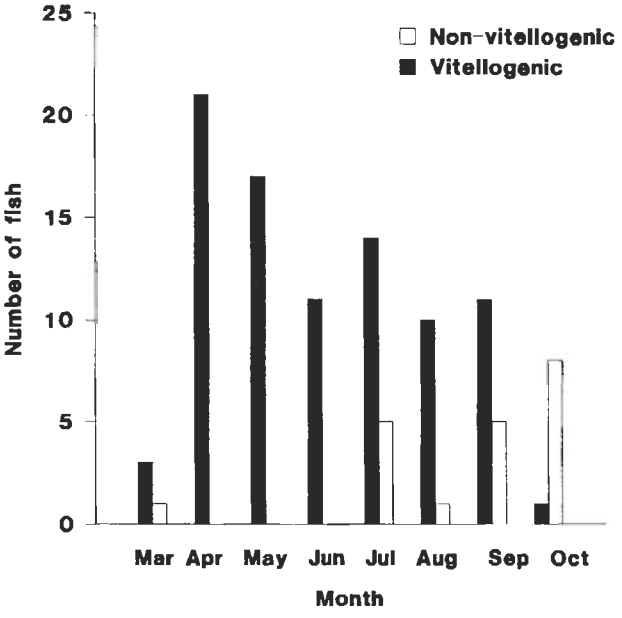


Figure 2. Monthly pattern of vitellogenic and non-vitellogenic female cobia, *Rachycentron canadum*, ≥ 834 mm FL. Figure represents a composite of years 1989-1990.

Not all fish could be correctly classified by their vitellogenic activity solely on the basis of oocyte size-frequency distributions. Seven fish which were initially classified as having undeveloped ovaries on the basis of oocyte frequencies were determined to be undergoing vitellogenesis based upon histological examination. No fish with their largest oocytes less than 150 µm were found to be developing. However, fish with their largest oocytes between 150 µm and 250 µm were either non-vitellogenic or undergoing vitellogenesis. All fish with at least one oocyte larger than 250 µm possessed oocytes with accumulated vitellin. Some of these fish could have represented post-spawning fish with residual vitellogenic oocytes.

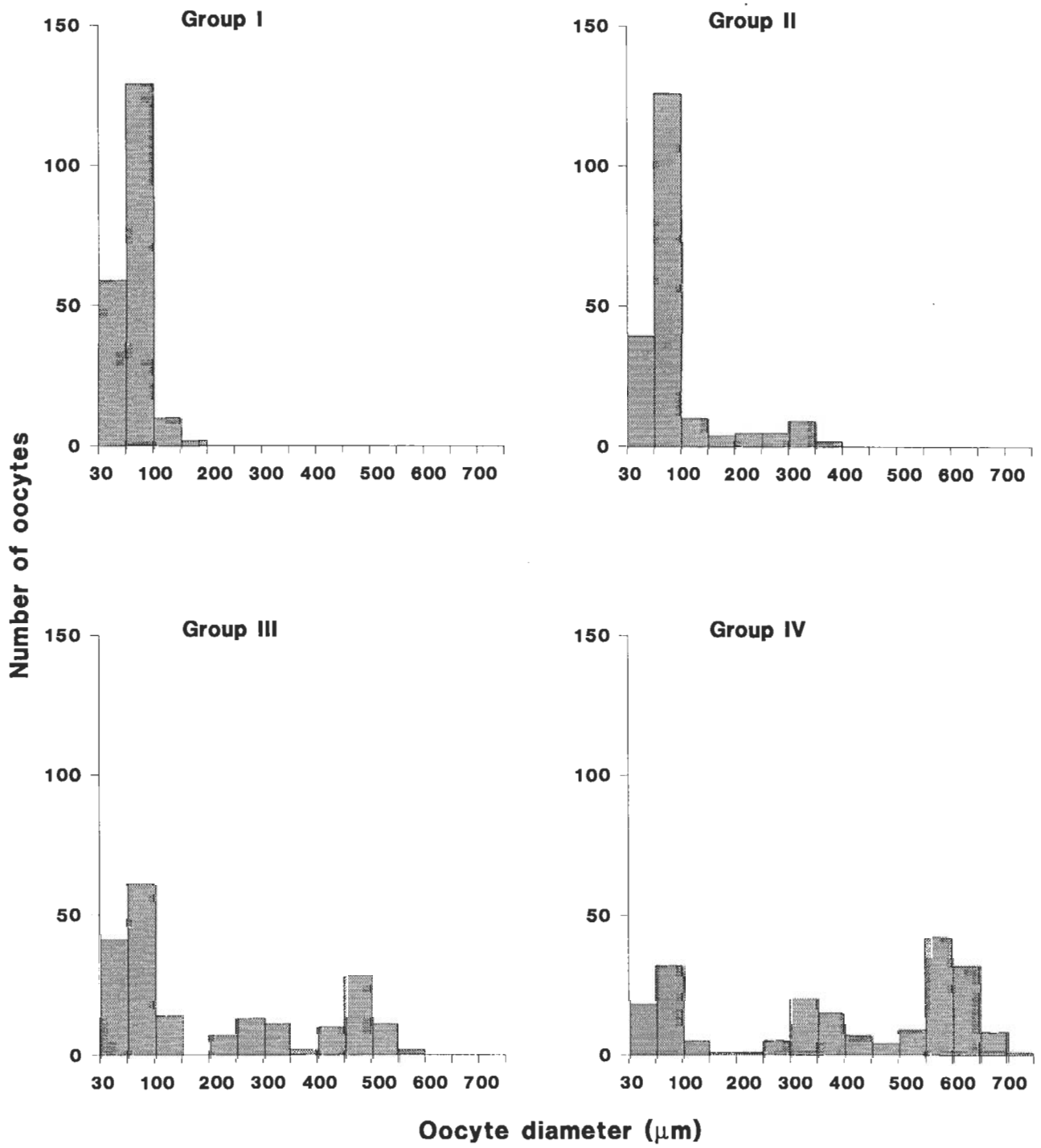


Figure 3. Oocyte size-frequency distributions of cobia, *Rachycentron canadum*. Group I: undeveloped ovary, Group II: early developing ovary, Group III: mid-developing ovary, Group IV: late developing ovary. Only oocytes 30 µm or greater were included in the groups.

