OOCYTE GROWTH AND DEVELOPMENT IN THE STRIPED MULLET, *MUGIL CEPHALUS*, DURING SEASONAL OVARIAN RECRUDESCENCE: RELATIONSHIP TO FECUNDITY AND SIZE AT MATURITY

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ABSTRACT

Oocyte growth and development in the striped mullet, *Mugil cephalus*, were examined during the period of rapid ovarian recrudescence that precedes spawning in coastal waters of northeast Florida. Based on the de novo appearance of yolk proteins detected by polyacrylamide gel electrophoresis, the oocyte size corresponding to the onset of vitellogenic growth was determined to be 0.18 mm (diameter). Through in vitro studies of oocyte responsiveness to steroid stimulation of meiotic maturation, the minimum prematuration oocyte size was determined to be 0.60 mm; largest prematuration oocytes collected during the study were 0.72 mm. Females with vitellogenic oocytes were first collected in Matanzas Inlet in late September. Females with prematurational oocytes were first observed in mid-October. Minimum size at sexual maturity for female striped mullet in northeast Florida ranged from 23 to 27 cm SL.

Oocyte size-frequency profiles led to the development of a staging system for striped mullet ovaries that can be related to simpler measurements of reproductive condition such as the gonadosomatic index and the largest oocyte diameter. According to this system, females with prespawning ovaries first appeared in Matanzas Inlet during mid-October, then disappeared from the Inlet in either mid-December (1985-86 season) or mid-January (1984-85 season). Females with ovaries in spawning condition were not observed in the Inlet during the 2 years of this study, supporting the commonly held assumption of offshore spawning. A few females with postspawn ovaries were collected as early as late November.

The potential fecundity of the striped mullet in northeast Florida was calculated from the size of the single clutch of developing oocytes, and related to both body weight and standard length.

The striped mullet, *Mugil cephalus*, is a euryhaline teleost with a nearly worldwide distribution in marine and estuarine waters. Economically important as a food and bait fish in many areas, the world commercial catch of mullet from 1979 to 1983 averaged nearly 185,000 t (metric tons) per year (FAO 1984). During this time, a yearly average of almost 14,000 t (nearly 8% of the world catch) were caught in the United States, more than in any other single country. Most were caught in the state of Florida, for an average of almost 12,000 t yearly in 1981 and 1982 (National Marine Fisheries Service preliminary data, in Comp and Seaman 1985), equivalent to 87% of the United States and 6% of the worldwide catch. In addition to being the basis of large natural fisheries, *M. cephalus* has been reared for aquacultural purposes in brackish and freshwater ponds (Bromhall 1954; Thomson 1966; Pien and Liao 1975) and has been the subject of induced breeding in the laboratory (Kuo et al. 1973, 1974a, b; Kuo 1982). General information on the biology of *M. cephalus* and related species of mullet can be found in Anderson (1958), Stenger (1959), and Thomson (1966).

Striped mullet have one breeding cycle per year lasting from 2 to 5 months depending on the location (Jacot 1920; Breder 1940; Bromhall 1954; Anderson 1958; Arnold and Thompson 1958; Stenger 1959; Tang 1964; Zhitenev et al. 1974; Pien and Liao 1975; Timoshek and Shilenkova 1975; Finucane et al. 1978; Apekin and Vilenskaya 1979; Azoury and Eckstein 1980; Chubb et al. 1981; Dindo and MacGregor 1981). In coastal waters of the southeast United States, spawning has been reported to occur from October through February as determined from the time of appearance and size of larvae and fry (Anderson 1958; Arnold and Thompson 1958), from the presence of migrating mullet with "developing" ovaries (Breder 1940; Arnold and Thompson 1958; Stenger 1959), and by monthly gonadosomatic index (GSI) changes (Dindo and MacGregor 1981).

In view of the extensive interest in the mullet, it

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is somewhat surprising that so little information concerning its reproduction, other than the seasonal spawning time, is available. For instance, little attention has been paid to the dynamics of oocyte development and ovarian recrudescence in natural striped mullet populations. A staging system for the ovary itself that would simply and meaningfully represent the extent of ovarian recrudescence in this species is lacking. And, although there have been numerous references to size of age at sexual maturity (Jacot 1920; Stenger 1959; Timoshek 1973; Apekin and Vilenskaya 1979), these are generally not based on comprehensive sampling. Similarly, although there have been equally numerous reports of striped mullet fecundity (Nash et al. 1973; Pien and Liao 1975; review by Alvarez-Lajonchere 1982), most derive from counts of eggs (or oocytes) in only a very limited number of fish, while body size-related data are virtually nonexistent.

We recently initiated several studies dealing with the reproduction of *M. cephalus* in coastal waters of northeast Florida and related topics. The specific purposes of this investigation include describing the patterns of oocyte growth during seasonal ovarian recrudescence, developing an ovarian staging system based on these patterns, and providing definitive determinations of both the size at maturity and the size-specific fecundity of female striped mullet in the area.

MATERIALS AND METHODS

Fish

Female striped mullet approximately 18 cm standard length (SL) or larger were captured by cast net from the Matanzas River Inlet south of St. Augustine, FL. Collections were made periodically from October 1984 through January 1985, and again from August 1985 through January 1986, for a total sample of 340 fish. Standard length and fork length (FL) were determined to the nearest 0.1 cm, and body weight (BW) to the nearest 0.1 g for all fish. Ovaries of most (248) fish ≥20 cm SL were removed and transferred to a buffered salt solution (FO: Wallace and Selman 1978), and any adhering non-ovarian tissue was removed. Whole ovaries were then patted dry and weighed to the nearest 0.01 g. The GSI for each fish was calculated as GSI = (ovary weight/body weight) × 100.

Oocyte Size-Frequency Profiles

Oocyte size-frequency profiles were constructed for each fish in the following manner. A representative (see below) piece was gently teased free from the middle of each newly collected ovary, patted dry, weighed to the nearest 0.1 mg, and placed in a petri dish containing FO solution. Pieces weighed from 1 to 9 mg and contained from 100 to 500 oocytes >0.10 mm in diameter. Individual follicle-enclosed oocytes were manually measured to the nearest 0.02 mm with an optical micrometer mounted on a dissecting microscope. Oocyte size-frequency profiles were not determined for fish with largest oocyte diameters (LODs) <0.10 mm, although their LODs were noted.

Profiles and LODs derived from a sample of ovary can be considered representative because oocyte development is known to occur uniformly throughout the mullet ovary (Ochiai and Umeda 1969; Shehadeh et al. 1973; Timoshek and Shilenkova 1975).

Oocyte Stages

The oocyte size at which vitellogenesis (the period of protein yolk accumulation) begins in the striped mullet was determined by the appearance of specific yolk protein bands in oocyte homogenates subjected to polyacrylamide gel electrophoresis (see Greeley et al. 1986b). Groups of small oocytes with mean diameters of 0.14, 0.16, 0.18, and 0.20 mm were isolated from surrounding ovarian tissue, homogenized in a sodium dodecyl sulfate (SDS) containing buffer solution, and heated at 100°C for 5 minutes. Samples were loaded onto a 0.75 mm thick polyacrylamide gradient gel (gradient: 3.5-20.4%) and were electrophoresed in SDS buffer with a constant applied current of 30 mAmps until the bromophenol blue marker migrated from the gel. Protein fixation, visualization with Coomassie blue, and molecular weight determinations were conducted as in Wallace and Selman (1985). Biochemicals and reagents were highest available grades from Sigma Chemical Company[3] and Bio-Rad Laboratories.

The minimum oocyte size competent to resume meiotic maturation (leading to the development of a mature fertilizable egg) in response to steroid hormone stimulation was determined in vitro by the methods of Greeley et al. (1986a). Larger follicle-enclosed oocytes were isolated in FO and assigned to one of four pools with mean diameters of 0.52, 0.56, 0.60, and 0.64 mm. Oocytes from each pool were randomly subdivided into treatment (steroid

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

added) and control groups, and then transferred into separate 35 × 10 mm petri dishes containing 3 mL of a 75% L-15 culture medium (Sigma) at pH 7.5. A solution containing 3 μg of 17α-hydroxy-20β-dihydroprogesterone in 10 μL of 95% ethanol was added to each treatment dish (for a final steroid concentration of 1 μg/mL); 10 μL of 95% ethanol was added to each control dish. Oocytes were incubated at 20°C for 48 hours and then examined for germinal vesicle breakdown (GVBD), which was easily noted by the absence of the germinal vesicle (GV) or nucleus in the highly transparent postmaturation oocyte, as an indicator of the resumption of meiotic maturation in response to the hormone.

Fecundity

Potential fecundity was estimated for each fish whose oocyte size-frequency profile demonstrated that recruitment of oocytes into vitellogenesis had ceased as potential fecundity = [(number of recruited oocytes in ovary piece) × (weight of whole ovary)]/(piece weight).

RESULTS

Gonadosomatic Index (GSI)

Ovarian recrudescence in the striped mullet occurs during autumn and early winter in coastal waters of northeast Florida, as indicated by seasonal changes in the GSIs of females collected from the Matanzas River Inlet (hereafter referred as Inlet) during the 1984-85 and 1985-86 breeding seasons (Fig. 1). In August of 1985, the earliest month of collections for this study, GSIs were <1 in all sampled fish. Ovaries represented by these low GSIs were quite small and were either translucent and colorless (smallest and least developed ovaries) or opaque with a red hue resulting from extensive vascularization (slightly larger and more developed ovaries). Similar low GSIs continued to be found in all females collected through mid-September.

In late September the GSIs of a portion of the collected females rose sharply, to nearly 9. The much larger ovaries represented by these high GSIs were

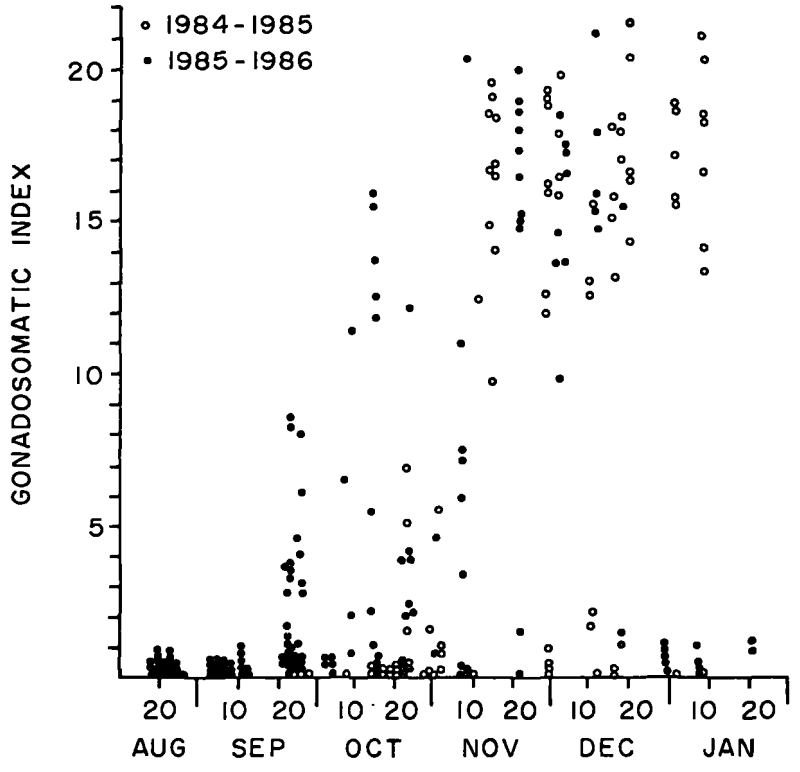

FIGURE 1.—Variation in the gonadosomatic index (GSI) of female *Mugil cephalus* ≥20 cm SL during prespawning ovarian recrudescence along the northeast Florida coast.

now yellow to golden in color because of a preponderance of yellow yolky oocytes in the ovarian lamellae. This initial phase of GSI increases continued through early November, at which time two distinct groupings of females within the population became apparent: those with GSIs remaining <2, and those with GSIs ranging between 10 and 21. Average GSIs in the latter group rose only slightly thereafter, ranging from 13 to 22 by early December. The lower GSIs of the rest of the population remained unchanged throughout the remainder of this study.

During the 1984-85 season, females with high GSIs continued to be caught until mid-January, at which time larger females (≥20 cm SL) apparently left the Inlet because none were caught during the 3 additional weeks of intensive collecting. In contrast, during the 1985-86 season, females with high GSIs were only collected through mid-December. Although a few mid-size females were collected through late January of this year after the expenditure of considerable collecting time and effort, all proved to have small GSIs.