Group III, Mid-Developing. Figure 3 depicts the oocyte size-frequency distribution of a fish belonging to the mid-developing group. Fish in this group exhibited oocyte-diameter distributions with a major mode at 50-100 µm. A distinct second mode was recognized at 400-450 µm or 450-500 µm. Some fish in this group displayed a third mode in the 250-400 µm range. The mean GSI of these females was 4.20 (S.E.=0.29). The mean oocyte density was 2.21×10^6 oocytes per gram (S.E.=5.40×10^5), with a mean number of oocytes per female of 1.47×10^8 (S.E.=2.47×10^7).

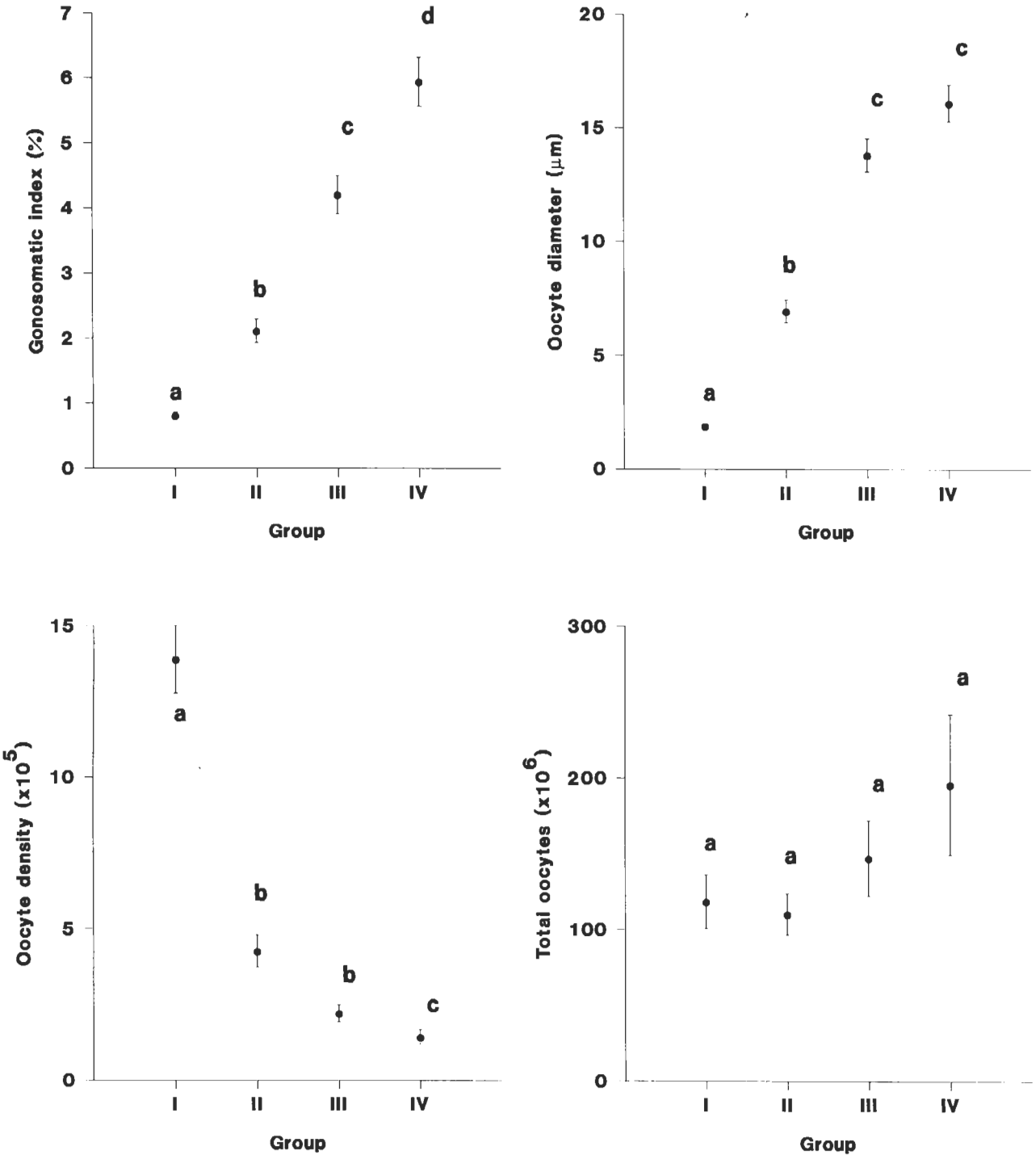


Figure 4. Comparisons of GSI, oocyte diameter, oocyte density and total number of oocytes among the four stages (groups) of ovarian development in cobia, *Rachycentron canadum* (means ± 1 standard error of the mean). Groups I-IV are as in Fig. 3. Means which share a letter are not significantly different from one another.

Group IV, Late Developing. The late developing group of fish possessed the most well-developed ovaries and were considered to be close to spawning (Figure 3). Twenty-three fish were placed in this group. The frequency distribution was distinctly bi-modal or tri-modal, with the most advanced mode in the 500 to 650 µm range. No running-ripe females or fish with hydrated oocytes were observed during our study. The mean GSI of these fish was 5.94 (S.E.=0.38), with a mean oocyte density of 1.40×10^6 (S.E.=2.34×10^5) per gram of ovarian tissue. The mean number of oocytes per female was 1.96×10^8 (S.E.=4.64×10^7).

A comparison of the four groups shows the relative size of the gonads, as a proportion of body weight (GSI) of female fish, increased as maturation proceeded (Figure 4). The differences among the four groups were statistically significant ($p<0.05$). Figure 4 further illustrates that the mean diameter of oocytes increased as maturation proceeded. The differences were significant between undeveloped (Group I) and early developing (Group II) ovaries, as well as between early (Group II) and mid-developing (Group III) ovaries, but the differences between mid-developing (Group III) and late developing (Group IV) ovaries were not statistically different. The density of oocytes per gram of ovarian tissue decreased as maturation proceeded and oocyte size increased (Figure 4). However, as Figure 4 indicates, there were no statistically significant differences among the groups in total number of oocytes per adult fish. Therefore, as maturation proceeded, there was little recruitment of new oocytes, and the size of the gonads increased to accommodate the increasing size of vitellogenic oocytes.

Histology

From some initial histological material not used for other analyses, we compared developmental stages in the left and right gonad of nine females and one male and found no significant differences in the stages of an individual. From four of those female fish, representing individuals with different stages of oocyte development, we examined histological sections from the anterior, middle and posterior of both ovaries and found no significant differences in the presence of stages among those sites. Each ovary contained previtellogenic and one or more vitellogenic stages, occasionally with a somewhat patchy distribution of oocytes in specific stages. In the case of the mature male, the tubules were especially filled with spermatozoa in the central portion. The walls of the efferent ducts contained more ripening germinal cysts with early developing stages in the periphery of the middle section of the testes than in those at either end. In summary, any section from a gonad provides a good indication of its developmental stage.

Figures 5-18 illustrate the developmental stages and features in cobia ovaries, and Figures 19-24 show those in cobia testes. Of the gonads of 94 females and 49 males examined histologically, those of 14 females and 9 males were from fish 860 mm FL or less. Four of the females and two of the males were fish caught in the Gulf off the Florida Keys 18-19 January 1991; two of those females and one male fit into the <860 mm FL category.

Some females were ripe May through September. Atretic oocytes occurred from July through October in nine fish from southeast Louisiana to northwest Florida, and degenerating hydrated oocytes occurred in three fish in July and October from the same location. However, a few fish in mid-June through July had ovaries in the resting state (containing both Group I and atretic oocytes), indicating they would produce eggs again because all females in August and September had high numbers of well-developed eggs. All four fish collected in January from the Florida Keys had atretic oocytes and degenerating hydrated oocytes.

Size at Maturity

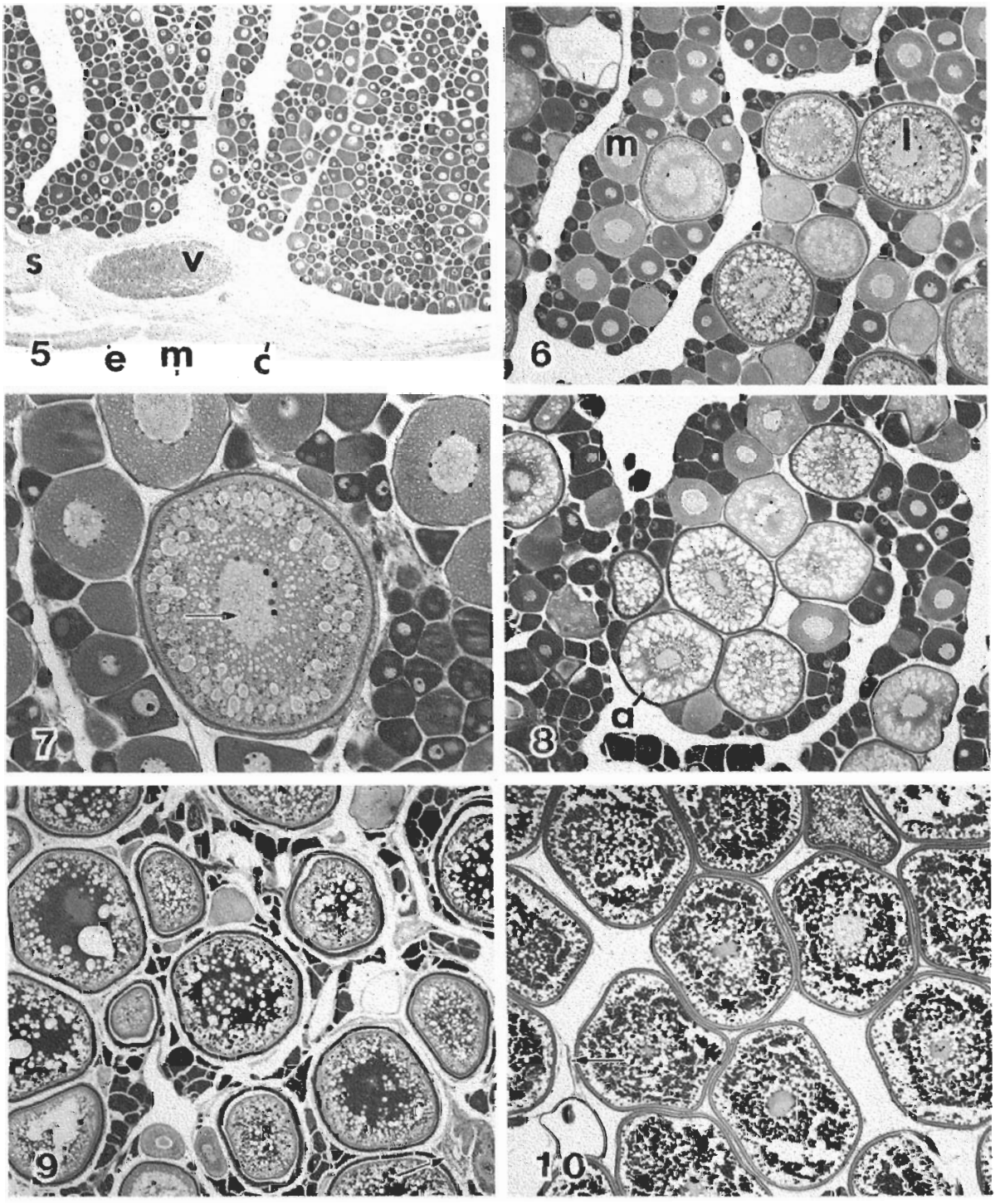
The smallest female exhibiting developing oocytes measured 834 mm FL and was determined to be 2 years old on the basis of otolith evaluation. Histologically, this was the smallest female fish with oocytes with a zona radiata and exhibiting vitellogenesis. Nine females (640 to 860 mm FL) examined histologically did not have developing oocytes. The smallest male exhibiting evidence of spermatogenesis was 640 mm FL. This particular male, 1 year old based on otolith evaluation, was not undergoing spermatogenesis although the tubules were filled with sperm. Actual onset of spermatogenesis may have occurred when this and other fish were smaller than 640 mm FL because no smaller male was examined histologically.