Oocyte Stages

A primary purpose of this project was to document the oocyte growth and development that accompanied these GSI changes during prespawning ovarian recrudescence. Two oocyte development stages of primary interest to us were 1) the stage at which yolk accumulation or vitellogenesis begins, and 2) the stage during, or following, vitellogenic growth when the oocyte first becomes competent to resume meiotic maturation and thereby develop into a fertilizable egg.

We previously showed two large proteins at M_r = 104,000 and 90,000 to be the major yolk proteins present in mullet oocytes at the end of vitellogenic growth (Greeley et al. 1986b). As evidenced by the de novo appearance of these marker proteins (Fig. 2), vitellogenesis in the striped mullet begins in oocytes that are between 0.16 and 0.18 mm in diameter. In this study, oocytes 0.16 mm in diameter and smaller are thus considered to be previtellogenic, a broad classification including both primary growth- and yolk vesicle-stage oocytes as described

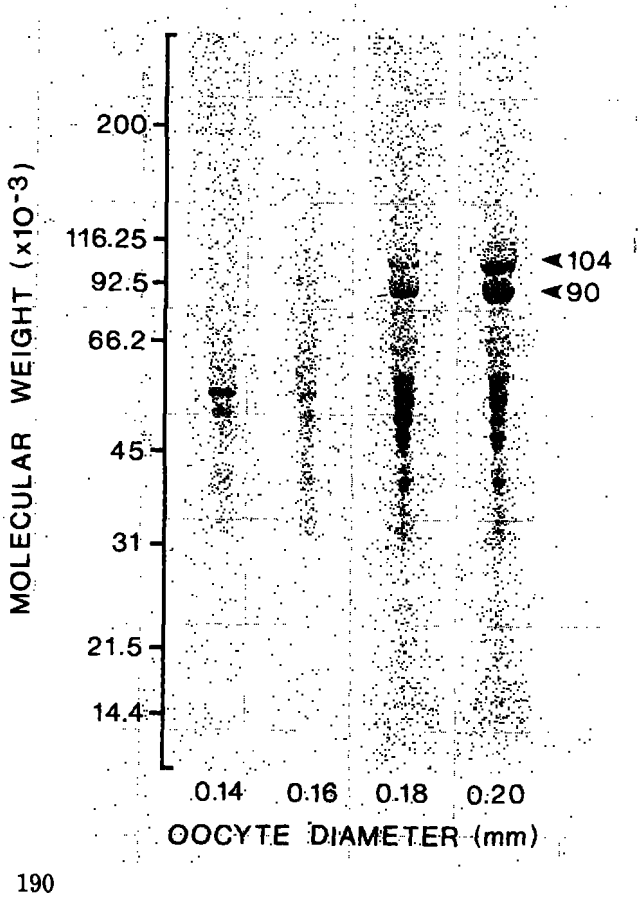

FIGURE 2.—Polyacrylamide gel electrophoresis of oocyte proteins indicating de novo appearance of yolk proteins in vitellogenic oocytes (0.18 mm in diameter) of the *Mugil cephalus*, with comparison to smaller previtellogenic and larger vitellogenic oocytes (see text). Molecular weight standards are indicated on the left for comparison; the molecular weights of the two major yolk proteins are shown on the right (arrows).

by Wallace and Selman (1981); oocytes 0.18 mm in diameter and larger are considered to be actively vitellogenic.

Late vitellogenic oocytes become competent to resume meiotic maturation and develop into a fertilizable egg in response to an in vitro steroid challenge at 0.60 mm in diameter, with larger oocytes being only marginally more responsive (Table 1). Therefore, oocytes 0.60 mm and larger that do not exhibit any signs of maturation such as yolk "clearing" or hydration are considered to be in a prematuration stage of development.

Striped mullet eggs following meiotic maturation are even larger (0.90 to 1.00 mm in diameter) (Abraham et al. 1966; Nash et al. 1973; Pien and Liao 1975; Finucane et al. 1978; Greeley et al. 1986b), nearly transparent (yolk has cleared), hydrated, float in full-strength seawater, and thus are easily distinguished from the smaller and more opaque prematuration oocytes. Females with mature eggs were not collected during this study.

TABLE 1.—Percentage of different-sized striped mullet oocytes undergoing germinal vesicle breakdown (GVBD) in vitro in response to treatment with 17α-hydroxy-20β-dihydroprogesterone (1 μg/mL) or an ethanol control.

Oocyte diameter (mm)	Treatment	N	(%)GVBD
0.52	control	55	0
	steroid	50	0
0.56	control	50	0
	steroid	45	0
0.60	control	326	0
	steroid	301	65
0.64	control	196	0
	steroid	198	71

Largest Oocyte Diameter (LOD)

The relationship of oocyte diameter to stages of oocyte development is clearly delineated in Figure 3. Three major developmental stages are shown: 1) previtellogenic growth, including both the pri-