Spawning

The bi- and tri-modal oocyte size-frequency distributions observed for Groups III and IV (Figure 3) suggest that oocytes continued to be matured throughout the spawning season; however, the exact number of spawns and spawning frequency could not be estimated from these data.

The size of a batch spawn was estimated from the group of oocytes around the most advanced mode of oocytes in the 23 fish in the late developing group. The proportion of oocytes which were represented by the most advanced batch of oocytes ranged from 11 to 60% (mean = 28%; S.E.=3%). The estimated batch fecundity ranged from 2.6×10^6 to 1.91×10^8 oocytes (Mean=4.8×10^7; S.E.=9.8×10^6).

Among spawning fish, larger fish produced larger spawns. This is depicted by the significant positive linear relationship between the logarithm of batch fecundity and fork length and between the logarithm of batch fecundity and fish weight as measured for fish belonging to the late developing group (Figs. 25a, 25b). In addition, spawning fish with larger ovaries produce larger spawns. Figures 25c and 25d illustrate the significant linear relationship between the logarithm of batch fecundity and GSI, and between the logarithm of batch fecundity and total gonad weight of fish in late development.

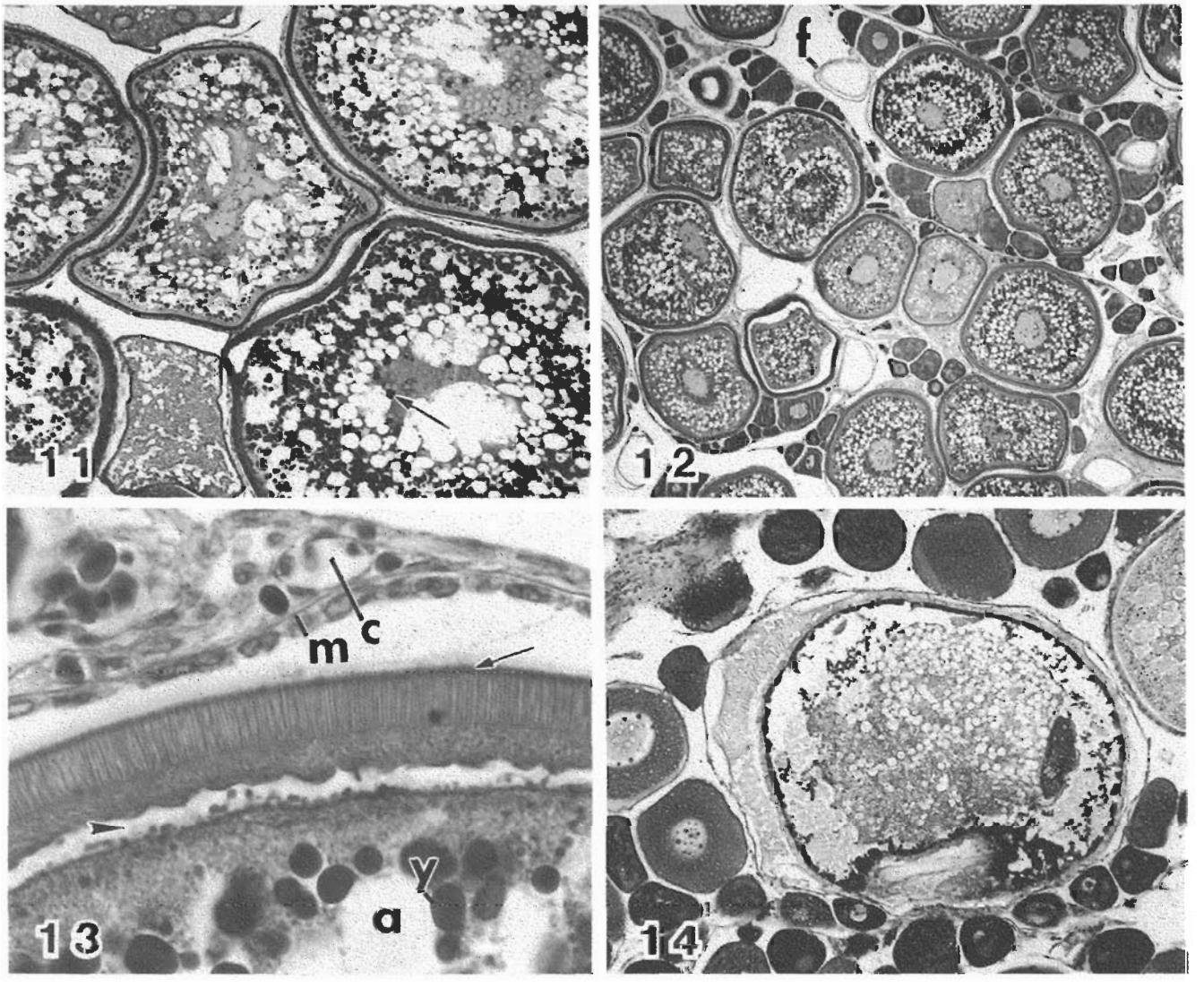


Figures 5-10. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers included in all figure legends that precede fish data are slide numbers. 5. Ovigerous lamella of immature fish lined by an ovary wall (tunica albuginea). Note mesovarium (m) and thin outer squamous epithelium (e) bordering collagen layer (c) and thick layer of smooth muscle (s) containing large blood vessel (v) (2703, July, 710 mm FL, 3.4 kg, 18.6 gm ovaries, 1 year old). 6. Lamellae of early ripening ovary showing medium-sized (m) oocyte starting vitellogenesis (small cortical alveoli and peripheral nucleoli extruding from nucleus into cytoplasm) and relatively large (l) later staged oocytes among various sized oocytes and small oogonia (2671, April, 1000 mm FL, 12.4 kg, 92.0 gm ovaries, 2 years old). 7. Close-up of same ovary. Note lampbrush chromosomes (arrow) in nucleus of more developed oocytes. 8. Ovary in similar stage as that shown in Fig. 7 but with more extensive cortical alveoli (a) (2672, April, 1230 mm FL, 465.0 gm ovaries). 9. Ripe ovary with nuclei beginning to migrate marginally. Note postovulatory follicle (arrow) of released egg (2716, August, 1320 mm FL, 566.6 gm ovaries, 4 years old). 10. Ripe ovary similar to that in Fig. 9 but with few oocytes in early stages. Note ovigerous fold covered by squamous epithelium (arrow) (2839, August, 920 mm FL, 330.0 gm ovaries).

DISCUSSION

Our study suggests, as did those of Thompson et al. (1992) and Biesiot et al. (1994), that the reproductive season for cobia in the northcentral Gulf of Mexico is protracted and extends from April through early October with greatest activity occurring in the spring and early summer. This also parallels the reproductive activity of other Gulf of Mexico coastal pelagic fishes such as *Scomberomorus maculatus*, the spanish mackerel (Finucane and Collins 1986) and *Scomberomorus cavalla*, the king mackerel (Finucane et al. 1986).

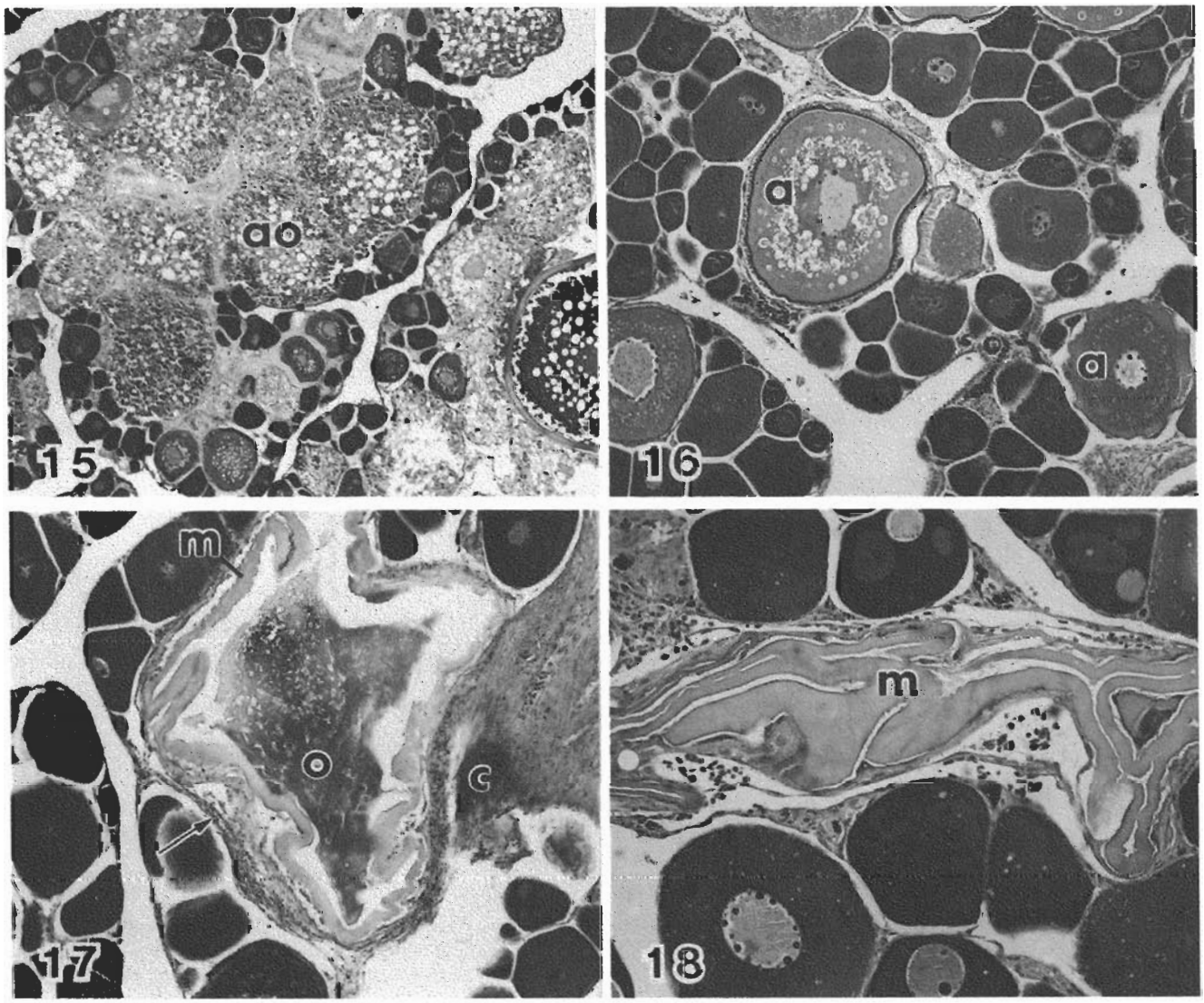
Richards (1967) reported the smallest mature female he examined from Chesapeake Bay was 696 mm FL, which is 138 mm shorter than the smallest mature female we observed. This discrepancy may reflect a slower growth rate for cobia in the cooler waters of the Chesapeake Bay area, rather than a regional difference in the age at maturation. Based upon scale