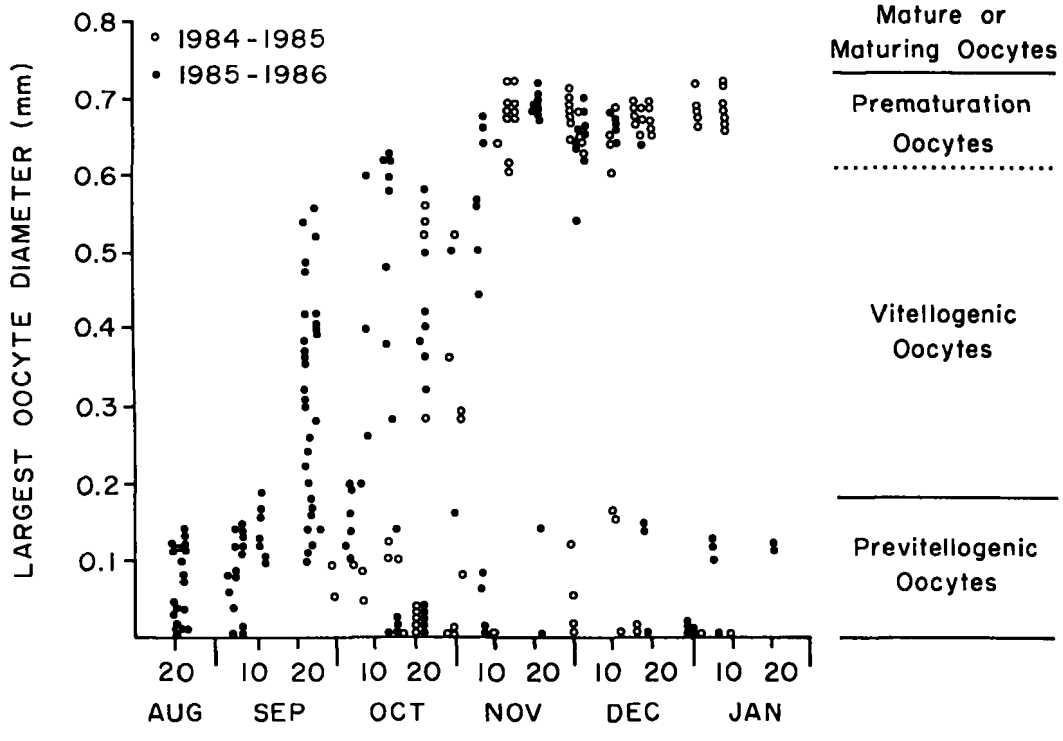

FIGURE 3.—Variation in the largest oocyte diameter (LOD) of female *Mugil cephalus* ≥20 cm SL during prespawning ovarian recrudescence along the northeast Florida coast. Presented on the right are oocyte developmental stages corresponding to these oocyte sizes. Broken line between vitellogenic and prematuration designations signifies uncertainty concerning whether the latter is actually a substage of the former. The category "previtellogenic" encompasses both primary growth and yolk vesicle oocytes.

mary growth and yolk vesicle phases as defined by Wallace and Selman (1981), 2) vitellogenic growth, and 3) maturation. An additional developmental category—prematuration—may or may not be considered a substage of late vitellogenesis; although oocyte growth appears to level off at this time, we have no direct evidence that the oocyte completely ceases to take up yolk precursors at this time.

Changes in the LOD (Fig. 3) during the period of seasonal ovarian recrudescence were similar to changes in the GSI. LODs were small in August and early September, representative of still previtellogenic oocytes. LODs then rose sharply in mid-September as vitellogenic oocytes began to appear in females captured in the Inlet. The range of LODs in the population at this time varied considerably, from <0.01 mm in some fish to nearly 0.56 mm in others. LODs continued to rise in one portion of the population through mid-November, before leveling off between 0.60 and 0.72 mm; in other females, LODs remained below 0.18 mm through the end of each study period. Females with high LODs were last collected in mid-December during the 1985-86 breeding season and in mid-January during the 1984-85 season.

Body Size at Maturity

An examination of the LODs as a function of standard length on a month to month basis (Fig. 4) reveals several body size-related trends that bear on the results presented in Figure 3. During August, the first month of the collections, there was little difference between the LODs of different-sized females, all being low and representative of previtellogenic oocytes. Only a few large striped mullet were collected during this month. This changed in September, with larger females over 32 cm SL becoming more prevalent in the Inlet and exhibiting a tendency to have higher LODs than smaller females. By October, smaller females (to a lower limit of 28 cm SL) also began to acquire vitellogenic oocytes, although their average LODs were still lower than those of larger females. During November, LODs of larger females leveled off at 0.60 to 0.72 mm, but a few smaller females (now to a lower limit of 26 cm SL) continued to have intermediate LODs indicative of oocytes in the early stages of vitellogenesis. By December and January, recruitment of smaller females into sexual maturity apparently ceased because LODs were now uniform—either greater than the 0.60 mm prematurational oocyte size or less than the minimum 0.18 mm vitellogenic size—in all females regardless of body size.

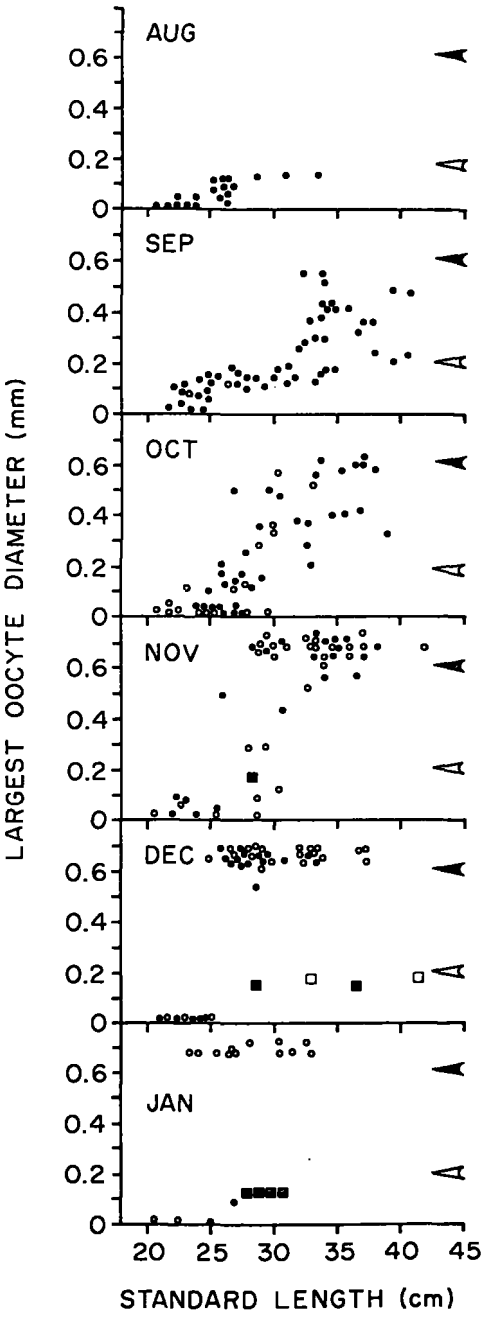

FIGURE 4.—Monthly variation in the relationship of the largest oocyte diameter (LOD) to standard length (SL) in female *Mugil cephalus* during prespawning ovarian recrudescence along the northeast Florida coast. Circles represent data points from prespawning females (open: 1984-85; closed 1985-86); squares represent data points from postspawn females (open: 1984-85; closed 1985-86). Open arrows indicate oocyte size at beginning of vitellogenesis; solid arrows indicate minimum prematuration oocyte size.

During November, December, and January, a few larger females with LODs less than the minimum 0.18 mm vitellogenic size were collected from the Inlet. This suggested that seasonal ovarian recrudescence does not occur in all large females every year. However, other criteria (see below) indicated that these were actually postspawn ovaries.