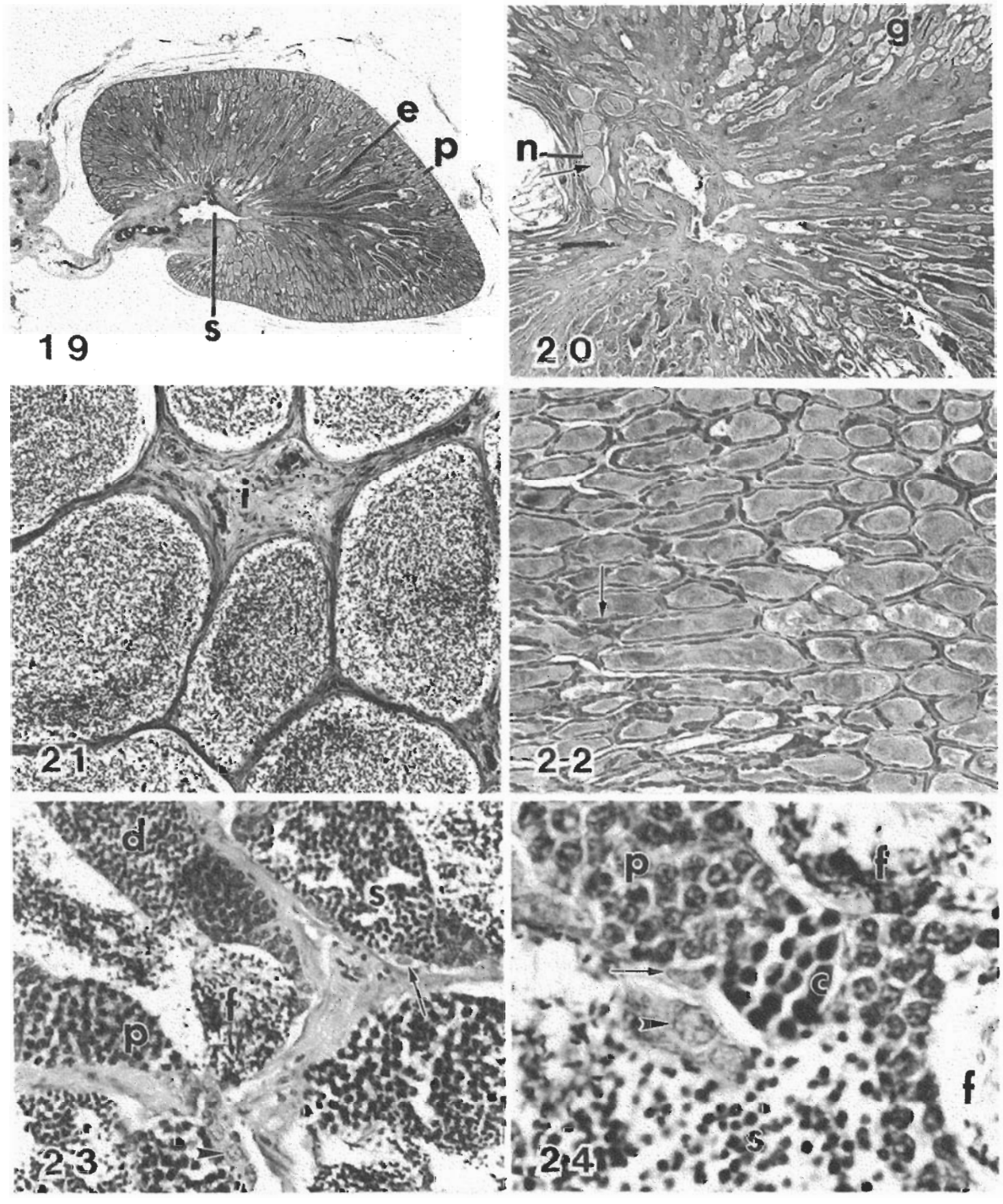
Figures 11-14. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 11. Ripe ovary at beginning of spawning period showing ripe non-hydrated oocytes with irregularly shaped nuclei with extruding nucleoli (arrow) (2670, April, 1225 mm FL, 21.5 kg, 772.7 gm ovaries, 3 years old). 12. Ripe ovary in period between two apparent major periods of spawning. Note numerous empty follicles (f) (2700, July, 1231 mm FL, 21.8 kg, 584.6 gm ovaries, 3 years old). 13. Close-up of oocyte with the striated zona pellucida (zona radiata) separating (arrowhead) from oocyte cytoplasm. Note follicular wall consisting of outer granulosa containing lipid droplets and inner zona pellucida consisting of darker thin outer layer (arrow) and thick pale inner layer. The separated peripheral cytoplasm of the oocyte contains darkly staining yolk droplets (y) and clear cortical alveoli (a). External to the granulosa and divided by a conspicuous basement membrane (m) is the theca externa containing capillaries (c) (2700, July, 1231 mm FL, 21.8 kg, 584.6 gm ovaries, 3 years old). 14. Atretic oocyte in ovary of fish before initial spawning period. Note the degenerated marginal nucleus (2671, April, 1000 mm FL, 12.4 kg, 92.0 gm ovaries, 2 years old).