Minimum female size at maturity by the end of the season ranged from 23 to nearly 27 cm SL. Three size-categories of females can now be recognized: 1) those smaller than 23 cm SL that are obligatory immatures; 2) those 23 to 27 cm SL that may enter into maturity before the end of the spawning season; and 3) those larger than 27 cm SL that are always sexually mature sometime during the spawning season. The minimum body size at sexual maturity can also be expressed as a function of fork length (27 to 31 cm) or body weight (232 to 383 g) with the use of the equations provided in Figure 5.

Ovary Stages

LODs are useful criteria for following the course of ovarian recrudescence in the striped mullet and for determining the size or age of females at sexual maturity. Yet the LOD by itself cannot distinguish between sexually immature and postspawn females,

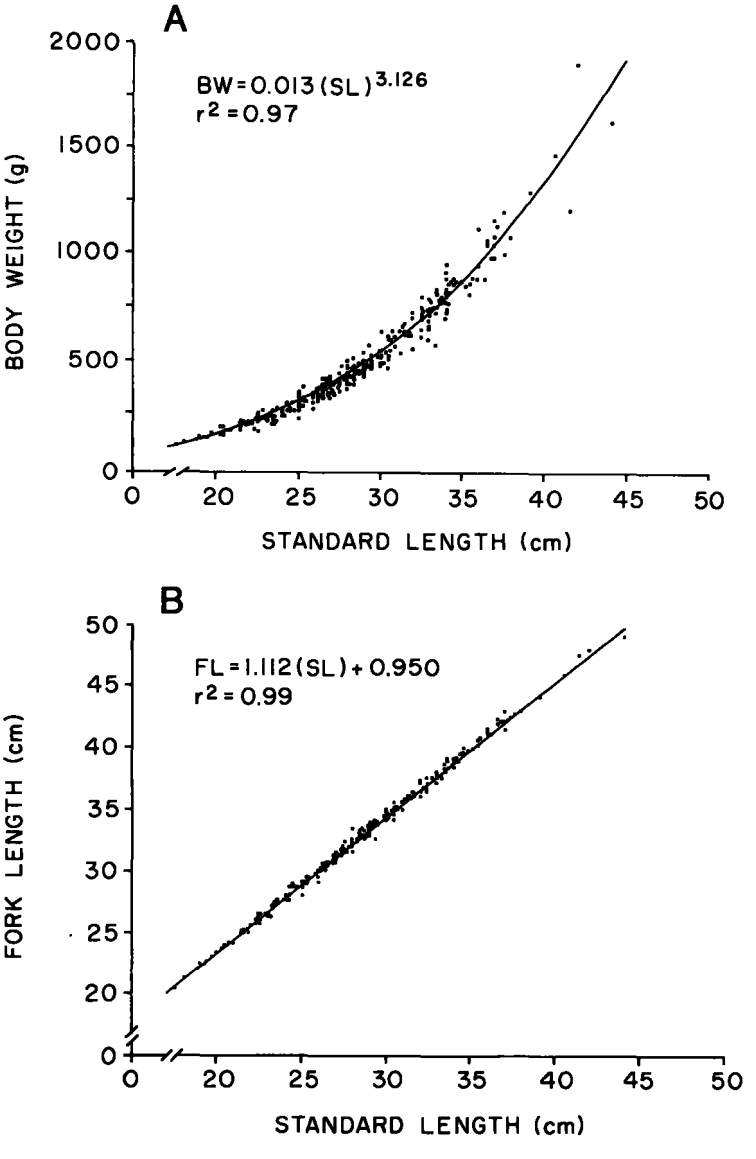

FIGURE 5.—Relationship of (A) body weight and (B) fork length to the standard length of female *Mugil cephalus* (≥20 cm SL) during prespawning ovarian recrudescence along the northeast Florida coast. Lines were fitted by least squares regression.

nor does it provide information about the range of oocyte sizes to be found in individuals. To better understand the pattern of oocyte development leading to the formation of a clutch of mature eggs, it is necessary to examine comprehensive oocyte size-frequency profiles. Representative profiles of Figure 6 illustrate both the pattern of oocyte development during ovarian recrudescence in the striped mullet and an ovarian staging system based upon these profiles.

In ovarian stage I (previtellogenesis), yolk accumulation by developing oocytes has not yet begun, as all oocytes are less than the minimum previtellogenic size of 0.18 mm in diameter. An oocyte size-