annuli, Richards (1967) surmised that females of 700 mm FL were 2 years old (in their third year of life). In the Gulf of Mexico, it is unlikely that a 700 mm FL female would be 2 years old, since 2-year-old females examined in this study averaged 850 mm FL (Franks et al. 1991a). Thus, 700 mm FL mature females collected in Chesapeake Bay by Richards (1967) may have been the same age as fish measuring about 850 mm FL in our study. The smallest mature male we found was 640 mm FL (age 1). Richards (1967) reported earliest maturity in males at 518 mm FL and age 1. Apparently males can mature when they are 1 year old, whereas females are not mature until 2 years of age.

The results of our study support Richards' (1967) suggestion that cobia spawn more than once during the spawning season. Richards (1967) reached his conclusion on the basis of finding fish with partially spent ovaries. We reached our conclusion because we observed group-synchronous oocyte maturation in fish collected during the spawning season, characterized by the presence of at least two distinct size groups of oocytes that had undergone vitellogenesis in the same ovary as well as postovulatory follicles (empty follicles) and atretic hydrated eggs in a few ovaries from July through October (and in January from the



Figures 15-18. Sectioned ovarian tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 15. Ovary of different fish than in Fig. 12 but during same interspawning period, showing a resting ovary with an abundance of clusters of atretic oocytes (ao) (2695, July, 944 mm FL, 8.7 kg, 171.1 gm ovaries, 2 years old). 16. Early phases of atresia (a) of some oocytes in ovary of post-spawned fish after end of spawning (2723, September, 940 mm FL, 9.1 kg, 60.9 gm ovaries, 2 years old). 17. Degenerating hydrated egg in post-spawned resting ovary, showing fibrous capsule (c) of atretic follicle containing the hydrated oocyte (o) with its membrane (m) and containing an abundance of inflammatory macrophages and fibrocytes (arrow) (2731, October, 1110 mm FL, 141.2 gm ovaries, 3 years old). 18. Resting ovary of fish in winter with atretic follicle containing hydrated egg. Note homogeneous egg membrane (m) (2826, January, 991 mm FL, 10.9 kg, 120.0 gm ovaries, 2 years old).



Figures 19-24. Sectioned testicular tissue from cobia, *Rachycentron canadum*. Numbers preceding fish data are slide numbers. 19. Cross-section through area of ripe testis containing spermatic duct (s), radiating efferent ducts (e) filled with spermatozoa, and peripheral tubules (p) (2708, July, 640 mm FL, 2.4 kg, 5.8 gm testes, 1 year old). 20. Close-up of mature testis showing engorged efferent ducts and associated nerve bundles. Note enveloped capillaries (arrow) within some bundles (n) (2317, 960 mm FL, 8.2 kg, 92 gm testes). 21. Cross-section of mature tubules no longer lined with germinal cysts. Note interstitial tissue (i) among tubules containing different aspects of capillaries (2708, July, 640 mm FL, 2.4 kg, 5.8 gm testes, 1 year old). 22. Cross-and tangential-sections through tubules in developing testis. Note germinal cysts (arrow) lining sperm-filled tubules (2689, 1330 mm FL, 28.8 kg, 1192.3 gm testes, 7 years old). 23. Germinal cysts in various developmental stages in tubules surrounded by interstitial tissue associated with capillaries and Sertoli cells (arrow). Note spermatogonia (arrowhead), relatively large primary spermatocytes (p), relatively small secondary spermatocytes (s), smaller spermatids (d), and small spermatozoa (f) both in the cysts and in the lumen (2691, May, 970 mm FL, 12.4 kg, 290.5 gm testes, 4 years old). 24. Higher power showing spermatogonia (arrowhead), Sertoli cell (arrow), vacuolated primary spermatocytes (p), primary spermatocytes dividing into secondary spermatocytes (c), secondary spermatocytes (s), and spermatozoa with their streaming flagella (f) (2687, May, 1260 mm FL, 29.1 kg, 795.2 gm testes, 9 years old).

Florida Keys). However, the GSI values we obtained, along with those of Biesiot et al. (1994) and Thompson et al. (1992) during most years, did not support the late summer spawning activity.

Our estimates of batch fecundity are considerably larger than Richards' (1967) estimates of total fecundity. We estimated the size of a batch spawn to be between 2.6×10^6 and 1.91×10^8 eggs, with an average of 4.8×10^7 eggs per batch. Richards (1967) estimated total fecundity to be from 2×10^6 to 5×10^6 eggs per female, based on the total number of oocytes greater than 500 µm in diameter in the ovaries of six cobia. In our study, we used Gilson's fluid rather than formalin as in Richards' study (1967). Since we determined that oocytes shrink 11% more in Gilson's than