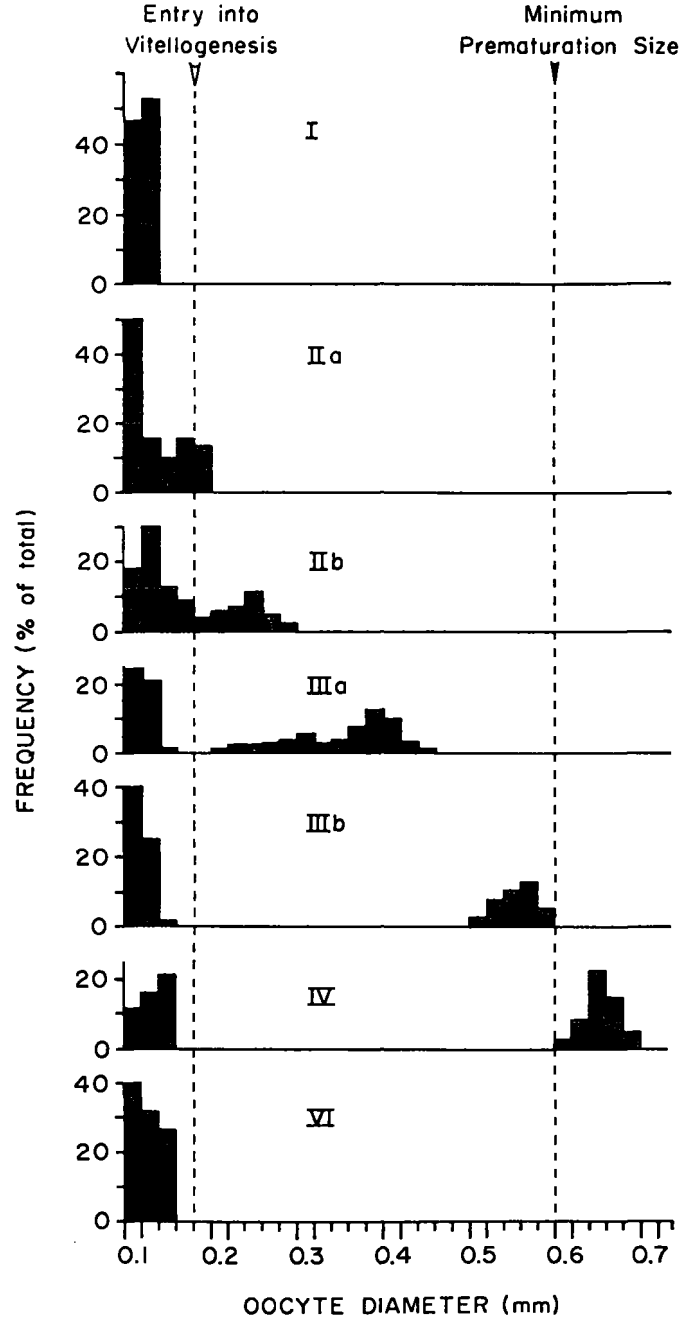

FIGURE 6.—Representative oocyte size-frequency profiles corresponding to stages of ovarian recrudescence in *Mugil cephalus*. Dashed line on left represents the transition between previtellogenic and vitellogenic oocytes; dashed line on the right delineates the minimum prematuration oocyte size. See text for explanation of stages.

frequency profile from an ovary in this stage is shown in Figure 6. GSIs of females with stage I ovaries ranged up to 0.8.

Stage II (early vitellogenesis) is characterized by recruitment of previtellogenic oocytes into vitellogenesis. A single clutch of vitellogenic oocytes starts to form, becoming distinct from the remaining mass of previtellogenic oocytes as the recruited oocytes increase in diameter due to yolk accumulation. This stage can be further divided into two substages on the basis of the appearance of the profiles. In stage IIa, recruitment into vitellogenesis has just begun; a clear separation between the developing (vitellogenic) and nondeveloping (previtellogenic) oocytes is not yet discernible. In stage IIb, the developing clutch forms a distinct peak (or peaks) as recruitment and subsequent vitellogenic growth continues. LODs of females with stage II ranged from 0.18 to 0.56 mm; GSIs varied from 0.3 to 8.5.

In stage III (mid- to late-vitellogenesis), recruitment into vitellogenesis ceases, although oocytes in the recruited clutch continue to increase in diameter due to further yolk accumulation. This stage can also be divided into two substages. In stage IIIa, recruitment has just ended: the recruited clutch is spread out in size, and multiple size-frequency peaks are apparent. In stage IIIb, the recruited clutch tightens into a single peak; oocyte diameters continue to increase. LODs of females with stage III ovaries varied from 0.40 to 0.59 mm; GSIs ranged from 2.1 to 12.1.

In stage IV (prespawning), oocytes in the recruited clutch reach the minimum prematuration size of 0.60 mm in diameter and become capable of resuming maturation in response to a hormonal signal. Late in the stage, as shown in Figure 6, all oocytes in an ovary will be at, or above, the minimum prematuration size. LODs of females with stage IV ovaries were 0.60 and 0.72 mm; GSIs were more scattered, from a low of 11.4 to a high of 21.2.

Stage V (spawning: not shown) is characterized by the presence in the ovary of maturing oocytes or mature eggs. No fish with ovaries in this stage were caught in the Inlet; however, this stage was produced in the laboratory by injection of human chorionic gonadotropin into prespawning females (Greeley et al. 1986b).

Stage VI (postspawn) ovaries, upon gross examination, are small, red, and flaccid in appearance with a thickened ovarian wall. Spawning is apparently complete—at least by the time the females return to the Inlet—as no partially spawned ovaries were collected, nor were large atretic oocytes or eggs ever observed. LODs of postspawn females were 0.12 to 0.14 mm; GSIs ranged from 0.1 to 1.6.

Monthly changes in the relative frequency of these stages in females collected from Matanzas Inlet during ovarian recrudescence in 1985-86 are shown in Figure 7. The variation in these stages is similar to the variation observed in the LOD, although the ovarian stages provide a somewhat different infor-

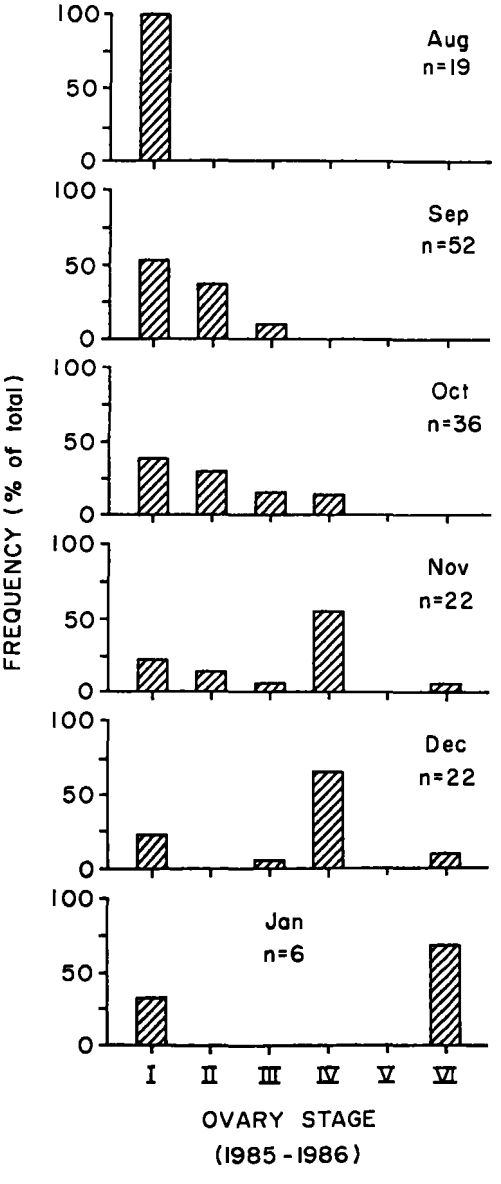

FIGURE 7.—Monthly variation in ovarian stages of adult *Mugil cephalus* (≥20 cm SL) during prespawning ovarian recrudescence along the northeast Florida coast. Data presented for the 1985-86 season only (see text).

mation. In August, only fish with previtellogenic ovaries (stage I) were collected. During September and October, a variety of ovary stages were observed, from previtellogenic (stage I) to prespawning (stage IV). Ovaries with active recruitment of oocytes into vitellogenesis (stages IIa and b) were not observed after October. The first postspawn fish (stage VI) was caught in late November, and by January only previtellogenic or postspawn fish were caught. Variation in ovary stages during the 1984-85 season (not shown) was similar, except that in this year females with prespawning (stage IV) ovaries were collected as late as mid-January.

Fecundity

Because oocyte size-frequency profiles indicate that only a single clutch of developing oocytes proceeds through vitellogenesis in a season, and that this single clutch is eventually spawned in its entirety, it is possible to calculate the individual fecundity of a female striped mullet by counting the number of vitellogenic oocytes in stages IIIa-IV ovaries (in which recruitment of oocytes into developing clutches has ceased). The annual potential fecundity, or number of eggs available to be spawned in a single breeding season, was thus found to be linearly related to body weight and geometrically related to standard length (Fig. 8). The lowest fecundity observed was 0.25×10^6 eggs in a fish 264 g BW and 23.5 cm SL, and the highest fecundity was 2.2×10^6 eggs in a fish 1,627 g BW and 44 cm SL.

DISCUSSION

The present results indirectly confirm that the spawning season of *M. cephalus* in coastal waters of northeast Florida extends from at least late November (when the first postspawn female was collected during the 1985-86 season) through mid-January (when the last prespawn female was collected during the 1984-85 season). However, there is probably a certain amount of year-to-year variation within this range: the first postspawn female was not observed until December during one season (1984-85), while the last prespawn in another (1985-86) was collected in December rather than January.

It is also probable that these dates are in reality only a conservative estimate of the actual range of the striped mullet spawning season in this area. Available evidence from other studies strongly suggest that striped mullet spawn offshore (Anderson 1958; Arnold and Thompson 1958; Finucane et al. 1978). If this is also true of striped mullet in northeast Florida, then postspawn females collected in November may have traveled extensively between spawning at offshore sites and their eventual capture in the Inlet. Likewise, the prespawn females collected in January would have required some time to reach offshore spawning sites after leaving the Inlet. Therefore, adding a month to each end of the observed range to conservatively account for such migrations may be appropriate and would make our dates consistent with previously published reports of spawning times for striped mullet in the southeast United States ranging from October through February (Broadhead 1956; Anderson 1958; Dindo and MacGregor 1981).

Do striped mullet of northeast Florida actually spawn offshore? The best evidence for offshore spawning migrations in this study was our failure to collect from the Inlet any females with spawning ovaries or even partially spent ovaries, suggesting that spawning probably occurred some distance from the Inlet. Further evidence for an offshore spawning site were the abrupt disappearances from the Inlet of fish with prespawning ovaries during both years of the study (mid-January 1984-85 and mid-December 1985-86), as this behavior suggested that mass spawning migrations to offshore waters occurred at these times.

If these disappearances did represent mass offshore spawning migrations, then how can we explain the earlier appearances in the Inlet of a few postspawn fish? Perhaps there are actually multiple spawning migrations, possibly by different populations of striped mullet moving through the Inlet at intervals throughout the period. Or perhaps some inshore spawning also occurs: staff at the Whitney Laboratory[4] have occasionally observed what they considered to be striped mullet spawning activity in the Intracoastal Waterway near the Inlet, and there are a few anecdotal accounts of inshore spawning in the literature (Breder 1940; Gunter 1945; Timoshek and Shilenkova 1975). However, if inshore spawning does occur, it must be limited in scope; otherwise, we would have collected females with spawning ovaries in the Inlet during our own studies. It may be that striped mullet can spawn either inshore or offshore, with offshore spawning favored, depending on factors—such as salinity, temperature, winds, currents, tides, or some combination thereof—which vary from locale to locale and from year to year.

[4]W. Raulerson, Whitney Laboratory, University of Florida, Route 1, Box 121, St. Augustine, FL 32086, pers. commun.

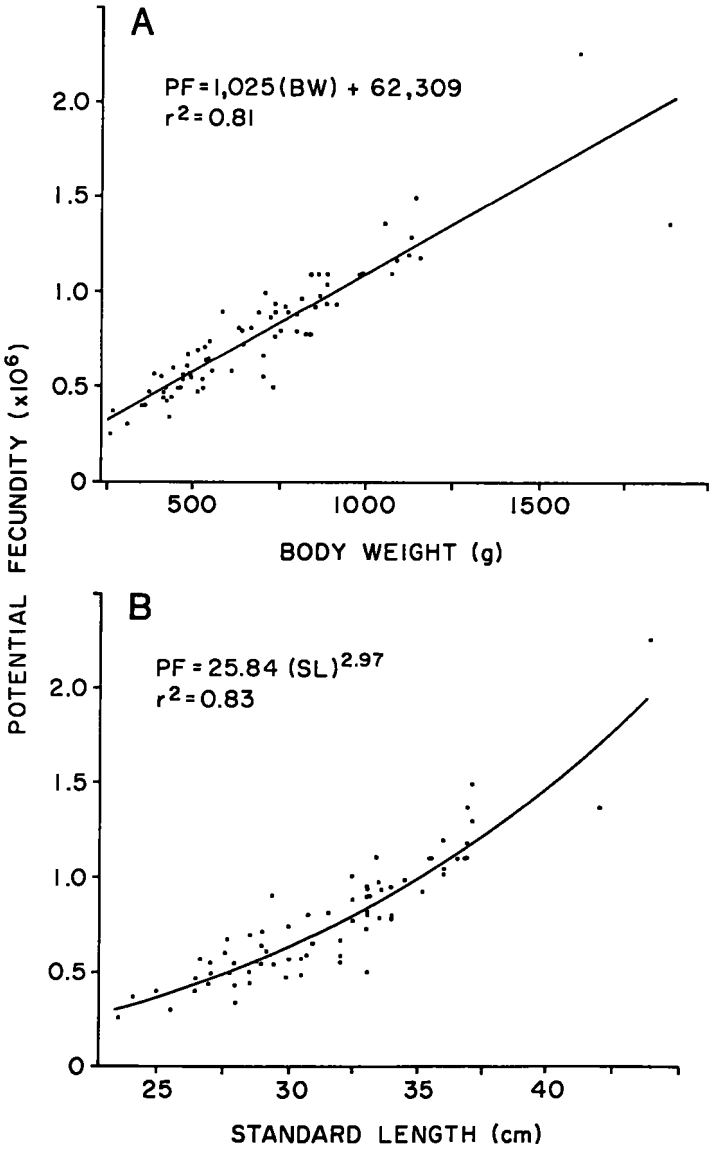

FIGURE 8.—The relationship of the potential annual fecundity of *Mugil cephalus* from northeast Florida to (A) body weight and (B) standard length. Lines are drawn from regression equations.