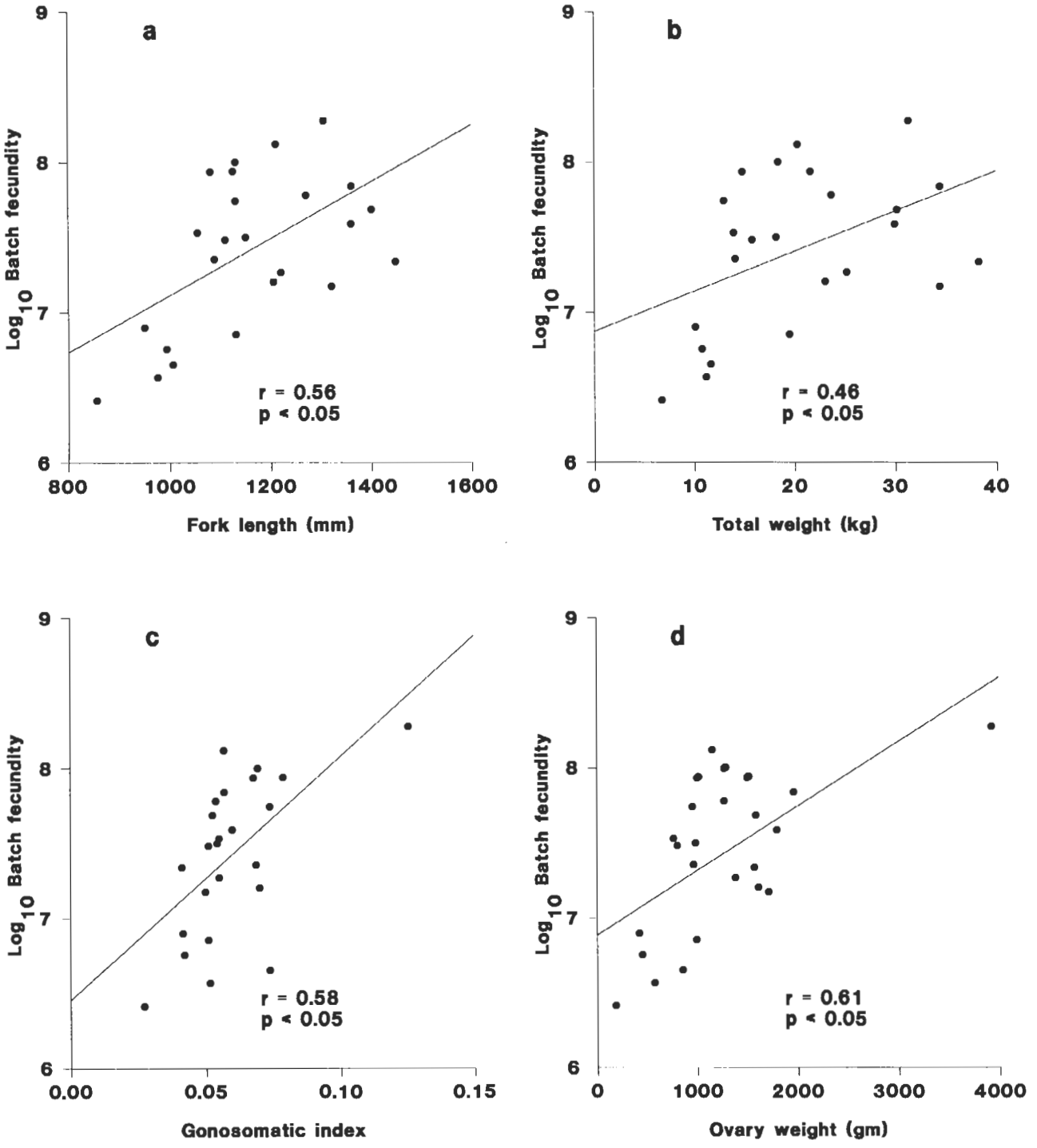


Figure 25. Relationship between batch fecundity and fork length (a), total weight (b), GSI (c) and ovary weight (d) for female cobia, *Rachycentron canadum*.

in formalin, we would probably have counted the same oocytes as Richards if we only consider eggs greater than 550 µm across. In late developing fish, oocytes greater than 550 µm generally constituted those used for our batch fecundity estimates. Those estimates are based on the advanced model group of developing oocytes and could overestimate fecundity if all eggs in the group are not released.

It is not straightforward to estimate the total seasonal fecundity of fish that spawn more than once per season without knowledge of the number of spawns per season. Although nonsynchronous formation of oocytes in the ovaries was observed, presenting strong evidence for multiple spawning (Hunter et al. 1992), we were unable to calculate spawning frequency (Hunter and Goldberg 1980; Hunter and Macewicz 1985; Hunter et al. 1985) because of the lack of recently hydrated oocytes and the relatively small sample sizes of fish from a single location over a one-year period. There appear to be some yearly variations in spawning, probably controlled in part by year-to-year temperature and locality fluctuations. Even though we never observed more than two advanced modes of developing oocytes over 30 µm in an ovary at one time by measurement, we are unable to conclude that cobia only spawn twice in a season.

This study showed that the total number of oocytes remained nearly constant as fish matured through the four gonadal developmental stages and spawned. In addition, the histological data and the oocyte size-frequency distributions indicated that some fish were close to spawning throughout the protracted spawning season. Therefore, it is assumed that recruitment of primary oocytes throughout the reproductive season and possibly continual transformation of primary oocytes into vitellogenic oocytes occurred throughout most of the reproductive season.

Whether cobia spawn during the day or at night is not well understood. Ditty and Shaw (1992) postulated that cobia spawn during the day because all larvae (N=74) examined from the Gulf, with one exception, were in similar late stages of development when collected during mid-morning. Behavior believed to be daytime spawning of cobia was observed off Panama City, Florida (Shaffer and Nakamura 1989). In the present study, we observed no fish with recently hydrated eggs, even though all examined fish were captured (by hook-and-line) during daylight hours. One explanation for the lack of fish with hydrated eggs in our study is that spawning cobia do not feed, as suggested by Richards (1967), and thus are not subject to capture by baited hook., Another explanation is that at least some cobia spawn at night. Some cobia may spawn far offshore as suggested by the abundance of eggs found in the Gulf Stream offshore from North Carolina (Hassler and Rainville 1975) and by reported observations of cobia spawning approximately 80 km off the South Carolina coast (Shaffer and Nakamura 1989). Cobia examined in our study were caught near mainland and barrier island beaches, in ship channels, over shallow water wrecks, and at petroleum structures located no further than 40 km offshore.

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