In fact, the apparent timing of the hypothetical final spawning migrations from the Inlet in each of the two seasons of the present study suggests there might be a tidal involvement in these events: one coincided with a set of new moon spring tides, and the other to a set of full moon spring tides. Such a tidal or lunar connection to spawning migrations of striped mullet has been proposed previously (Bromhall 1954), although supporting evidence is still inconclusive. Others have alternatively suggested wind and currents might be contributing factors to the onset of spawning migrations (Apekin and Vilenskaya 1979); further work is needed to clarify these issues.

Most workers agree that individual female *M. cephalus* spawn only once a year (Zhitenev et al. 1974; Timoshek and Shilenkova 1975; Chubb et al. 1981). Our results support this assumption, as we never observed more than a single clutch of developing oocytes proceeding through vitellogenesis dur-

ing the fall period of prespawning ovarian recrudescence. Our failure to collect any partially spawned females is also consistent with a single seasonal spawn.

In this study, both the GSI and the LOD proved to be adequate, although not completely satisfactory, indicators of the reproductive condition of female striped mullet. However, of these two indices the LOD would appear to be preferable. Determination of the LOD requires only the biopsy of a small piece of ovary (see Shehadeh et al. 1973) which can be easily accomplished without harm to the fish, while determination of the GSI requires the sacrifice of the fish. Furthermore, the validity of the GSI has been questioned (deVlaming et al. 1982) as to its accuracy in correcting for body size in a consistent manner over all reproductive stages.

On the other hand, the speed and ease of obtaining the LOD are its only advantages over a more comprehensive indicator of reproductive condition —the oocyte size-frequency profile. Such a profile also requires only the biopsy of a small piece of ovary and is a much more accurate indicator of ovarian stage, especially during the active vitellogenic growth of the ovary when a developing clutch may be quite spread-out in size.

An adequate understanding of the functional relationship between oocyte size and stage is, of course, required for correct interpretation of either LODs or oocyte size-frequency profiles. Of particular interest to us during this study were the sizes of the oocyte at 1) the beginning of vitellogenesis and 2) the prematuration stage of development. Our data indicate that oocytes of the striped mullet are able to grow to a point immediately prior to vitellogenic growth, then temporarily arrest at that stage. In contrast, once vitellogenic growth begins, further development leading to a subsequent clutch of mature eggs is apparently ensured. Thus knowledge of this transition point is extremely important to investigators attempting to predict the future reproductive status of these fish. Likewise, clearly identifying the prematuration stage of oocyte is important because at this stage the oocyte is competent to resume meiotic maturation culminating in the formation of a fertilizable egg.

We define the beginning of vitellogenesis in the striped mullet oocyte by the initial appearance of yolk proteins detectable by electrophoretic techniques and the prematuration stage of development by in vitro culture techniques. Our resulting prematuration stage is in essential agreement with the "functional maturity" stage of Kuo et al. (1974b), as is our 0.18 mm initial vitellogenic stage with the initial "yolk globule" stage (0.20 mm) of these authors. We did not examine smaller oocytes in detail and thus did not attempt to establish specific stages for previtellogenic oocytes.

The oocyte size-frequency profiles of this study, plus the additional data relating oocyte sizes and stages, demonstrate that *M. cephalus* has a group-synchronous type of oocyte development, as originally defined by Marza (1938) and reiterated by Wallace and Selman (1981). In such an ovary, a single developing clutch of oocytes coexists with an apparently asynchronous pool of previtellogenic oocytes. However, it must be pointed out that in the mullet this pattern is not always straightforward. The movement of oocytes into vitellogenesis is quite prolonged in time, so that a truly discrete clutch, as characterized by the cessation of additional recruitment into the clutch, does not become apparent until the more developed oocytes within the clutch are already well into vitellogenic growth. Furthermore, even after recruitment into vitellogenesis ceases, the developing clutch can be quite heterogenous in size (see oocyte size-frequency profile for ovarian stage IIIa, Figure 6) and thus may not appear to be undergoing synchronous growth until ovarian stage IIIb. Such a pattern of oocyte development could be very difficult to characterize without examination of ovaries from females collected throughout the period of ovarian recrudescence.

To the best of our knowledge, the ovary staging system put forth in this paper is the only such comprehensive system developed specifically for the striped mullet. Based on well-defined and physiologically significant criteria, it is presented with the purpose of standardizing future studies dealing with reproduction in the striped mullet.

The range (23 to 27 cm SL) we present for the size at maturity of striped mullet in northeast Florida is similar to the only other published report for the area (25 cm SL by Stenger 1959), but much lower than the values (30 to 46 cm SL) reported by Jacot (1920), Thomson (1951), Ochiai and Umeda (1969), Timoshek (1973), and Apekin and Vilenskaya (1979). Most investigators agree that female mullet reach sexual maturity at the end of their third year (Thomson 1951; Timoshek 1973). We made no attempt to age the fish because methods available were not always agreed upon (see Thomson 1966). However, according to the growth schedules of Thomson (1966), adapted from various primary sources, the age at maturity in this study apparently ranged from 2¼ years (23 cm SL) to 2½ years (27 cm SL).

It thus appears that female *M. cephalus* attain sex-

ual maturity in northeast Florida at a smaller size, and probably at an earlier age, than anywhere else in the world. However, caution must be taken in making such comparisons. Our results demonstrate that smaller fish typically lag behind larger fish in reaching a sexually developed state by as much as 2 months on a seasonal basis. In consequence, the determinations of size or age at maturity by other investigators, if based on only a limited number of samples or on samples obtained at only a limited number of dates within the prespawning period of ovarian recrudescence, might not be truly comparable to our results.

The annual fecundity of *M. cephalus* has been reported to be from 1.2×10^6 to 2.8×10^6 by most authors (Thomson 1966), although estimates ranged from as low as 0.5×10^6 to as high as 14×10^6 (from review by Alvarez-Lajonchere 1982). Unfortunately, the methods whereby these values were obtained are often not given, so many reports have to be considered suspect. In addition, data concerning size-related trends in the fecundity of striped mullet are nearly nonexistent. By contrast, our estimates of fecundity (0.25×10^6 to 2.5×10^6) are well-documented and demonstrate a clear and highly predictable relationship between individual fecundity and body size.

In conclusion, this study describes the pattern of oocyte development during seasonal ovarian recrudescence in the striped mullet, proposes an ovarian staging system based on oocyte stages and size-frequency profiles, gives a range of values for the female size at maturity, and presents the only comprehensive examination of size-related fecundity for *M. cephalus* to date.

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